BSP trypanosomiasis and leishmaniasis seminar

(followed by a mini-symposium celebrating prof. Alan Fairlamb's achievements)

September 1–4, 2025

České Budějovice, Czechia

INSTITUTE OF PARASITOLOGY
Molecular Biology and Biochemistry of Protists

BIOLOGY CENTRE ASCR

Welcome

Dear colleagues and friends,

It is our great pleasure to welcome you to České Budějovice, the venue of the BSP Trypanosomiasis and Leishmaniasis Seminar 2025. We greatly appreciate that you have made such a long journey. For our local team, this is the third opportunity to host this conference. From all we know, both meetings were well accepted by the participants, so we can only hope that also the upcoming meeting will be both scientifically exciting and socially entertaining.

This year's venue is the Clarion Hotel, the only hotel in České Budějovice able to accommodate all 156 participants. It seemed ideal, as it brings everything under one roof: from breakfast to the scientific program, lunch, posters, and even evening entertainment. While the immediate surroundings may not be the most picturesque, the historic center of České Budějovice is only a 10-minute walk away—provided you choose the right direction. If you take the other direction, you'll find yourself at the city's main shopping center, which for some may also be an attractive destination.

Our chosen excursion is certain to leave a lasting impression. This time, we will travel to Český Krumlov, a UNESCO World Heritage Site with a rich history and timeless charm. There you will be free to wander its winding streets and climb towards the castle, where the former riding hall (Zámecká jízdárna)—now a cultural venue—stands within its baroque gardens. For dinner and lively conversation, we will be accompanied by Swing Trio Avalon, a local band renowned for its spirited jazz and swing improvisations.

Thanks to your participation, the scientific program promises to be equally rich and inspiring. We look forward to hearing new stories, exploring new approaches, discovering new opportunities, meeting new colleagues, and sharing the vibrant atmosphere that always marks gatherings of this kind. We are fortunate to belong to such a friendly and welcoming community—open to challenges and novelties, yet steadfast in its original spirit of helping those less fortunate, which lies at the heart of research on neglected tropical diseases.

We are honored by the opportunity to organize this meeting for the BSP community and beyond. We very much look forward to welcoming you here and hope that the days ahead will bring both inspiration and fond memories.

Alena Zíková, Zdeněk Paris and Julius Lukeš

Maps

Gala Evening in Zámecká Jízdarna in Český Krumlov

Wednesday, Sept 3rd

Time schedule:

15.30 – 15.45	Departure from the Clarion hotel
16.20	Arrival to BUS STOP Český Krumlov
	Free time
17.30	Zámecká Jízdárna is opened
19.00	Reception/dinner
19.30	Jazz Trio Avalon
22.00	First departure to České Budějovice
23.30	Final departure to České Budějovice

Suggested walk:

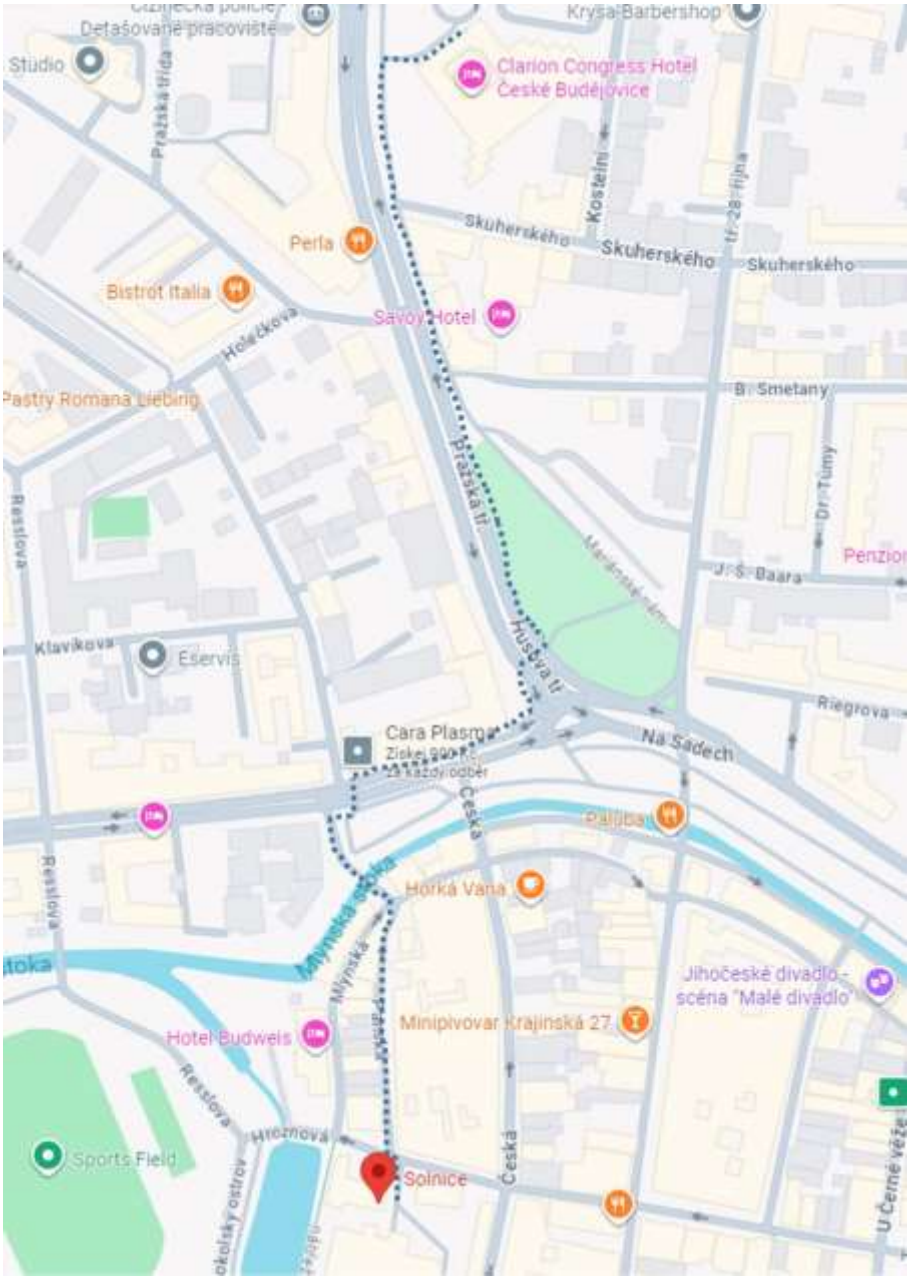
Mini-symposium celebrating prof. Alan Fairlamb's achievements

Thursday, Sept 4th

Restaurant and brewery Solnice, Piaristrické náměstí 3, České Budějovice

Time schedule:

15.00	Registration
15.30	Session I
17.00	Coffee break
17.30	Session II
19.00	Reception/dinner
23.00	End



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Oral Abstracts

Monday 1st September

Plenary - (Meeting room Rožmberk (1st Floor))

1-September-2025, at 18:00 (30 mins)

A Surprise

Prof Petr Slavíček, , *University of Chemistry and Technology*

18:30 (30 mins)

Extant Genetic Hybridization in Human-Infective Kinetoplastids

Dr Michael E. Grigg, *SENIOR INVESTIGATOR, Laboratory of Parasitic Diseases/NIAID/NIH*

Tuesday 2nd September

Session I: Mapping the Molecular Landscape of Kinetoplastids: Organelle Proteomics and Evolution - (Meeting room (1st Floor))

2-September-2025, at 08:30 (30 mins)

How do Leishmania swim forwards?

Dr Richard Wheeler, *University of Edinburgh*

Leishmania are motile parasites whose movement is crucial for their life cycle. Like all kinetoplastids, motility is driven by a single flagellum which is a complex organelle with a core structure conserved across eukaryotic life but with parasite-specific adaptations. To control their swimming, *Leishmania* alternate between a symmetric flagellar beat for forward motion and an asymmetric beat for changing direction. A fundamental, unresolved question is how flagella define and switch between beat types, vital for their function yet poorly understood in any organism. To investigate this, we comprehensively mapped the composition and organisation of the kinetoplastid flagellum, guided by the genome-wide protein localization data from the *Trypanosoma brucei* TrypTag project. Using cryo-electron microscopy, we determined the high-resolution structure of the *Leishmania* flagellum, creating an atomic-resolution model by docking Alphafold predictions, incorporating lineage-specific improvements. We then systematically analysed the swimming behaviours of deletion mutants for all axoneme proteins to identify the machinery essential for effective swimming. Further targeted detailed analysis of dynein motor proteins and asymmetrically distributed proteins pinpointed those required for either symmetric or asymmetric beats and a normal waveform. This revealed a division of labour between the inner and outer dynein arm motor protein complexes in producing different beat types, with a specific inner dynein arm likely acting as a key coordinator. We also found that asymmetries along the flagellum length primarily involve the outer dynein arms and may dictate the direction of wave propagation. This is the first comprehensive compositional, structural, and functional analysis of a flagellum, offering key insights into the biomechanics that define flagellum beating patterns and, ultimately, how parasite cells can control where they swim.

09:00 (15 mins)

Understanding the mechanism of chromosome segregation in diplonemids and kinetoplastids

Dr Bungo Akiyoshi, *Senior Lecturer, Bungo Akiyoshi*

B Akiyoshi[1];
[1] *University of Edinburgh, UK*

Diplonemids are highly abundant marine microorganisms and are the sister group of kinetoplastids. Dissecting the biology of diplonemids is key to understanding their ecological importance as well as the origin of distinct biological processes and pathogenicity in trypanosomes and leishmanias. Interestingly, diplonemids apparently do not have any known structural kinetochore proteins. By examining the localization of >60 proteins, we recently discovered unique mitotic features in a model diplonemid *Paradiplonema papillatum*. We are currently using expansion microscopy and immunoprecipitation/mass spectrometry to characterize the mechanism of chromosome segregation in this marine plankton, which will shed light on the origin of unconventional kinetochores in kinetoplastids.

09:15 (15 mins)

Identifying protein subcellular localization in *Leishmania mexicana* and *Trypanosoma cruzi* using spatial proteomics.

Dr Eden Ramalho Ferreira, *Post doctoral research associate, University of York*

ER Ferreira[5]; U Dobramysl[2]; JL Reis-Cunha[1]; A Lima[4]; AA Dowle[1]; R Wheeler[3]; J Mottram[1];
[1] *University of York, UK;* [2] *University of Oxford, UK;* [3] *Institute for Immunology and Infection Research, University of Edinburgh., UK;* [4] *Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil;* [5] *Biology Department & York Biomedical Research Institute, University of York, UK*

To further understand the biology of *L. mexicana* and *T. cruzi*, information on subcellular localization of proteins is key to making testable predictions about protein function, organelle composition and cellular architecture. For this purpose, we are using Localisation of Organelle Proteins by Isotope Tagging after Differential ultracentrifugation (LOPIT-DC). We lysated cells by nitrogen cavitation and generated eleven fractions by differential ultracentrifugation. Next, fractions from four independent experiments were labelled with TMTplex for relative quantification and analysed by LC-Mass Spectrometry. Database searching resulted in the identification of 3782 *L. mexicana* proteins and 4801 for *T. cruzi,* common in all four samples. Using the resulting set of abundance data across all fractions, we employed a Gaussian mixture model to infer the localisation of proteins with unknown location. We trained this model with manually curated annotations of protein localisation taken from the literature and highly conserved T. brucei proteins from *tryptag.org* carried over to *L. mexicana* and *T. cruzi*. t-SNE and HDBSCAN showed well defined organelles clusters such as glycosome, flagellum, ribosome, mitochondrion, nuclear sub compartments, endocytic machinery, and cytoplasm, with a mixture of known and newly identified proteins, including hypothetical proteins assigned to the organelles. In addition, new sub-clusters were also identified including intraflagellar transport; proteasome core and regulatory clusters; and mitochondrion subdivided into matrix, intermembrane and mitochondrial ribosome. Interestingly we also identified a cluster of proteins indicative of acidocalcisomes and contractile vacuole and the latter being found in *Leishmania* and *T. cruzi*, but not in *T. brucei*. Bringing strength to our model, some of the clusters were successfully validated using CRISPR-Cas9 tagging of hypothetical proteins assigned to different compartments. These data provide a comprehensive overview of organellar proteomic organization and function and new insights into organellar evolution of parasitic trypanosomatids.

09:30 (15 mins)

Subcellular proteomics illuminates interdependence between and its bacterial endosymbiont

Prof Vyacheslav Yurchenko, *Professor, University of Ostrava*

V Yurchenko[3]; M Hammond[2]; L Chmelová[3]; N Kuenzel[4]; ER Ferreira[1]; V Puente[2]; AK Maurya[4]; LR Cadena[4]; K Záhonová[3]; AA Dowle[1]; E Nowack[4]; J Lukeš[2];
[1] University of York, UK; [2] Institute of Parasitology, Biology Centre, Czechia; [3] University of Ostrava, Czechia; [4] Heinrich Heine University Düsseldorf, Germany

The relationships between symbionts and Strigomonadinae appear to be well-integrated and mutualistic, as evident from their reduced genome sizes, coordinated cell cycles, association with glycosomes, and established metabolic cooperation. To gain a more comprehensive perspective on the host-endosymbiont interactions, we employed subcellular proteomics of *A. deanei*, resolving over 5,000 proteins and assigning 2,938 specifically to cell organelles as well as the endosymbiont, further identifying 7 new ETPs via predictive clustering. Our enzymatic localization demonstrates the endosymbiont's metabolic dependence on energy substrates provided by the glycosomes, underpinning their close association. We additionally used this dataset to identify a novel association between the endosymbiont and acidocalcisomes of Strigomonadinae that likely mediates calcium signaling between both compartments.

09:45 (15 mins)

A novel method for pooled gene tagging using CRISPR/Cas9 with stably integrated repair templates (Cas-Tag)

Dr James Budzak, *Post-doctoral researcher, Ludwig-Maximilians-Universität (LMU)*

J Budzak[1]; MR Schmidt[1]; C Weist[1]; YT Schnellbach[1]; TN Siegel[1];
[1] Ludwig-Maximilians-Universität (LMU), Germany

Endogenous gene tagging is an essential genetic tool for the study of protein function, but its throughput is limited. Recently, pooled gene tagging methods have been used to tag thousands of proteins simultaneously. However, current techniques for pooled gene tagging rely on non-homologous end joining (NHEJ) or prime editing, which can only insert small tags (<120 nt).

To address this challenge, we developed Cas-Tag, a novel method of pooled gene tagging that uses CRISPR/Cas9 with stably integrated repair templates that does not require NHEJ and can insert large tags. We demonstrate that Cas-Tag can be used to tag genes with high efficiency in *T. brucei* without the need for drug selection or the modification of endogenous UTRs. Cas-Tag can be used to transfect cells with plasmid libraries to tag many genes simultaneously. We anticipate this method will accelerate the scalability of genetic screens using gene tagging in kinetoplastids and many other organisms.

10:00 (15 mins)

LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Dr Ulrich Dobramysl, *Data Scientist, University of Oxford*

U Dobramysl[3]; E Ferreira[4]; RP Neish[1]; LD Davidson[9]; R Pereira[2]; R Etzensperger[2]; S Aellig[2]; M Young[5]; J Smith[5]; J Damasceno[7]; JD Sunter[6]; J Mottram[1]; E Gluenz[2]; R Wheeler[8];
[1] University of York, UK; [2] Institute of Cell Biology, University of Bern, Switzerland; [3] University of Oxford, UK; [4] University of York, Centre for Immunology and Infection, UK; [5] Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [6] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [7] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [8] Institute for Immunology and Infection Research, University of Edinburgh., UK; [9] Oxford Brookes University, UK

Success of Leishmania as a parasite is encoded in its genome, yet only a small proportion of the ~8,000 genes have been experimentally analysed. The LeishGEM project is addressing this, taking the 8,267 *L. mexicana* protein coding-genes and: 1) Determining the fitness of barcoded deletion mutants cell lines as promastigotes, axenic amastigotes, amastigotes in macrophages, and amastigotes in a mouse footpad infection; 2) Visualising the sub-cellular localisation of proteins, with high sequence dissimilarity to *T. brucei*, in promastigotes and axenic amastigotes by fluorescent protein tagging at the N and C terminus; 3) Analysing the sub-cellular localisation of all mass spectrometry-detectable proteins in promastigote using LOPIT-DC. The data generation phase of the LeishGEM project is almost complete, with the data available at browse.leishgem.org and leishtag.org. We will present our initial near genome-wide analyses of deletion mutant fitness and evolution of parasitism.

10:15 (15 mins)

Expansion microscopy as a tool to study and visualize the 3D cellular architecture of protozoan parasites

Dr Vladimir Varga, *PI, Institute of Molecular Genetics*

V Varga[1]; L Stepanek[1]; M Zelena[1];
[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Czechia*

Expansion microscopy is a super-resolution approach based on the physical expansion of a specimen and its imaging using fluorescence microscopy. It has numerous advantages, such as the ability to localize biomolecules in an entire cellular volume at significantly increased resolution, the ability to identify and image rare cells within a population, and it is rapid. We have been using the approach to study kinetoplastids, in particular *Trypanosoma brucei*, and observed, that it is well suited to characterize cellular architecture, its changes during differentiation, and biogenesis of organelles. Importantly, expansion microscopy data can be easily segmented, facilitating their 3D representation. Using these data we have been exploring possibilities of 3D printing scientifically accurate models of protozoan cells including their internal architecture. Our aim is to create a library of such 3D printed models, which can be used for educational purposes.

-Coffee Break-

Session II: Genetic Exchange, Hybridization, and Evolutionary Dynamics in Kinetoplastid Parasites - (Meeting room (1st Floor))

2-September-2025, at 11:00 (15 mins)

Identification of the *Trypanosoma brucei gambiense* genes necessary and sufficient for resistance to the human Trypanolytic Lytic Factor

Prof Mark Carrington, PI, University of Cambridge

N Minshall[1]; S Banerjee[1]; H Webb[1]; A Cook[2]; B Bakewell-Smith[2]; S Hester[4]; O Macleod[1]; M Taylor[3]; M Higgins[2]; M Carrington[1];
[1] *University of Cambridge, UK;* [2] *University of Oxford, UK;* [3] *London School of Hygiene and Tropical Medicine, UK;* [4] *Nuffield Department of Medicine, University of Oxford, UK*

Trypanosoma brucei brucei is killed by trypanolytic factors (TLFs) in human serum. Here, we identify a set of genetic changes present in *T. b. gambiense* that are sufficient to confer TLF resistance on T. b. brucei. Deletion of the previously identified TgsGP gene did not prevent proliferation in human serum. *T. b.brucei* Lister 427 cells were modified to express TgsGP and transfected with sheared *T. b. gambiense* genomic DNA, three clones that grew in human serum selected. RNAseq analysis showed all clones had included polymorphisms from

Tbg.972.2.1820. Manipulation of T. b. brucei Lister427 cell lines showed that expression of TgsGP and Tbg.972.2.1820 was sufficient for growth in human serum. Deletion of Tbg.972.2.1820 from *T. b. gambiense* revealed that the gene is not essential for growth in bovine serum but is necessary for growth in human serum. Thus, Tbg.972.2.1820 and TgsGP are sufficient and Tbg.972.2.1820 is necessary for growth in human serum.

11:15 (15 mins)

A story of ageing and rejuvenation: mitochondrial DNA dynamics in trypanosomatid parasites

Dr Achim Schnaufer, *Prof of Parasite and Mito Biology, University of Edinburgh*

Z Chen[2]; E Wadsworth[2]; S Cooper[2]; C Clucas[4]; A MacLeod[4]; M Geerts[3]; L Vermeiren[3]; P Monsieurs[3]; N Van Reet[3]; J Van Den Abbeele[3]; P Büscher[1]; F Van den Broeck[3]; N Savill[2]; A Schnaufer[2];
[1] Institute Tropical Medicine, Antwerp, Belgium; [2] Institute of Immunology and Infection Research, University of Edinburgh, UK; [3] Institute of Tropical Medicine, Antwerp, Belgium; [4] Wellcome Centre for Integrative Parasitology, University of Glasgow, UK

Mitochondrial kinetoplast DNA (kDNA) is a network of thousands of interlinked DNA rings: ~30 maxicircles encode subunits of the OXPHOS system and the mitoribosome; thousands of minicircles encode gRNAs, which direct the extensive post-transcriptional editing of the maxicircle-encoded pre-mRNAs. Parasite sex in the insect vector involves recombination of parental kDNA.
We assembled kDNA of 224 *Trypanosoma brucei* field isolates and demonstrate that the absence of sex in the sleeping sickness parasite *T. b. gambiense* type I (TbgI) results in loss of kDNA coding capacity. In contrast to the redundant kDNA content typical for T. b. brucei, TbgI isolates contain a minimal kDNA that, in extreme cases, is dysfunctional and incompatible with transmissibility. We conclude that sex is critical for maintaining trypanosome mitochondrial fitness and transmissibility. In its absence, parasite populations show mitochondrial ageing and dysfunction. We suggest that TbgI are on a path to extinction.

11:30 (15 mins)

How Sex Reshapes the Genome: Comparative Long-Read and Chromatin Conformation Analysis of Clonal and Sexual *Trypanosoma cruzi* Strains

Dr Kyrie Dickson, *Research Associate, University of Glasgow*

KP Dickson[1]; JA Costales[2]; P Schwabl[3]; R McCulloch[1]; MS Llewellyn[1]; T Otto[1];
[1] University of Glasgow , UK; [2] El Centro de Investigación para la Salud en América Latina (CISeAL), Ecudor; [3] Harvard T.H. Chan School of Public Health, , United States

Once believed to be a strictly clonal organism, *Trypanosoma cruzi* is now known to undergo hybridisation and recombination in natural populations - processes likely central to its adaptability and evolution. We generated high-contiguity genome assemblies for six Ecuadorian TcI isolates (three from clonal populations, three from sexually reproducing populations) using PacBio HiFi and Pore-C sequencing to investigate ploidy shifts and structural variants associated with reproductive strategy. Initial findings reveal substantial divergence within and between groups, suggesting dynamic genome evolution, along with features common across both populations (such as conserved polysomy) and features unique to each group (such as linkage decay and relative heterozygosity, shown in previous work). Our ongoing analysis aims to provide foundational insights into how sexual reproduction may shape genome diversity and organisation in *T. cruzi*, as well as potential implications for parasite fitness and pathogenicity.

11:45 (15 mins)

Mitochondrial minicircle loss impairs RNA editing and attenuates virulence in Leishmania

Dr Tiago Rodrigues Ferreira, Research Fellow, Laboratory of Parasitic Diseases, Division of Intramural Research, NIAID, NIH

T Ferreira[2]; Z Chen[1]; E Inbar[2]; C Catta-Preta[2]; M Chaves[2]; A Ivens[1]; N Savill[1]; A Schnaufer[1]; DL Sacks[2];
[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Laboratory of Parasitic Diseases, National Institutes of Health, NIAID, United States

The role of kinetoplast RNA editing in Leishmania infection in vivo remains elusive. Here, we show that spontaneous loss of specific minicircle classes in axenic culture attenuates virulence in cutaneous leishmaniasis. Using a genetic cross between two Leishmania major Fn clones differing in lesion severity, we generated hybrids via sand fly co-infection. Strikingly, hybrids segregated across hypervirulent to avirulent phenotypes in mice. NGS revealed significant variation in minicircle composition, with avirulent hybrids lacking specific classes targeting complex I (cI). RNA-seq confirmed increased or impaired cI mRNA editing efficiency in hyper- or avirulent hybrids, respectively. Deletion of a cI subunit recapitulated the attenuation phenotype in mice, while add-back restored virulence, linking cI to infection. We propose that the kDNA is a critical, heritable determinant of pathogenicity. Our work reveals the functional impact of minicircle composition in parasite fitness.

12:00 (15 mins)

Metagenomic analysis of *L. infantum* hybrid field isolates reveals antigenic variation as a possible driver of emerging visceral and cutaneous infection

Dr Gerald Spaeth, *Professor, Institut Pasteur*

G Späth[1]; F Bruno[3]; G Castelli[3]; B Li[1]; T Cokelaer[1]; J Pipoli da Fonseca[1]; S Reale[3]; E Carra[4]; S Varani[2]; F Vitale[3]; S Scibetta[3]; C Rugna[4];
[1] Institut Pasteur, France; [2] University of Bologna, Italy; [3] IZS Sicily, Italy; [4] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", Italy

Inter-species *Leishmania* hybridization has been proposed as a major driver of genome evolution and phenotypic innovation. Our recent discovery of *L. infantum/L. donovani* hybrid isolates causing emerging, human visceral leishmaniasis (VL) in northern Italy established a firm role of *Leishmania* sexual reproduction in shaping the clinical outcome of infection (Bruno et al., 2024). Here, applying Illumina short-read and Nanopore long-read sequencing analyses, we report the comparative genomics analysis of four novel *L. infantum* field isolates causing atypical cutaneous leishmaniasis in immunocompetent patients across the Italian peninsula.

Based on SNP profile, these isolates fall into two distinct clusters that were separate from the previously identified VL-causing hybrids, suggesting their independent origin and the frequent emergence of inter-species hybrids with increased pathogenic potential. Adapting the Kraken metagenomic sequence classification system on the analysis of *Leishmania* hybrid genomes uncovered a significant percentage of reads uniquely mapping to the *L. donovani* (over 4%) and *L. tropica* (over 2%) reference genomes. This suggests a complex hybridization history involving possible multiple hybridization events, followed by recombination and gene conversion processes that have markedly reduced linkage disequilibrium. Gene-level assessment revealed a striking enrichment of *L. donovani*- and *L. tropica*-derived hybrid genes encoding for various families of surface proteins, including amastins and gp63. Further evolutionary analyses across the *Leishmania* genus identified amastins as a hyper-evolvable gene family, underscoring their potential role in immune escape. Conceivably, the expression of *L. donovani* and/or *L. tropica* proteins on the surface of *L. infantum* parasites as well as the hyper-variability of genes that are crucial for the immune response could facilitate immune evasion during

transmission in *L. infantum*-exposed populations, with the potential to cause the unusual spectrum of cutaneous and visceral disease phenotypes observed for these hybrids.

Together, our results highlight the role of inter-species hybridization in enhancing *Leishmania* fitness during clinical infection and reveal a novel form of antigenic variation that may confer selective advantages right after transmission rather than operating during chronic infection.

Reference

Bruno F, Castelli G, Li B, Reale S, Carra E, Vitale F, Scibetta S, Calzolari M, Varani S, Ortalli M, Franceschini E, Gennari W, Rugna G, Späth GF. Genomic and epidemiological evidence for the emergence of a *L. infantum*/*L. donovani* hybrid with unusual epidemiology in northern Italy. mBio. 2024 Jul 17;15(7):e0099524. doi:10.1128/mbio.00995-24. Epub 2024 Jun 4. PMID: 38832792

12:15 (10 mins)

2 Min Speed talks (see Poster abstracts for further details)

Rokyay Ahmad, Jorge Arias del Angel, Jovana Sádlová, Sergio Araujo and Osvaldo Pompilio de Melo

-Lunch-

Poster Session I (odd numbered posters) (Hallway, meeting room Svět)

13:30 (120 mins)

-Coffee Break-

Session III: Structural and Molecular Innovations in Kinetoplastid Cell Biology - (Meeting room (1st Floor))

2-September-2025, at 16:00 (30 mins)

SL RNA recognition by the unusual trypanosomatid cap-binding complex

Dr Eva Kowalinski, *EMBL, Grenoble*

E Kowalinski[1];
[1] *EMBL, Grenoble, France*

The RNA 5'-cap-binding complex is central to the fate of eukaryotic RNAs. Trypanosomatids are eukaryotic protozoans, bearing unusual RNA biogenesis pathways. The processing of their primary expressed pre-mRNA polycistrons requires the coupled action of trans-splicing and polyadenylation to yield monogenic mature mRNAs for all genes. As a result of the process, all mature mRNAs carry a unique and identical sequence, the SL RNA mini-exon with a hypermethylated cap-4 structure at their 5'-ends. The SL RNA is bound by the unusual trypanosomatid cap-binding complex (CBC). CBC is vital for the cell and necessary all mRNA processing. In difference to mammals and yeast, trypanosomatid CBC consists of four subunits, three of which lack sequence homology to annotated proteins.
Here, we present the structural and functional characterization of the Trypanosoma brucei CBC. We reveal the function of its subunits. Through binding assays with fully modified synthetic cap-4 SL RNA, we define the molecular SL RNA binding properties of trypanosomatid CBC. Our refined understanding of trypanosomatid CBC will now enable further detailed studies of RNA processing pathways in these parasites. Due to the non-conserved nature of the complex, it may serve as a future anti-infective drug target.

16:30 (15 mins)

Filling in the missing pieces of the trypanosomal cytochrome c reductase complex: Overlooked microprotein subunits are revealed by complexome profiling

Mr Hassan Hashimi, *Associate Professor, Biology Centre, Czech Academy of Sciences*

C Benz[2]; R Khan[3]; U Kobler[1]; LR Cadena[2]; M Vrbacký[3]; H Hashimi[2];
[1] Faculty of Science, University of South Bohemia in České Budějovice, Czechia; [2] Biology Centre, Institute of Parasitology, CAS, Czechia; [3] Institute of Physiology, Czech Academy of Sciences, Czechia

The ubiquitous respiratory chain (RC) multiprotein complexes conduct oxidative phosphorylation, the coupling of nutrient combustion with ATP production. Thus, resolving the molecular composition of the RC from various eukaryotes is paramount to understanding their evolution. RC complexes incorporate microproteins, which are often overlooked due to their small size (>150 amino acids). Here, we employ complexome profiling (CP) for the first time in *Trypanosome brucei* to find four microproteins that assemble into cytochrome c reductase, also named Complex III. CP works on the principle that subunits of the same complex will exhibit the same separation pattern when resolved by Native-PAGE. We show that these four microproteins are essential for both essential and conversely rely on proper Complex III assembly. Thus, we have validated CP as a way to find novel subunits of mitochondrial (and other) complexes filling in the missing pieces of macromolecular mosiacs.

16:45 (15 mins)

Metabolic Disruption of the Branched-Chain Amino Acid Degradation Pathway in Trypanosoma cruzi: Exometabolomic Insights from Gene Knockouts

Dr Sabrina Marsiccobetre, *Post Doctoral Researcher, University of São Paulo*

S Marsiccobetre[1]; MS Ballari[1]; MB Alencar[1]; AC Mengarda[1]; HF Santos Souza[1]; JF Nascimento[1]; AM Silber[1];
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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, remains a major public health concern across the Americas. *T. cruzi* exhibits remarkable metabolic versatility, relying on amino acids not only as an alternative to glucose for carbon and energy sources but also for key physiological processes, including life cycle progression, adaptation to environmental stress, and successful parasite-host cell interaction. In this study, we investigated how different amino acid metabolic pathways in *T. cruzi* interconnect with the branched-chain amino acids (BCAA: leucine, isoleucine, and valine) degradation pathway and influence biological processes. To this end, we derived the exofluxome, the flux of excreted metabolic products, from the parasite exometabolome, with a particular focus on comparing knockout lineages for specific enzymes with the wild-type and parental (Cas9) strain. We conducted proton nuclear magnetic resonance (^{1}H-NMR)-based exometabolomic analysis of knockout strains deficient in enzymes involved in the BCAA degradation pathway. The knocked-out genes encoded subunits of the branched-chain α-keto acid dehydrogenase complex (E1, E2, E3), isovaleryl-CoA dehydrogenase (IVDH), and enoyl-CoA hydratase (ECH). Key findings include the increased excretion of propylene glycol and formate (e.g., in E3a KO and IVDH KO strains), the reduced acetone excretion (e.g., in E1α KO and IVDH KO strains), and altered levels of isobutyrate and 2-hydroxyisovalerate in the E1α KO strain incubated with valine. While most alterations aligned with the targeted pathways, some metabolites, such as formate and propylene glycol, showed unexpected changes, suggesting metabolic cross-talk with other pathways or compensatory mechanisms requiring further investigation. These findings support the existence of metabolic interdependence between amino acid pathways in *T. cruzi* and underscore the biological significance of BCAA degradation enzymes. Ultimately, this knowledge may aid in identifying novel therapeutic targets against the parasite.

17:00 (15 mins)

Three paralogs of Oxa1 insertases in *Trypanosoma brucei* show specific functions in biogenesis of mitochondrial membranes

Mr ONDŘEJ GAHURA, *Researcher, Institute of Parasitology, Biology Centre CAS*

JW Wong[1]; A Zikova[1]; O Gahura[1];
[1] *Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czechia*

Oxa1 is an insertase of proteins into the inner mitochondrial membrane (IMM) essential for the biogenesis of OXPHOS complexes. *Trypanosoma brucei* encodes three putative Oxa1 paralogs, TbOxa1-1, 1-2 and 1-3. In the bloodstream form, which relies on ATP synthase to maintain IMM potential, knock-out of TbOxa1-2 caused reduced membrane potential and a decreased ATP synthase levels, due to defects in assembly of the nuclear encoded c-ring. In procyclic *T. brucei*, KO of TbOxa-1 and 1-2 resulted in growth arrest and loss of cI and cIV, and cIII and ATP synthase, respectively. Proteomic analysis of submitochondrial fractions showed distinct alterations across mitochondrial compartments, together indicating specific roles of TbOxa1 paralogs in the IMM biogenesis. The ablation of TbOxa1-2 led to accumulation of IMM proteins in matrix, suggesting its role in conservative sorting. We identified a rhomboid peptidase-like protein that facilitates ATP synthase assembly together with TbOxa1-2.

17:15 (15 mins)

Regulation of a constitutive kinetochore protein, KKT3 in unconventional trypanosome kinetochores

Dr Midori Ishii, *postdoc, University of Edinburgh*

M Ishii[1]; B Akiyoshi[1];
[1] *University of Edinburgh, UK*

The kinetochore is a macro-molecular protein complex which links the centromeric DNA to spindle microtubules during mitosis. Although kinetochore components are highly conserved among eukaryotes, kinetoplastid species do not have any canonical kinetochore proteins. Instead, a number of kinetochore proteins (KKT1-25 and KKIP1-12) have been identified in *Trypanosoma brucei*. It remains unknown how trypanosomes assemble kinetochores specifically at the centromere. KKT3, a core protein for kinetochore assembly, localizes constitutively at centromeres. Unexpectedly, we found that the centromere localization of KKT3 depends on the KKT16 complex (KKT16/KKT17/KKT18), which has similarities to the meiotic synaptonemal complex and localizes transiently at the kinetochore. Pulse-chase experiments showed that KKT16 complex was important for the recruitment of KKT3 at the kinetochore. We are currently working towards understanding the regulatory mechanism between KKT16 complex and KKT3.

17:30 (15 mins)

A central pair assembly complex revealed by combinatorial RNAi and ultrastructure expansion microscopy

Dr Samuel Dean, *Assistant Professor, University of Warwick*

A Paterou[1]; S Dean[1];
[1] *Division of Biomedical Sciences, Warwick Medical School, UK*

The African trypanosome flagellum is essential for its motility, infectivity and transmission. During flagellar biogenesis, the probasal body (pBB) elongates and remodels to become the basal body (BB), transition zone

(TZ) and axoneme. Axoneme beating is orchestrated by a central pair (CP) of microtubules that emerge from the "basal plate", an electron-dense structure at the distal end of the TZ. Previously, we defined a fully validated TZ proteome and showed basalin and TZP103.8 were basal plate components essential for nucleating the CP (Dean et al., 2016; Dean et al., 2019).

Here, we use a novel RNAi screen to identify two additional CP Assembly Proteins (CPAPs). Using ultra-expansion microscopy (UExM) co-localisation, we show that basal plate CPAPs form a complex arrangement of toroids that surround a TZP103.8 core. Targeting CPAPs for RNAi in tagged cell lines reveals “themelin” is the CPAP master recruiter at the pBB, and that the final step of basal plate maturation is co-recruitment of TZP103.8 and gamma tubulin to form a "central pair assembly complex" (CPAC). We propose that the pBB contains a "pre-basal plate" that matures to form the TZ basal plate.

17:45 (15 mins)

New insights into the kinetoplast, within the mitochondrion of Trypanosomatids

Dr Michael Hammond, *Post-Doc Researcher, Institute of Parasitology Czech Acadamy of Science*

M Hammond[3]; M Pendlebury[2]; J Lukes[1];
[1] Institute of Parasitology, Biology Centre, ASCR, Czechia; [2] Institute of Parasitology, Biology Centre, Czechia; [3] Institute of Parasitology Czech Acadamy of Science, Czechia

The mitochondrial DNA (called kinetoplast DNA or kDNA) of trypanosomatids is represented as a singular concentration of interconnected DNA circles, itself within the singular mitochondrion of these organanisms. Here we outline our advances in resolving ‘kinetoplast proteins’, which coordinate the associated processes involved in maintaining this complex arrangement within *Trypanosoma brucei*. We show the recent identification of the first kinetoplast DNA segregation factor, as well as a category of kinetoplast proteins involved in a newly described kDNA association mechanism. We extrapolate demonstrated mitochondrial translocation and association implications across the parasitic clade of trypanosomatids to provide insight into the import innovations developed by this group of parasitic protists.

Wednesday 3rd September

Session IV: Drug Targets, Mechanisms, and Resistance in Kinetoplastid Parasites - (Meeting room (1st Floor))

3-September-2025, at 08:30 (30 mins)

Functional genomics in Leishmania

Dr Tom Beneke, *Junior Group Leader, University of Wuerzburg*

Functional genomics is a key approach to understanding how genetic variation shapes critical processes in Leishmania biology, including drug resistance. While mechanisms of resistance have been extensively studied, other genetic factors influencing drug response remain poorly defined, such as those underlying collateral sensitivities and persister-like behaviour. This knowledge gap limits our understanding of drug interactions and hinders the development of improved therapies, such as combination treatments. To address this, we generated a genome-wide map of genotype–phenotype associations linked to drug response using a CRISPR/Cas9 cytosine base editing screen. This functional genomics approach also enabled systematic mapping of single- and multi-copy genes affecting parasite fitness in both promastigotes and amastigotes across Leishmania species. In my talk, I will present key findings on gene fitness, drug response, and species-specific differences, and highlight the complexity and interconnectivity of the underlying genetic content in *Leishmania*.

09:00 (15 mins)

Targeting a unique mRNA decapping enzyme for trypanosomatid infectious disease drug discovery

Dr Martin Zoltner, *Head of Drug Discovery, Charles University in Prague*

EA Agbebi[2]; M Grechnikova[2]; L Pereira[1]; D Dzadz[6]; AA Amaral[1]; N Karolak[6]; M Warminski[3]; M Bednarczyk[3]; J Jemielity[4]; P Sacha[5]; J Kowalska[3]; MW Gorna[6]; SK Kramer[1]; M Zoltner[2];
[1] Biozentrum der Universität Würzburg, Germany; [2] BIOCEV, Department of Parasitology, Charles University, Czechia; [3] Division of Biophysics, Faculty of Physics, University of Warsaw, Warsaw, Poland, Poland; [4] Centre of New Technologies, University of Warsaw, Warsaw, Poland, Poland; [5] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic, Czechia; [6] Structural Biology Group, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Warsaw, Poland, Czechia

Trypanosomatids encode no homologues of canonical decapping enzymes, but employ the ApaH-like phosphatase ALPH1, belonging to a phosphatase family absent in mammalian systems, for the essential process of mRNA decapping. We have shown that T. brucei ALPH1 functions within a decapping complex and thorough characterisation, both biochemically and in the cellular context, renders ALPH1 a robust drug target. We set out to conduct a screening campaign to identify inhibitors with the potential to become highly selective candidate drugs for trypanosomatid caused diseases, including African sleeping sickness, Chagas Disease and the leishmaniases. We have developed a robust assay strategy to monitor inhibition of ALPH1 activity, relying on the quantification of ADP liberated from a dinucleotide cap analog. Here we present novel mechanistic insights into the trypanosome decapping complex and report the preliminary outcome of our pilot screen encompassing 12760 small molecules.

09:15 (15 mins)

Structure and Activity of the Essential Deubiquitinase DUB16 from Leishmania donovani

Prof Anthony Wilkinson, *Professor, University of York*

AJ Wilkinson[1]; JA Brannigan[1]; M Kamran[2]; NG Jones[1]; EM Nightingale[1]; SA Ejazi[2]; J Mottram[1]; N Ali[2];
[1] University of York, UK; [2] CSIR-Indian Institute of Chemical Biology, India

In Leishmania parasites, the ubiquitin proteasome system is important for cell viability. DUB16 is one of four cysteine protease type deubiquitinases (DUBs) essential for parasite survival in the promastigote stage. The crystal structure of DUB16 from *Leishmania donovani* reveals a recessed active site to which substrate access is restricted by a crossover loop. The enzyme efficiently hydrolyses C-terminal aminocoumarin and rhodamine conjugates of ubiquitin, while exhibiting very low but selective deubiquitinase activity towards Lys11, Lys48 or Lys63 linked diubiquitins. With much higher turnover numbers, DUB16 cleaves the ubiquitin-ribosomal L40 fusion protein to give the mature products pointing to an important role in ribosome biogenesis. A DUB-targeting cysteine-reactive cyanopyrrolidine compound, IMP-1710, inhibits DUB16 activity and exhibits parasite killing activity with comparable potency to the anti-leishmanial drug, miltefosine. *L. mexicana* parasites engineered to overproduce DUB16 showed a modest increase in resistance to IMP-1710, providing preliminary evidence for on-target inhibition *in vivo*.

09:30 (15 mins)

How the Structure of a *T. cruzi* Dehydrogenase Facilitates Understanding, Predicting, and Engineering of NAD/NADP Specificity in Enzymes Across All Kingdoms

Prof Erik Debler, *Associate Professor, Thomas Jefferson University*

EW Debler[2]; H Hashimoto[2]; IH Mawn[2]; W Escobar-Arrilaga[1]; L Nguyen[1]; LA Madigan[1]; G Antuono[1]; T Rossy[1]; J Sojati[1]; A Mienko[2]; JP Palenchar[1];
[1] Villanova University, United States; [2] Thomas Jefferson University, United States

NAD and NADP are common redox cofactors in biology and are primarily used in catabolic and anabolic reactions, respectively. NAD/NADP specificity is highly defined by each enzyme; bacterial β-hydroxybutyrate dehydrogenases (HBDHs) strictly exclude NADP usage, while HBDHs of trypanosomes – global agents of devastating parasitic diseases – utilize both NAD(P) or solely NADP. Here, we will present the mechanism of strict *Trypanosoma cruzi* HBDH NADP specificity and describe how we altered its cofactor specificity and increased its catalytic rate tenfold through mutagenesis, providing guidelines for increasing activity and engineering cofactor specificity in HBDHs and related NAD/NADP-dependent enzymes. This mechanism of cofactor selectivity constitutes a new HBDH class. Our structures of trypanosomal HBDHs will be valuable for investigating their enigmatic functions and facilitate drug discovery towards parasitic diseases.

09:45 (15 mins)

A common mechanism of resistance to the benzoxaboroles among pathogenic animal trypanosomes

Dr Federica Giordani, *Research Associate, University of Glasgow*

F Giordani[1]; V Suresh[1]; G Hamilton[1]; M Bakari-Soale[3]; B Wickstead[3]; C Gadelha[3]; R Young[4]; P Steketee[4]; L Morrison[4]; C Lapsley[1]; M Krasilnikova[1]; R McCulloch[1]; M Pearce[2]; M Barrett[1];
[1] University of Glasgow , UK; [2] GALVmed, UK; [3] University of Nottingham, UK; [4] The Roslin Institute, University of Edinburgh, UK

The development of a new class of trypanocides would represent an extraordinary step forward for the control of the neglected disease African Animal Trypanosomosis, especially where resistance to current drugs is found. Benzoxaboroles belonging to the valinate amide subclass (OX) show great promise in this respect, but thorough analysis of the risk and mechanisms of resistance to these compounds is essential during early evaluation. We previously showed that in *Trypanosoma brucei brucei* these compounds behave as prodrugs that, once inside the parasite, are cleaved to a highly potent derivative by the action of carboxypeptidases (CBP1s). We also showed that deletion of these activating enzymes from the genome is associated with resistance to the OXs. Similar results were found for *T. congolense*. However, the higher complexity of the CBP1 locus in this latter species did not allow an accurate analysis by Illumina WGS. Here we present additional experimental results aimed at deepening our understanding of resistance to the OX in pathogenic animal trypanosomes. Nanopore sequencing was used to re-analyse the CBP1 locus in *T. congolense* wild type and in an array of OX-resistant lines. The analysis revealed that the locus in *T. congolense* wild type contains more genes than previously known. In the OX-resistant lines major rearrangements were observed, with deletions and duplications of specific gene forms, suggesting that not all genes within the tandem array contribute equally to the resistance phenotype. We also showed that in the other pathogenic veterinary species *T. vivax*, the single CBP1 copy is not deleted, but point mutations disrupt its expression in laboratory-selected resistant lines. Amplification of the main OX biological target, the Cleavage and Polyadenylation Specificity Factor (CPSF3), was also found to be a possible contributor to resistance, at least in T. brucei. Finally, we discuss preliminary findings regarding OX resistance in *T. evansi.*

10:00 (15 mins)

Acoziborole resistance-associated mutations in TbCPSF3

Dr Marketa Novotna, *Research assistant, University of Dundee*

M Ridgway[1]; M Novotna[1]; S Altmann[1]; M Tinti[1]; D Horn[1];
[1] *University of Dundee, UK*

Acoziborole is a safe, single dose, oral therapy, suitable for treatment of both early and late-stage human African trypanosomiasis, and also currently under development for paediatric application. Oxaboroles also show efficacy against other trypanosomatids, apicomplexans and fungi. Acoziborole targets cleavage and polyadenylation specificity factor 3 (CPSF3; see PMID: 30185555), but others have suggested CPSF3-independent modes of action. To determine whether CPSF3 is the primary target of acoziborole, we used Oligo-Targeting (PMID:35524555) for saturation mutagenesis around the putative acoziborole binding-site. Among >1000 edits tested, only $Asn^{232}His$ edits conferred three-fold resistance to acoziborole. By targeting multiple codons simultaneously, however, we found that an $Asn^{232}His$, $Tyr^{383}Phe$, $Asn^{448}Gln$ triple-mutant conferred thirty-fold resistance to acoziborole. These results highlight the versatility of Oligo-Targeting and demonstrate that CPSF3 is indeed the primary target of acoziborole. We find that all three edits above are homozygous and present on the same allele and we are currently exploiting these strains to further probe acoziborole mechanism of action.

10:15 (15 mins)

Towards cidal drugs for *Trypanosoma cruzi*: new assays, new targets, new molecules

Prof Pascal Mäser, *Unit Head, Parasite Chemotherapy, Swiss Tropical and Public Health Institute*

P Mäser[1];
[1] *Swiss Tropical and Public Health Institute, Basel, Switzerland*

While breakthroughs have been made in the development of new drugs for human African trypanosomiasis, drug discovery for Chagas disease has been much less successful. At present, benznidazole and nifurtimox remain the only options for the chemotherapy of Chagas disease. The lessons from the meagre output of the

antichagasic drug R&D pipeline have been the following: (i) the need for new drugs is higher than ever; (ii) new drug targets have to be identified in *Trypanosoma cruzi* to broaden the antichagasic target space; (iii) drug efficacy tests that only measure endpoints such as 50% inhibitory concentration are inadequate to prioritize the hits from screening campaigns – pharmacodynamics and cidality tests are needed. Here I summarize our recent research along these lines. In collaboration with DNDi, Novartis and academic partners, we are improving antichagasic *in vitro* assays, identifying new drug targets, and further developing molecules that are fully cidal to *T. cruzi*. The most promising of these are the cyanotriazoles. These molecules are highly selective for trypanosomatid parasites, and the leads cure the mouse models of *T. brucei* and *T. cruzi* chronic infection by oral application. The cyanotriazoles are topoisomerase II poisons that covalently bind to a cysteine residue that is highly conserved among the kinetoplastids but absent in other eukaryotes. The cidal action of cyanotriazoles against *T. cruzi* was reproduced in cell culture, using macrophages as host cells that had been derived from human induced pluripotent stem cells. These so called iMAC can be reverse-genetically manipulated and produced in large numbers, offering great opportunities for drug efficacy testing and to study host-pathogen interaction.

-Coffee Break-

Session V: Gene Regulation, Immune Evasion, and Survival Strategies in Trypanosomatids - (Meeting room (1st Floor))

3-September-2025, at 11:00 (15 mins)

Transcriptomic and proteomic analysis of quiescent *Trypanosoma cruzi* epimastigotes: a resource for investigating persistence.

Prof John Kelly, *Professor of Molecular Biology, London School of Hygiene and Tropical Medicine*

F Olmo[2]; A Hesketh[4]; H Hayat[4]; JM Monneuse[4]; MC Taylor[1]; FC Costa[1]; C DaSilva[4]; F Bequet[4]; D Mercer[4]; A Saliou[4]; F Escudie[3]; E Chatelain[3]; A Josephine[4]; JM Kelly[1];
[1] London School of Hygiene and Tropical Medicine, UK; [2] Universidad de Granada, Spain; [3] Drugs for Neglected Diseases initiative (DNDi), Switzerland; [4] BIOASTER, Lyon, UK

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 transiently-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. 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.

11:15 (15 mins)

Novel Leishmania trans-regulators control pathogen surveillance, infectivity and virulence

Dr Pegine Walrad, *PI, University of York*

N Teles[1]; E Parry[1]; RP Neish[1]; M Care[2]; AA Dowle[3]; A Droop[2]; F Pais[2]; S James[4]; K Newling[4]; JC Mottram[1]; PB Walrad[1];

[1] *YBRI, Dept of Biology, University of York, UK;* [2] *Data Science Lab, Bioscience Technical Facility, Dept of Biology, University of York, UK;* [3] *MAP Lab, York Bioscience Technical Facility, Dept of Biology, University of York, UK;* [4] *Genomics Lab, York Bioscience Technical Facility, Dept of Biology, University of York, UK*

Leishmania gene regulation is mostly post-transcriptional with RNA binding proteins (RBPs) the main regulators. We created and screened a BarSeq CRISPR-RBPome knockout (RBP KO) cell library. Notably, 60% of RBP-encoding genes appear essential in promastigotes. A progressive, competitive lifecycle screen revealed essential roles of individual RBPs in differentiation and capacity to infect macrophages and mice. Essential roles for individual RBPs in macrophage infection were validated. 15 RBPs were endogenously tagged, uv-crosslinked and immunoprecipitated to identify associated candidate RNA targets and protein cofactors. Correlative interactome modelling groups networks implicit in parasite infectivity. GO terms of target RNAs highlight relevant mechanisms. We overlap interactome models to reveal common RNA targets and examine the impact of RBP KO upon parasite transcriptomes. This may reveal gene regulatory pathways inherent to pathogen survival and virulence.

11:30 (15 mins)

The in vivo dynamics of antigenic variation in *Trypanosoma brucei* in natural hosts

Mr Stephen Larcombe, *Postdoctoral Researcher , University of Edinburgh*

S Larcombe[3]; J Munday[1]; G Oldrieve[5]; C Duffy[2]; M Mugnier[6]; AP Jackson[2]; L Morrison[4]; R McCulloch[7]; K Matthews[3];
[1] *University of Glasgow , UK;* [2] *University of Liverpool, UK;* [3] *University of Edinburgh, UK;* [4] *The Roslin Institute, University of Edinburgh, UK;* [5] *Institute for Immunology and Infection Research, University of Edinburgh., UK;* [6] *John Hopkins, United States;* [7] *School of Infection and Immunity, University of Glasgow, UK*

In vitro studies and high parasitaemia acute infections in rodents have established the current paradigms for antigenic variation by progressive expression of antigenically distinct variable surface glycoproteins (VSGs) in *Trypanosoma brucei*. However, disease-relevant bovine infections are characterised by sustained low parasitaemias and chronicity. We compared the infection dynamics of isogenic parasites in mice and cattle during early and chronic infection, quantitating antigen expression diversity within and between hosts. This revealed enhanced antigenic diversity in cattle but with a surprisingly reproducible temporal hierarchy for the expression of shared antigen types between independent infections. Genomic analyses demonstrated an unexpected and preferential dominance of a particular telomeric VSG expression site in both mice and cattle. Surprisingly, DNA recombination mutants that limit antigenic variation could sustain chronic infections in mice.

11:45 (15 mins)

A non-coding role for trypanosome VSG transcripts in allelic exclusion

Dr Douglas O Escrivani, *Postdoctoral Research Assistant, University of Dundee*

D Escrivani[4]; S Hutchinson[3]; M Tinti[4]; JE Wright[4]; CA Marques[1]; J Faria[2]; A Trenaman[4]; D Horn[4];
[1] *University of Glasgow , UK;* [2] *University of York, UK;* [3] *Institut Pasteur, Paris, France;* [4] *University of Dundee, UK*

Bloodstream-form African trypanosomes display mono-telomeric expression of a Variant Surface Glycoprotein (VSG) gene in an inter-chromosomally bridged transcription and splicing compartment, such that the dominant gene produces 10,000 times more transcript than excluded VSG genes. Here we show that the VSG transcript impacts allelic competition. We induced either specific translation blockade by recruiting MS2 coat protein to the active VSG 5'-untranslated region, or VSG transcript depletion using RNA interference. Neither perturbation substantially compromised exclusion of native VSGs, as determined by transcriptomic analyses. In contrast, exclusion of a VSG transgene was compromised when the native transcript was transiently depleted. Notably, cells with two active VSGs also displayed two VSG transcription (VEX2) compartments. We conclude

that the VSG transcript is a bi-functional coding and non-coding RNA that participates in allelic competition to establish exclusion.

12:00 (15 mins)

An updated map of the trypanosome pore and its associated factors and export pathways

Prof Susanne Kramer, *PI, Biozentrum der Universität Würzburg*

BG Gabiatti[3]; SS Siqi Shen[2]; JK Krenzer[1]; TK Krüger[1]; SB Braune[1]; LJ Jekic[1]; SK Kramer[1]; M Zoltner[2];
[1] Department of Cell & Developmental Biology, Biocentre, University of Würzburg, Würzburg, Germany, Germany; [2] BIOCEV, Department of Parasitology, Charles University, Czechia; [3] EMBL, Grenoble, France

The current model of the trypanosome nuclear pore complex lacks asymmetry, failing to explain how directionality of export is achieved. We used expansion microscopy and BioID to identify basket- and cytoplasmic-site specific subcomplexes. With asymmetric components in hand, we employed BioID data from baits across the pore to predict the sub-pore localisation of NUPs and all proteins with TrypTag-assigned pore localisation and to analyse export pathways. We identified many novel proteins at the basket site, and confirmed their localisation with expansion microscopy. Many of these are hypothetical proteins, indicative of a non-conserved architecture of the trypanosome basket. Strikingly, we did not identify any new components at the cytoplasmic site, in particular no homologue to Dbp5, that in all other eukaryotes remodels the exported mRNP complex. Instead, we will present new data that are in agreement with the previously suggested Ran-based mRNA export system.

12:15 (15 mins)

Codon biased translation mediated by Queuosine tRNA modification is essential for the virulence of *Leishmania mexicana*

Dr Julie Kovarova, *postdoc, Biology Centre CAS*

J Kovarova[1];
[1] Biology Centre CAS, Czechia

The complex life cycle of the human parasite *Leishmania mexicana* requires rapid translational adaptation for survival in different hosts. We show that Q-tRNA modification at the anticodon position 34 provides a novel mechanism for regulation of gene expression. Q-tRNA levels increase substantially during Leishmania differentiation from the insect stage to the mammalian-infective stage. Mutant cells lacking the enzyme responsible for Q incorporation exhibit substantial changes in the proteome, specifically, downregulated proteins were enriched in Q-tRNA-dependent NAU codons, whereas upregulated proteins contained more NAC codons. Subsequently, the parasite virulence is decreased, as demonstrated by independent infections of macrophages and mice. Taken together, these results highlight the critical role of Q-tRNA modification in maintaining translational balance and reveal a novel layer of gene expression regulation, which facilitates parasite adaptation to changing

12:30 (8 mins)

2 Min Speed talks (see Poster abstracts for further details)

Isabelle Louradour, Nathalia Ballesteros, Simone Altman, Francisco Olmo

-Lunch-

Poster Session 2 (Even numbered posters) (Hallway, meeting room Svět)

13:30 (120 mins)

-Coffee Break-

-Depart for Český Krumlov -

Thursday 4th September

Session VI: Environmental Sensing and Developmental Transitions in Kinetoplastid Parasites - (Meeting room (1st Floor))

4-September-2025, at 09:00 (30 mins)

In-host DRiF-Seq reveals novel virulence factors not acting through canonical quorum sensing.

Dr Catarina Gadelha, *Reader/Group Leader, University of Nottingham*

C Gadelha [1]
[1] *University of Nottingham*

High-throughput methods for gene function analysis are powerful tools to understand parasite biology, but have to date only been applied in one culture-adapted stain of Trypanosoma brucei. This means that significant aspects of infection biology cannot be directly addressed. Our Direct RNAi-Fragment Sequencing (DRiF-Seq) method provides means to generate and quantitatively follow massively parallel libraries of 1000s of RNAi mutants and can be applied to different strains of *T. brucei* and also *T. gambiense, T. congolense*, and *T. vivax*.

To characterize the role of the invariant cell surface in infection, we created a specific library of ~25,000 independent barcoded cell surface mutants and measured their fitness in vivo during establishment/growth, peak parasitaemia and subsequent waves. This uncovers novel virulence factors whose silencing substantially increases parasitaemia, but not as a result of changes in growth rate, production/sensitivity to classical quorum sensing signals, or modified invasion of tissues. As these factors are part of a multigene (GR)ESAG family, we developed a new synthetic library (SynDRiF-Seq) to isolate the effects of individual family members/subfamilies and predicted interactors. This demonstrates that all impact on virulence is from a non-expression site subfamily, but also reveals downstream pathway components directly involved in tissue tropism.

In addition to revealing novel modifiers of parasite virulence, these data demonstrate the power of highly-parallel quantitation of mutant fitness – including more effective means to study gene function in vivo that massively reduces animal usage while also increasing experimental power and sensitivity.

09:30 (15 mins)

Using a proximity labeling approach to identify interactors of an Haptomonad Differentiation Kinase in *Leishmania mexicana*

Ms Laryssa de Liz, *Postdoc, University of York*

L de Liz[1]; J Sadlova[2]; C Taylor[3]; AA Dowle[1]; P Volf[2]; J Mottram[1]; N Baker[1];
[1] *University of York, UK;* [2] *Charles University, Prague, Czechia;* [3] *Department of Biology, University of York, UK*

Leishmania parasites differentiate into several forms (e.g. amastigotes, metacyclic, haptomonads) in response to pH and temperature. To investigate sensing mechanisms, we screened a kinome deletion library (164 lines) for survival in low pH. We identified a CAMK kinase required for metacyclic differentiation, which we named Haptomonad Differentiation Kinase 1 (HDK1). HDK1-null mutants form haptomonad-like cells at low pH and fail to colonise the stomodeal valve. HDK1 relocalises along the flagellum at low pH, peaking its expression after two days. Transcriptomic and proteomic analysis suggest HDK1-null mutant parasites adopt energy-saving adaptations after exposure to low pH, similar to AMPK-activated cells. To explore how HDK1 senses pH,

we are performing proximity labeling. Initial pull-downs at pH 7.4 revealed candidate interactors, which will be compared to those from low pH conditions.

09:45 (15 mins)

Translation control is key to *Trypanosoma cruzi* differentiation

Mr Bernardo Papini Gabiatti, *Post-doc, EMBL Grenoble*

BG Gabiatti[1]; ER Freire[2]; SP Bispo[2]; FB Holetz[2];
[1] EMBL, Grenoble, France; [2] Carlos Chagas Institute, FIOCRUZ, Brazil

Nutritional stress triggers the differentiation of *T. cruzi* epimastigotes into infective metacyclic trypomastigotes. We determined how mRNA translation contributes to differentiation by analysing epimastigotes, epimastigotes under acute nutritional stress (2 hours), and metacyclic trypomastigotes using Ribo-seq. We discovered a pre-programmed mechanism in translation that is triggered as fast as 2 hours. This mechanism leads to global translation arrest while allowing ribosomal association of only specific mRNAs involved in infection. We used de novo proteomics (SILAC) to confirm that only a small pool of proteins is translated in metacyclics, and are directly implicated in infection (flagellar, membrane transport proteins, proteases). What is the molecular mechanism in translation underpinning differentiation? In trypanosomes, six paralogues of the translation initiation factor eIF4E exist, a factor with known roles in recruiting mRNAs to translation in eukaryotic cells. We established a method to sequence mRNAs bound to each (RIP-seq). We identified two that function primarily in epimastigotes, regulating the translation of mRNAs involved in cell growth and metabolism. However, these two are inhibited during stress and in metacyclics, indicating a function of a third eIF4E factor in differentiation. Together, we demonstrate that translational control is key to differentiation and provide first hand insight into the mechanism by the function of the different eIF4E factors. While global translation is arrested, the specific translation of a pool of mRNAs prepares the parasite for infection.

10:00 (15 mins)

Genome-wide fitness profiling by DRiF-Seq identifies novel regulators acting early in the *Trypanosoma brucei* quorum sensing signaling pathway

Dr Kirsty McWilliam, *PDRA, University of Edinburgh*

KR McWilliam[1]; S D'Archivio[3]; F Rojas[2]; P Kim[1]; O Dluzniewska[1]; C Gadelha[3]; KR Matthews[1];
[1] University of Edinburgh, UK; [2] Lancaster University, UK; [3] University of Nottingham, UK

Within its infected mammalian host, *Trypanosoma brucei* undergoes a density-dependent developmental transition that is reminiscent of bacterial quorum-sensing (QS). Proliferative 'slender' cells that establish infection differentiate to cell-cycle arrested 'stumpy' cells that are competent for transmission into the tsetse fly vector. This differentiation is triggered in response to an increasing concentration of extracellular oligopeptides that are transported into the cell via the parasite surface transporter *Tb*GPR89.

A previous genome-wide RNAi screen identified molecules that form a QS signal transduction pathway regulating slender to stumpy differentiation. However, since this screen used a cell-permeable selective tool, *Tb*GPR89 and the molecules immediately downstream of the transporter were bypassed. Thus, the molecules that detect the oligopeptide signal and activate the previously characterised signaling pathway components remain unknown.

To identify these molecules we have used **D**irect **R**NA**i** **F**ragment Sequencing, DRiF-Seq, to perform a genome-wide quantitative fitness profiling of cells exposed to the physiological oligopeptide differentiation signal. Our screen identified both previously known quorum-sensing components, such as ZC3H20 and HYP2, as well as several novel molecules, as regulators of oligopeptide sensitivity.

We have characterised two kinases identified by the screen, AKT-like kinase and an AGCK family kinase. We have found that knock-down or knock-out of either kinase reduces slender to stumpy differentiation both *in vitro* and *in vivo*, as well as in response to synthetic dipeptides. Furthermore we have demonstrated that these kinases operate upstream of all the previously identified QS signal transduction pathway components. A comprehensive panel of mutants has revealed the residues important for AKT-like's regulation and activity and, furthermore, we have demonstrated differential phosphorylation of the kinase's substrates during differentiation *in vivo*. Ongoing work aims to use quantitative phosphoproteomics to link AKT-like to the previously identified QS signal transduction pathway components.

Our results present an exciting insight into the earliest events regulating bloodstream *T. brucei* differentiation, revealing novel signaling molecules that control parasite development.

10:15 (15 mins)

Mitochondria in Transition: The Journey from Simple to Complex in *Trypanosoma brucei*

Ms Michaela Husová, *PhD student, University of South Bohemia*

M Husová[5]; R Gerova[2]; M Zoltner[4]; MC Field[3]; B Wickstead[2]; C Gadelha[2]; A Zíková[1];
[1] Institute of Parasitology, Biology Centre, ASCR, Czechia; [2] University of Nottingham, UK; [3] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK; [4] BIOCEV, Department of Parasitology, Charles University, Czechia; [5] University of South Bohemia, Ceske Budejovice, Czechia

The single mitochondrion of *Trypanosoma brucei* undergoes extensive structural and functional remodelling throughout the parasite's life cycle. In the proliferative bloodstream form (BF), the organelle is tubular and metabolically constrained. However, during differentiation to the insect-stage procylic form (PF), it transforms into a highly branched, metabolically active structure that plays a central role in cellular bioenergetics. To characterize this transition, we employed a multi-omic approach that incorporated whole-cell proteomics, high-throughput quantitative fitness measurements for RNAi mutants targeting mitochondrial metabolic pathways, and an assessment of ultrastructural changes. Our data identify the metabolic nodes that are essential at different times during the progression from proliferative BF to quiescent stumpy, and then to PF, highlighting differences in the TCA cycle, amino acid metabolism, and ATP production. They also reveal an early upregulation of the mitochondrial proteome in stumpy parasites, but show this to be independent of a specific functional need at this stage. Major structural reorganization, including increased mitochondrial branching and cristae formation, was observed between 12 and 24 hours of differentiation into PF. This was followed by significant metabolic rewiring around the 48-hour mark. The integration of proteomic and functional genomics approaches enabled us to resolve the temporal sequence of mitochondrial remodelling and identify key bioenergetic transitions critical for the parasite's adaptation to the insect-stage environment.

10:30 (15 mins)

Genes essential for surface attachment of the insect parasite *Crithidia fasciculata*

Dr Megan Povelones, *Associate Professor, Villanova University*

J Quatse[1]; A Quezada[3]; S Kapila[3]; S Denecke[1]; R Hoang[3]; PA Yates[4]; M Povelones[2]; M Povelones[2];
[1] University of Pennsylvania, United States; [2] Villanova University, United States; [3] Haverford College, United States; [4] Oregon Health & Science University, United States

Crithidia fasciculata is a monoxenous trypanosomatid parasite that infects mosquitoes. Like all trypanosomatids, it uses its single flagellum to attach to tissues in its insect host, facilitating differentiation, colonization, and transmission. How parasites sense and respond to their environment, resulting in attachment at a particular time and place, is not yet clear. Insect parasites such as *C. fasciculata* are powerful models for this process since they attach readily *in vitro* and produce robust infections in laboratory colonies of mosquitoes. We have used this system to uncover a key role for cyclic AMP (cAMP)-based signaling, which is

organized in flagellar microdomains, in the transition from swimming to attached cells. We now demonstrate that *C. fasciculata* can perform RNAi-mediated knockdown of target genes through constitutive expression of stem-loop RNAs from episomal plasmids. Using this approach, we show that knockdown of the flagellar central pair protein PF16 blocks motility without changing the growth rate. Surprisingly, *C. fasciculata* parasites undergoing PF16 RNAi are also deficient in attachment *in vitro*, indicating that normal flagellar structure is important for this process. To determine if attachment-deficient parasites also fail to colonize mosquitoes, we established a nanoLuciferase (nLuc)-based quantitative system to measure intensities of infection in different mosquito tissues. When parasites containing the PF16 RNAi construct in the nLuc background are used to infect mosquitoes, we observe a marked decrease in parasites found in the mosquito hindgut compared to nLuc cells harboring a control stem-loop plasmid. We have also used RNAi to demonstrate that individual knockdown of two cAMP phosphodiesterases completely blocks attachment *in vitro*. Further studies will untangle how flagellar structure, motility, signal transduction, and attachment contribute to successful trypanosomatid colonization of insects.

10:45 (15 mins)

Extended conserved gene family with an essential role in Leishmania-sand fly interactions

Mr Owino Barrack Omondi, *Student, Oxford Brookes University*

B O OWINO[4]; R Yanase[1]; K Pruzinova[3]; A Marron[5]; S Vaughan[4]; P Volf[2]; JD Sunter[4];
[1] University of Nottingham, UK; [2] Charles University, Prague, Czechia; [3] Charles University, Czechia; [4] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [5] Center for Bioimaging, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK

Successful Leishmania transmission requires parasite adhesion to the sand fly stomodeal valve via a complex adhesion plaque. While three kinetoplastid-insect adhesion proteins (KIAPs) are known, the overall plaque proteome is obscure. Using TurboID-tagged KIAP3 combined with proteomics and light microscopy, we identified novel plaque components that localised to the adhered flagellum, including multiple members of a large gene family, containing a P-loop NTPase domain that is conserved across the kinetoplastids. Phylogenetic analysis of the evolution of this family, supported a model of ancestral paralogy followed by lineage-specific diversification. Deletion of the top-hit protein from this family severely impaired haptomonad adhesion in vitro, and the mutants were unable to colonise the sand fly stomodeal valve. Our work provides more detail of the molecular machinery essential for vector colonisation, opening new avenues for developing transmission-blocking strategies.

-Coffee Break-

Session VII: Persistence, Transmission, and Control Strategies for Trypanosomatid Diseases - (Meeting room (1st Floor))

4-September-2025, at 11:00 (15 mins)

House-hunting: *Trypanosoma cruzi* style. Utilising microscopy to capture highly dynamic processes in the *T. cruzi* intracellular lifecycle

Mr Richard Atherton, *PhD Candidate, London School of Hygiene and Tropical Medicine*

RL Atherton[1]; JM Kelly[1]; MC Taylor[1]; F Olmo[1];
[1] *London School of Hygiene and Tropical Medicine, UK*

Trypanosoma cruzi is a promiscuous parasite able to infect any nucleated mammalian cell. Host-cell invasion has been well characterised, however egress is less understood. Making use of fluorescent *T. cruzi* strains we have employed a microscopy-based approach to study infection-replication-egress dynamics. We found a surprising variety of phenotypes during the dynamic period of parasite differentiation, egress, and subsequent reinvasion, which could have different outcomes for host and parasite. Importantly, our observations have shown the line between invasion and egress is blurred; invasion can be rapidly followed by egress with the exiting trypomastigote coated in host cell membrane. This process of invasion and rapid egress can be repeated a number of times before the parasite differentiates to amastigotes and begins its replicative cycle. This cell-to-cell transmission could have a role in parasite dissemination and immune evasion, and could act to sustain host infection.

11:15 (15 mins)

Insights in cellular sanctuary and parasitic quiescence from preclinical Leishmania infection models

Miss Cassandra Present, *PhD student, University of Antwerp: Laboratory of Microbiology, Parasitology and Hygiene*

C Present[1]; L Dirkx[1]; R Ahmad[1]; S Wong[1]; S Hendrickx[1]; A Baeza Garcia[2]; G Caljon[1];
[1] University of Antwerp, Belgium; [2] University of Antwerp: Laboratory of Microbiology, Parasitology and Hygiene, Belgium

For a plethora of infectious diseases, host sanctuary and pathogen quiescence are increasingly understood to determine treatment outcomes. Infection of rodents with bioluminescent and fluorescent transgenic parasites revealed that stem cells in the bone marrow act as a source of relapse in visceral leishmaniasis. We also identified these stem cells as a unique cellular niche where parasites enter into quiescence. Dissecting the underlying mechanisms by immunophenotyping, transcriptomics and reverse genetics identified a unique transcriptional '*StemLeish*' signature and coordinated pathways involved in regulating *Leishmania* infection. Transcriptional studies were conducted to compare the quiescent and non-quiescent parasite stages. As the current R&D pipeline does not yet specifically explore aspects of sanctuary and quiescence, initiatives will be presented for the discovery of novel assays, targets and drugs to reduce the risk of relapse and subsequent propagation.

11:30 (15 mins)

Immunomodulatory role of SAPA repeats present in *Trypanosoma cruzi* Trans-sialidases

Prof SANTUZA MARIA Teixeira, *PROFESSOR, Federal University of Minas Gerais*

N Aprigio-Santos[1]; C Almeida-Junior[1]; M Ricci[1]; LL Reis-Cunha[2]; GA Burle-Caldas[1]; FS Machado[1]; SM TEIXEIRA[1];
[1] FEDERAL UNIVERSITY OF MINAS GERAIS, Brazil; [2] York Biomedical Research Institute, Department of Biology, University of York, UK

Proteins containing repeat domains are widespread among protozoa parasites. *T. cruzi* has a family of surface proteins named trans-sialidases (TS), responsible for transferring sialic acid from host glycoconjugates to parasite mucins. TS, an essential enzyme required for parasite survival in the mammalian host, contains a C-terminal immunogenic domain with amino acids repeats named SAPA. We investigated the role of SAPA repeats by immunizing mice with recombinant TS proteins with and without repeats as well as with RNA formulations encoding TS sequences with and without SAPA. Besides confirming the immunodominance of the SAPA domain, after challenging immunized animals with *T. cruzi*, we showed that vaccination protocols with the antigen with or without repeats promotes similar protection. However, as a vaccine component, the presence of SAPA is desirable since it resulted in increased production of anti-inflammatory cytokines and less tissue damage.

11:45 (15 mins)

Comparative transmission of Leishmania major to *P. papatasi*: natural reservoir (Meriones shawi) vs. BALB/c mice

Mrs Barbora Bečvářová, *Postdoc, Katedra Parazitologie, Přírodovědecká fakulta, UK*

B Becvarova[3]; T Becvar[3]; N Mekarnia[4]; D Frynta[2]; KB Benallal[5]; Z Harrat[6]; P Volf[1]; J Sadlova[1];
[1] Charles University, Prague, Czechia; [2] Department of Zoology, Faculty of Science, Charles University, Czechia; [3] Faculty of Science, Charles University, Czechia; [4] UR 7510 ESCAPE - USC Anses, School of Pharmacy, Université de Reims, France; [5] Arboviruses and Emergent viruses, Institut Pasteur d'Algérie, Algeria; [6] Algerian Academy of Science and Technology, Algeria

Cutaneous leishmaniasis caused by Leishmania major is transmitted by sand flies and maintained in rodents. While hosts with lesions are considered the main infection source, the role of asymptomatic hosts and parasite distribution within the skin is poorly understood. We examined L. major transmissibility in natural and laboratory hosts, focusing on parasite distribution and minimal infectious dose required to establish sand fly infection. Using xenodiagnosis, microbiopsies, and qPCR, we found that M. shawi, regardless of symptoms, were highly infectious to *P. papatasi*. Parasite distribution was heterogeneous, with lesion margins being critical sites for transmission. As few as 2–10 amastigotes could infect sand flies feeding at these sites. In contrast, BALB/c mice required 1,500–10,000 amastigotes. This highlights the importance of using natural host models to assess transmission dynamics and suggest that asymptomatic carriers may play a large role in *L. major* epidemiology.

12:00 (15 mins)

Dermotropic *Leishmania donovani* in Nepal: emergence of 'prudent' parasites and threat for elimination?

Prof Jean-Claude DUJARDIN, *, Institute of Tropical Medicine*

P Monsieurs[1]; T de Gooyer[1]; K Cloots[1]; K Choukri[1]; G Van der Auwera[1]; S Uranw[2]; N Bhattarai[2]; MR Banjara[3]; P Ghimire[3]; E Hasker[1]; MA Domagalska[1]; JC Dujardin[1];
[1] Institute of Tropical Medicine, Antwerp, Belgium; [2] BPKIHS, Dharan, Nepal; [3] Tribhuvan U, Nepal

Visceral leishmaniasis (VL) in the Indian sub-continent is due to *L. donovani* and is lethal in the absence of treatment. During the last epidemic of VL in Nepal, prior to the commencement of the current elimination initiative in 2005, the disease was confined mostly to the Terai region in the southeast of the country. Although reported VL case numbers are now much lower than before, a shift in foci to hilly regions in western Nepal has been observed. There has been a concurrent emergence of *L. donovani*-associated cutaneous leishmaniasis (CL), a form of the disease that was not reported in the past, mostly also distributed across western Nepal. We aimed to (i) track the evolutionary origin of these dermotropic *L. donovani* and (ii) understand to what extent parasites causing CL may be contributing to persistence of VL in the Indian subcontinent. To do this, we sequenced the genome of *L. donovani* parasites directly from DNA extracted from clinical samples from VL and CL patients in western Nepal and assessed relatedness with published 'pre-elimination' genomes from the region. Our main findings were: (1) In Nepal, elimination seems to have been associated with replacement of the historically predominant viscerotropic parasite populations by new (rare) variants that expanded in the last decade; (2) Their progeny constituted at least two new genetic groups, (pseudo-)clonally propagating, but hybrids were observed; and (3) both epidemic 'clones' and hybrids were associated with CL and VL. Genomes of contemporary and closely related CL and VL variants are being scrutinized to find potential functional differences between the two forms and results will be presented at the conference. We hypothesize that from a main pre-elimination population of aggressive *L. donovani* (causing lethal VL), less virulent parasites emerged and spread; this kind of 'prudent' parasites would spare their host and contribute to transmission to other hosts. These parasites seem to keep the potential to cause VL, hence

we strongly recommend implementing genomic surveillance in the region and including CL management in the elimination program.

12:15 (15 mins)

ECLIPSE: an intervention programme to improve patient journey and reduce stigma for people with cutaneous leishmaniasis

Prof Helen Price, *Professor of Parasitology, Keele University*

H Price[1]; S Agampodi[5]; TC Agampodi[5]; PR Machado[3]; A Mulugeta[4]; L Trad[3]; L Dikomitis[2];
[1] Keele University, UK; [2] University of Warwick, UK; [3] Federal University of Bahia, Brazil; [4] Mekelle University, Ethiopia; [5] Rajarata University of Sri Lanka, Sri Lanka

Cutaneous leishmaniasis (CL) affects highly marginalized and underserved communities across the globe. While there are WHO initiatives to address skin-related NTDs, effective control will require a clearer understanding of the barriers to accessing healthcare for CL and the wider challenges and effects of the disease on individuals and their communities. I will present findings from the five-year interdisciplinary ECLIPSE programme which aimed to improve the CL patient journey and reduce stigma in the most underserved communities in Brazil, Ethiopia and Sri Lanka. We used qualitative, quantitative and creative approaches to investigate the impacts of CL on individuals and communities, levels of disease awareness and the barriers and facilitators for accessing diagnosis and treatment. Through dedicated Community Advisory Groups and Communities of Practice we ensured that our approach, research tools and interventions are appropriate for each context and that all activities are underpinned by stakeholder involvement. Findings from over 200 interviews and 2,500 surveys across three countries showed that understanding of the causes and transmission of CL was low and that misinformation contributed to fear and stigmatization of affected individuals and, in some cases, of whole communities. Barriers to accessing healthcare included distance of travel to clinics, lack of childcare, fear of drug side-effects, inability to work, and low disease awareness in communities and healthcare professionals. We found the burden of CL to be particularly high in Tigray, Ethiopia, where the ECLIPSE team evidenced the devastating impact of the recent conflict on healthcare systems, together with a sharp increase in CL cases due to population displacement to caves and greater contact with rock hyrax, a reservoir host for *Leishmania aethiopica*. Understanding and awareness of CL was very low in affected communities. There was a view at primary healthcare level that the disease was untreatable, which impacted on referral for diagnosis and treatment. In the absence of biomedical treatments, the use of traditional remedies was very high. There was evidence of severe stigma of individuals, which was linked to local beliefs about CL. The ECLIPSE team addressed identified challenges through the co-production and implementation of bespoke community-facing interventions, with shared learning across country teams. Interventions include awareness campaigns, community-based films and books, a traditional masked folk theatre, video animation, television and radio programmes, podcasts, and training courses for healthcare professionals. We have engaged with policymakers across multiple sectors and influenced policy change around decentralisation and increasing capacity and access for CL diagnosis and treatment. Work with the Tigray Regional Health Bureau, Ayder and HEWO Hospitals resulted in the establishment of a new inpatient centre and seven new outpatient treatment centres for CL in Tigray. Over 2,000 people have been treated in these new centres since November 2023.

-Lunch and Symposium close-

Alan FairLamb Mini Symposium I - (Restaurant Solnice)

4-September-2025, at 14:00 (30 mins)

Lessons from the Malaria Drug Accelerator: Targets, resistance mechanisms and their conservation across species

Dr Elizabeth Ann Winzeler, *Professor & Associate Dean, University of California, San Diego, United States*

14:30 (60 mins)

Targeting a novel proteasome binding site for malaria treatment

Prof Meg Phillips, *Prof, UT Southwestern*

-Coffee Break-

Alan Fairlamb Mini Symposium II - (Restaurant Solnice)

4-September-2025, at 16:00 (30 mins)

Virtual Presentation - One of the biggest unanswered questions in TB research

Carl Nathan, *Prof, Weill Cornell Medicine*

Tuberculosis spreads by aerosol. Transmission of Mycobacterium tuberculosis (Mtb) has been well studied epidemiologically, but mechanistic studies have been limited to exposing experimental animals or filters to patients' exhalations. Here I will describe an in vitro model for studying Mtb's transmission biology under physiologically relevant conditions that allows assessment of the potential contribution of each gene of the pathogen at each stage of the process from necrotic cavities to healthy lungs.

16:30 (30 mins)

Virtual Presentation - Evolution of HIV treatment: Paving the way for innovations in infectious disease care.

Felix Calderon, *Scientific Director, ViiV Healthcare*

17:00 (30 mins)

Alan Fairlamb - An appreciation

Prof Michael Ferguson, *Regius Professor of Life Sciences, University of Dundee*

-Dinner-

Friday 5th September

Alan Fairlamb Mini Symposium III - (Meeting room Bezdrev (1st Floor))

5-September-2025, at 09:00 (15 mins)

Leishamania impacts on macrophage immunometabolism

Prof Michael Barrett, University of Glasgow,UK

09:15 (15 mins)

Arginine sensing in Leishmania.

Prof Dan ZILBERSTEIN, *Professor, Technion-Israel Institute of Technology*

09:30 (30 mins)

Virtual Presentation - Bonding: Electrons are FREE

Emily Fong, *Dr, University of Dundee*

Emily is a medical artist exploring life and death, embodiment and emotion; the experience of existing in a human container. Her artistic practice is underpinned by the observation and communication of the life cycles of living things; growth, mortality and change from the micro to the macro. Through the mediums of drawing, painting, sculpture and writing, she seeks to highlight our similarities not only to one another but also to other species that occupy this planet. What are we made of? How are we structurally and emotionally connected beneath the skin? Her intuition is that, by going deep inside life, turning it inside out, she might discover new ways of observing and re-configuring the outside.

-Coffee Break-

Alan Fairlamb Mini Symposium IV - (Meeting room Bezdrev (1st Floor))

5-September-2025, at 11:30 (15 mins)

Reminiscences on trypanosomes, trypanothione, polyamines and biosafety in the Fairlamb lab.

Dr Mark Ariyanayagam, *H&S Manager / Biosafety Adviser, Queen Mary University of London (non-research)*

Posters (Hallway, meeting room Svět)

Poster 1 : Sand fly chemotherapy: A new way to control leishmaniases?

Dr Andreia Wendt, *Postdoc, Charles University Prague / Swiss TPH*

A Wendt[3]; M Pirelli[2]; B Vojtková[1]; T Bečvář[1]; J Sádlová[1]; P Mäser[2]; P Volf[1];
[1] Charles University, Czechia; [2] Swiss TPH, Switzerland; [3] Swiss TPH, Charles University Prague, Switzerland

In the absence of human vaccines, leishmaniases control relies on limited chemotherapy and in reducing contact with the insect vectors. Yet, vector control is undermined by cost, impracticality and growing insecticide resistance. Mass spraying and traditional toxic sugar baits pose significant risks to non-target organisms, such as pollinators, potentially causing serious ecological imbalances. A new approach targeting parasites within their insect vectors using parasiticides has recently been proposed and has the potential to be combined with antiparasitic sugar baits with minimal toxicity to non-target organisms. We aim to contribute to the development of a novel and better approach for screening anti-*Leishmania* compounds in infected sand flies, with minimal toxicity to the vector and other putative non-target organisms.

Poster 2* : Protector or defector? Identifying Leishmania host factors involved in LRV maintenance

Ms Donnamae Klocek, *PhD student, University of Ostrava*

D Klocek[1]; SA Reddy[1]; A Saura[1]; A Zakharova[1]; ES Gerasimov[2]; V Yurchenko[1];
[1] Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czechia; [2] Moscow State University, Moscow, Russian Federation

Leishmania RNA virus (LRV) is a virus of the family *Pseudototoviridae* that infects some strains of the parasite *Leishmania*, exacerbating the disease caused by the latter in humans. LRV is structurally simple: an icosahedral capsid made up of 120 units of capsid protein (CP) encloses a single copy of a nonsegmented, double-stranded RNA genome and two copies of RNA-dependent RNA polymerase (RdRP). Little is known about the interface between LRV and its host at the molecular level.

We identified *L. guyanensis* proteins that interact with its resident virus LRV1-4 via coimmunoprecipitation using LRV1-4 CP as bait, followed by mass spectrometry. Using a modified CRISPR-Cas9 protocol, we knocked out a few of these genes. Among the genes knocked out is a homolog of flagellar protein 1 binding protein (FLA1BP). The FLA1BP knockout line has a lower cell division rate, altered morphology, and altered LRV viral level compared to the wild type.

Poster 3 : Using natural products to decipher essential host-parasite interactions in visceral leishmaniasis.

Dr David Blake, *Professor, Fort Lewis College*

D Blake[1];
[1] Fort Lewis College, United States

Leishmaniasis is a vector-borne neglected tropical disease (NTD) caused by over 20 protozoan parasite species. Leishmaniasis is transmitted by sandflies and contracted by approximately 1 million people each year leading to more than 50,000 deaths, making it one of the most important NTD worldwide and the single most neglected in terms of new drug development. Our long-term goal is to identify natural products that modify host-parasite interactions required for parasite replication. Our overall objective is to identify the mechanism of action of sulforaphane (SFN) to determine its antileishmanial properties. Our data indicates that SFN is an effective, non-toxic, anti-parasitic compound that inhibits promastigote and amastigote replication through the role of NRF2 and autophagy within the innate immune response.

Poster 4 : Comparative genomics of *Trypanosoma cruzi* vectors (Reduviidae: Triatominae) as compass to direct targeted vector control

Ms Antonella Bacigalupo, *PhD Student, University of Glasgow, SBOHVM*

A Bacigalupo[1]; K Dickson[1]; KR Elmer[1]; MS Llewellyn[1];
[1] *School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK*

The Reduviidae family harbours entomophagous and haematophagous bugs. The latter conform the subfamily Triatominae and are vectors of Trypanosoma cruzi, which causes Chagas disease. Current triatomine control methods rely on insecticides that affect many arthropods. Insecticide resistance has been detected in some triatomine populations. New control methods should be specific, requiring extensive knowledge of the target species and others that could be affected. We sequenced, assembled and annotated one non-triatomine reduviid and eight triatomine genomes, and performed a comparison including published reduviid genomes. A total of 122 protein clusters were triatomine-specific. Nine protein clusters were significantly expanded in Triatominae. Many single copy orthologs showed positive selection in triatomines. Future studies should establish functionality of these proteins, especially of those potentially haematophagy-related, which could constitute control targets for *T. cruzi* vectors.

Poster 5 : Improved molecular diagnosis of visceral leishmaniasis (VL) using the mini direct on blood PCR Nucleic Acid Lateral Flow Immunoassay (dbPCR-NALFIA).

Dr Henk Schallig, *Senior scientist, Amsterdam University Medical Center*

H Schallig[1]; N van Dijk[1]; D Hagos[3]; S Menting[1]; D Huggins[1]; E Carrillo Gallego[2]; D Wolday[3];
[1] *Amsterdam UMC, Netherlands;* [2] *Instituto de Salud Carlos III, Spain;* [3] *Mekelle University, Ethiopia*

Current VL diagnostics (parasitology and serology) have limitations (safety and accuracy). Molecular tools have high accuracy, but complexity and costs limit their use in endemic areas. Simplified molecular diagnostics that can be used in resource limited settings are needed. Miniaturized direct-on-blood PCR nucleic acid lateral flow immunoassay (dbPCR-NALFIA) was developed as easy-to-use molecular assay for VL diagnosis. This platform circumvents DNA extraction and utilizes a small thermal cycler (solar-charged), enabling performing the test without any laboratory infrastructure. Test results are visualized by lateral flow strip. A lab evaluation of db-PCR-NALFIA using suspected VL patient samples (N=146) and PCR as reference test was done. Compared to PCR, mini db-PCR-NALFIA had a sensitivity of 95.8% and a specificity of 97.2%. LoD of the platform is 10 parasites/µl. db-PCR-NALFIA has a good diagnostic performance and is ready for evaluations in disease endemic countries.

Poster 6 : Metabolic Impact of Sulphur Amino Acid Pathway Disruption in *Trypanosoma cruzi*: A 1H-NMR Exometabolomic Approach

Dr Sabrina Marsiccobetre, Post Doctoral Researcher, University of São Paulo

MS Ballari[1]; S Marsiccobetre[1]; MB Alencar[1]; AC Mengarda[1]; AM Silber[1];
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Chagas disease, a parasitic illness classified among Neglected Tropical Diseases, poses a significant public health challenge across the Americas, particularly affecting economically disadvantaged populations. The causative agent, *Trypanosoma cruzi*, utilizes amino acids as a major carbon source and for essential biological processes, including differentiation, stress response, and host cell invasion. Cysteine (Cys), a sulphur-

containing amino acid, plays a critical role in protein structure, stability, and catalytic activity. In *T. cruzi*, Cys contributes to central metabolism through its conversion to pyruvate and provides -SH groups necessary for synthesizing sulphur-containing molecules. Additionally, sulphur amino acids are essential for macromolecule methylation and polyamine synthesis. Cys biosynthesis in *T. cruzi* occurs via two main pathways: 1) *de novo* synthesis from serine (Ser) in two steps, catalyzed by serine O-acetyltransferase (SAT) and cysteine synthase (CS), using H_2S as a sulphur source; 2) the trans-sulphuration pathway, in which Cys is synthesized from Ser and homocysteine (HCys). HCys, in turn, is derived from methionine (Met) through a three-step process. Although the Met biosynthetic pathway in *T. cruzi* remains unknown, the parasite can salvage Met from HCys via homocysteine S-methyltransferase (HMT) in a single step. This study aimed to elucidate the metabolic roles of Cys and Met by generating *T. cruzi* mutants with total (−/−) or partial (+/−) knockouts of genes involved in Cys biosynthesis—SAT, CS, and HMT—using CRISPR/Cas9 technology in a strain constitutively expressing Cas9 (parental lineage). To assess metabolic changes resulting from these gene deletions, we performed exometabolomic profiling using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Epimastigotes (CL Brener strain) were first subjected to nutritional stress by overnight incubation in phosphate-buffered saline (PBS), then exposed to individual carbon sources (5 mM glucose, Cys, Met, Ser, or PBS as control) for 6 hours at 28 °C. Then supernatants were analysed by ^{1}H-NMR. The analysis revealed significant alterations in metabolite excretion, particularly acetate, formate, alanine, and glycine. The $SAT^{-/-}$ strain showed markedly increased acetate excretion under nutritional stress, suggesting acetyl-CoA accumulation due to loss of SAT activity. In contrast, the $CS^{+/-}$ strain exhibited reduced acetate production across all carbon sources, indicating CS's key role in acetate generation via Cys biosynthesis. The $HMT^{+/-}$ strain also showed elevated acetate excretion under stress, likely reflecting a shift in carbon flux toward trans-sulphuration and downstream pyruvate-derived pathways due to impaired HCys-to-Met conversion. Overall, this approach revealed how disrupting sulphur amino acid metabolism reshapes the excreted metabolic profile in *T. cruzi*. These findings highlight the tight integration of sulphur metabolism with redox balance and cellular energy metabolism, emphasizing these pathways as promising targets for new therapeutic strategies.

Poster 7* : Unravelling the γδ t cell dynamics during naturally transmitted *Trypanosoma brucei* infections using multiparameter spectral flow cytometry

Miss Yentl Jacobs, *PhD student, LMPH - University of Antwerp*

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African trypanosomiasis, transmitted by tsetse flies, is increasingly eliminated as a public health risk but still threatens millions in 36 sub-Saharan African countries. The limited understanding of its immunology hinders vaccine development, resulting in no effective vaccine available despite promising laboratory results. Among the different immune cell populations that undergo dramatic expansion during trypanosome infection, γδ T cells remain largely underexplored. To investigate the γδ T cells' tissue-specific roles and their interactions with other immune cells during naturally transmitted *Trypanosoma brucei brucei* infections, a comprehensive 41-parameter panel was developed for analysis by spectral flow cytometry. This multi-parameter panel was designed based on selected markers for myeloid (eosinophils, neutrophils, dendritic cells, mast cells, and macrophages) and lymphoid cells (NK cells, αβ T cells, γδ T cells, and B cells), including various activation markers, immune checkpoint molecules, and intracellular cytokines. Fluorochromes were chosen based on antigen density, brightness, and spectral overlap, followed by a thorough antibody panel optimisation and quality control on a Cytek® Aurora flow cytometer. The developed panel was applied to study extravascular tissue responses of mice infected with *T. b. brucei* following a *Glossina morsitans morsitans* tsetse fly bite. The multiparameter cell profiling offered valuable insights into immune response dynamics in the lungs, liver, and spleen. Unsupervised analysis revealed significant changes in lymphoid cell populations and activation states following *T. b. brucei* infection. Notably, γδ T cells showed tissue-specific responses, with increases in Vγ1.1

cells in the lungs and Vγ6 cells in the liver, alongside varying levels of Vγ1.1 and Vγ2 cells in the spleen, indicating specialised roles across different tissues. These preliminary findings underscore the need for further studies to confirm and expand on these observations, and highlight the benefit of a multi-parameter panel to unravel the complex immune responses during *T. b. brucei* infections.

Poster 8 : Harnessing the Potentials of Propolis-Based Natural Products for the Integrated Control of Trypanosomatid Infections in Humans and Livestock: A One Health Approach

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Trypanosomatid parasites cause neglected tropical diseases in humans and livestock, with limited treatment options due to drug resistance and toxicity. Aligned with the One Health framework, we explored propolis—a bee-derived natural product—as a source of sustainable therapeutics. Propolis samples from diverse regions were chemically profiled using LC-MS and PCA, revealing broad compositional diversity. OPLS analysis linked a butyrate ester of pinobanksin to activity against T. brucei, and methyl ethers of chrysin and pinobanksin to *T. congolense*. Flavonoids, particularly phenolic compounds, emerged as the main bioactive class, displaying potent in vitro activity against Trypanosoma and Leishmania species, including drug-resistant strains, with no cytotoxicity to mammalian cells. These findings support propolis as a reservoir for novel, cross-species antiparasitic agents and advance One Health-aligned drug development strategies.

Poster 9 : Investigating the role of regulated cell death in visceral leishmaniasis in mice

Miss Silke Mortelmans, *PhD student, University of Antwerp*

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It is well established that Leishmania spp are capable of inhibiting apoptosis during early stages of infection, and other studies have suggested a role for pyroptosis in the development of cutaneous disease, either showing exacerbation of disease through the facilitation of cell-to-cell spread or claiming a protective role. However, the role of cell death in the development of disease in a model for visceral leishmaniasis remains unclear and largely unexplored. In this research we make use of gsdmd$^{-/-}$, casp1/11$^{-/-}$, casp8$^{-/-}$ and ripk3$^{-/-}$ mice models to explore the contribution of different host cell death pathways to infection kinetics of *L. infantum*. This is done in vitro by infection of primary bone marrow-derived macrophages and monitoring of their cell death response and in vivo, making use of PPyRe9 transfected *L. infantum* so infection burdens and spread of the disease can be followed up by bioluminescent imaging.

Poster 10 : QIQ1-dependent sensing in trypanosomes

Dr Anna Trenaman, *Senior PDRA, University of Dundee*

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Parasitic protists, such as African trypanosomes, occupy distinct environments in mammals and insects, and adapt energy metabolism accordingly. We used a genome-scale loss-of-function genetic screen to identify knockdowns associated with a gain-of-fitness, or a 'quick' growth phenotype, in bloodstream-form *T. brucei*. The top hit, Quick IQ-motif protein 1 (QIQ1, Tb927.8.6870), encodes a flagellum-localised protein with multiple IQ-motifs. In a competition assay, the density-dependent growth advantage of *qiq1*-cells was abrogated by calcium chelation. When cells were grown at lower density in serum, only bicarbonate restored the *qiq1* growth advantage. Proteomic analysis indicated that QIQ1 impacted carnitine acetyltransferase, suggesting a role in fatty acid metabolism, and a putative flagellar calmodulin. We conclude that trypanosomes sense and respond to their environment in a QIQ1-dependent manner, via signaling involving calcium, calmodulin, and carnitine.

Poster 11* : Characterization in the pathogen *Trypanosoma brucei* of an intriguing protein: BILBO3

Miss Chloé Lambert, *PhD student , MFP lab*

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Trypanosoma brucei is a flagellated parasite that causes human and animal trypanosomiasis. Its flagellum exits the cell body through the flagellar pocket (FP), the sole site of endo- and exocytosis, which is enclosed by the flagellar pocket collar (FPC), a cytoskeletal structure essential for FP integrity.

Our team previously identified BILBO1 and BILBO2, two key FPC proteins with structurally similar N-terminal domains (NTDs) that interact with FPC4.

We have now identified three additional proteins sharing ~30% NTD homology with BILBO1, including the previously uncharacterized BILBO3. In silico and in vitro data suggest these proteins form a trypanosome-specific family with related function. Here, I show that BILBO3 localizes to distinct cytoskeletal structures, implicating it in FPC biogenesis. Using BioID, I identified novel BILBO3 interactors, suggesting that FPC assembly and function involve a more complex network than previously appreciated.

Poster 12* : A Novel Iron-Responsive Glycosomal Protein Is Essential for Leishmania Growth and Pathogenicity

MSc Romário Boy, *PhD student, University of São Paulo*

RL Boy[1]; RA Zampieri[1]; JI Aoki[2]; LM Floeter-Winter[1]; MF Laranjeira-Silva[1];
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Leishmania parasites face critical conditions within macrophages, notably a scarcity of essential nutrients such as iron, which triggers the differential expression of various genes. Through in silico analyses of *Leishmania* genes modulated by iron deprivation, we identified a conserved gene containing predicted transmembrane domains, a glycosomal targeting motif, and a RhaT domain, previously associated with drug and metabolite transport. We confirmed the glycosomal localization of this protein and, using CRISPR/Cas9, we generated mutant lines. Notably total gene disruption was lethal unless complemented episomally, demonstrating essentiality. Partial knockout mutants revealed the role of this gene in promastigote and amastigote replication, iron and zinc homeostasis, and parasite in vivo virulence, highlighting its role in pathogenicity.

Poster 13* : Unravelling the role of *Leishmania mexicana* LmxTMP18, an ortholog of the Novymonas esmeraldas NesmTMP18

Miss Kate Sagoe, *Student, University of Ostrava*

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The shift from endosymbiont to organelle is pivotal in eukaryotic evolution; however, the molecular mechanisms supporting endosymbiont maintenance remain largely unknown. The recently discovered endosymbiotic association between *Novymonas esmeraldas* and *Candidatus* Pandoraea novymonadis offers a rare model to study early endosymbiosis. Notably, this system has revealed that a neo-functionalized duplication of the transmembrane protein 18 (NesmTMP18) has yielded appearance of its paralog NesmTMP18e, which plays an essential role in the spatial organization and vertical inheritance of the endosymbiont. To better understand the evolutionary context of this new functionality, we are investigating the role of NesmTMP18 ortholog in the model species, *Leishmania mexicana*. We have established knockout and add-back cell lines and found LmxTMP18 to localize perinuclearly, suggesting conserved roles that may have been adapted in *Novymonas esmeraldas*.

Poster 14* : Quantitative proteomics reveals cytoskeleton-associated proteins essential for stage differentiation in *Leishmania mexicana*

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Leishmania differentiate from extracellular promastigotes to intracellular amastigotes within mammalian macrophages. Understanding how they differentiate and evade the host defense system is crucial to understand different disease manifestations and to improve diagnostic tools and drug development. We therefore performed high-resolution proteome profiling of Leishmania spp. across three species during macrophage infection and identified distinct temporal expression patterns. To validate our dataset's relevance to understand the infection process, we investigated LmPAVE1 (LmxM.23.0080), a cytoskeletal protein previously described in T. brucei, which is upregulated early in infection. LmPAVE1 disruption impairs axenic amastigote formation, suggesting a role in differentiation. Two additional proteins, LmPAVE2 and LmAIR9, may interact with LmPAVE1 to support morphological changes. Our study explores the roles of these proteins in differentiation and intracellular survival.

Poster 15 : Endocytic, surface and secreted VSG family protein

Dr Rupa Nagar, *Postdoctoral Research Associate, School of Life Sciences, University of Dundee*

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T. brucei proteins related to Variant Surface Glycoproteins (VSGs) counter innate immune defences (SRA and TgsGP) and serve as (transferrin) receptors, but many VSG family proteins (VFPs) remain uncharacterised. We have focussed on a group of approx. 40 *VFP* genes located at the ends of polycistronic RNA polymerase II transcription units. Proteome and transcriptome data indicate that these VFPs are selectively expressed in

blood stream stage cells. Several VFPs lack GPI-anchor signals, and accordingly we identified several that were secreted, along with a cocktail of digestive enzymes. Myc-tagging, without disrupting potential signal sequences, revealed other VFPs localised to endocytic compartments and/or to the plasma membrane. Further studies will address functions at the mammalian host-parasite interface, in particular relating to immune defences or receptor functions.

Poster 16* : Characterising the role of the actin cytoskeleton in the *Leishmania mexicana* flagellum and endosomal organisation

Miss Yaimie López, *PhD Student, Oxford Brookes University*

YS López[1]; JD Sunter[1];
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The actin cytoskeleton of Leishmania is reduced compared to model organisms: only 29 canonical actin associated genes identified. Previous work in Leishmania indicated roles for actin in flagellum and endocytosis; however, these only investigated a subset of actin associated proteins. We present the results from screening localisation and function of all known Leishmania actin associated proteins. Each protein was tagged at their endogenous locus localising to the cytoplasm, flagellum and nucleus. We generated 26 deletion cell lines of actin and associated genes and measure their growth rate, motility, flagella length and endosomal organisation. There is a decrease in flagella length in actin-like protein (ALP)1 and ALP3 deletion mutants. These mutants also had defects in motility. Changes in lysosome morphology were seen in the actin and coronin deletion mutants. The actin cytoskeleton has an important role in the biology of flagella and endosomal organisation of *L. mexicana*.

Poster 17* : Inferring reliability of gel-based visualization of LRV2 dsRNA presence through transcriptomic analysis.

Mr Sai Kumar Mishra, *Phd. Student, University of Ostrava*

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Leishmania is known to host multiple species of viruses of the family *Pseudototivirudae*, such as *Leishmania* RNA viruses (LRVs). Viral presence has been linked to the increased chances of developing more severe mucocutaneous leishmaniasis over the cutaneous form, spread of the parasites, and elevated resistance to treatments. A traditional screening for RNA viruses relies on gel-based identification of dsRNA bands, yet it was not clear how sensitive this approach is. In this work, we addressed this issue by direct comparison of gel identification and RNA-sequencing, which represents the gold-standard unbiased approach currently used in the field. Our dataset included 40 *L. major* isolates from Central Asia, 8 of which were shown to contain dsRNA viruses by wet-lab screening. Whole transcriptome analysis also revealed LRV2 presence in the same sub-set of isolates testifying that gel-based identification is sensitive enough, at least in the case of *L. major*

Poster 18* : Intron distribution in genomes of Kinetoplastea: discovery of a novel intron-containing gene and the first eukaryote known to use solely trans-splicing

Miss Karolína Skýpalová, *PhD Student, University of Ostrava*

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In trypanosomatids, parasitic flagellates that include human parasites, *cis*-splicing has been reported in only two genes: poly(A) polymerase and an RNA helicase, while *trans*-splicing is nearly ubiquitous. We analyzed genomes of representatives of all described trypanosomatid genera as well as several non-trypanosomatid kinetoplastids and identified a third intron-containing gene encoding an RNA-binding protein conserved across Kinetoplastea. Remarkably, *Perkinsela* sp., an endosymbiont of an amoebozoan, is the first eukaryote known to completely lack *cis*-splicing while retaining *trans*-splicing. Our data also reveal patterns consistent with reverse transcriptase-mediated intron loss, conservation of 5' splice sites, and the presence of intronic coding RNAs. The three genes encode RNA-binding proteins, implying selective retention of *cis*-splicing, and provide new insights into intron evolution and gene regulation in eukaryotes.

Poster 19* : Dynamics of RNA viruses in *Leishmania spp*

Mr Sai Aditya Reddy, *PhD student, University of Ostrava*

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To better understand virus-host interactions, it is essential to analyze viral load dynamics in parasitic protists. In this study, we monitored the viral loads of three RNA viruses—LRV1, LRV2, and LBV—in three *Leishmania* species: *L. guyanensis, L. major, and L. braziliensis,* respectively. Daily measurement of viral load was conducted by RT-qPCR, normalized for parasite density. All virus-species combinations exhibited a comparable temporal pattern: viral levels increased during the early logarithmic phase, peaked in the late logarithmic to early stationary phase, and then dropped. This signifies a controlled connection between viral replication and host cell physiology, possibly linked to changes in host metabolism, replication rates, or immune evasion strategies. The findings provide crucial understanding of endosymbiotic virus regulation in *Leishmania*, potentially aiding future studies on virus effects on parasite virulence.

Poster 20* : Expanding CRISPR cytosine base editor toolbox for functional genomics in Leishmania: Moving toward multiplexed CRISPR screens

Miss Nicole Herrmann May, *PhD candidate, Department of Cell and Developmental Biology, University of Wuerzburg*

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Functional genomics in *Leishmania* is limited by its repetitive genome, inefficient DNA repair, and lack of RNAi. We demonstrate how cytosine base editing (CBE) facilitates the introduction of functional mutations, including homopolymers and STOP codons, through C-to-T conversion. Co-expression of T7 RNA polymerase and an AsCas12a variant allows efficient integration of large sgRNA libraries, enabling genome-wide loss-of-function screening. To move beyond single-gene perturbations, we developed a multi-guide array using a Cas12a-optimized direct repeat, allowing simultaneous targeting of two or more genes per parasite to investigate genetic interactions. This CBE toolbox supports both single and multiplexed gene targeting and provides a robust platform for functional genomics in *Leishmania*. It has the potential for uncovering genetic interactions and key drivers of drug resistance, fitness, and other phenotypes across species and life stages.

Poster 21* : TurboID proximity labelling identifies interactors at the *Trypanosoma brucei* replication fork

Miss Grace Gill, *PhD Student, University of Glasgow*

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In *Trypanosoma brucei* and all related trypanosomatids, the DNA replication fork proteome remains poorly understood. Investigation of replisome interactors may shed light on maintenance of DNA replication fidelity in the face of replication obstacles, both protein and genomic-landscape related. In this work, TurboID proximity labelling was used to profile proteins interacting with two core components of the active replication helicase (MCM2 and CDC45). Mass spectrometry revealed enrichment of most known replisome factors in addition to some pre-replication complex proteins. MCM2-TurboID recovered a broader array of replication associated proteins than CDC45-TurboID, perhaps reflecting the differing cell-cycle-dependent localisations of these bait proteins. Beyond canonical replication factors, proteins linked to transcription, chromatin remodelling, and DNA repair were identified, suggesting a complex replisome environment. This work begins to establish a network of interactors at the *T. brucei* DNA replication fork, with implications for all trypanosomatids.

Poster 22 : Dissecting the tRNA Thiolation Machinery in *Trypanosoma cruzi*

Mrs Marilene Souza Braga, *PhD student, University of São Paulo*

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The kDNA from *Trypanosoma cruzi* lacks tRNA genes; tRNAs are imported from the cytosol for mitochondrial protein synthesis. tRNAs undergo post-transcriptional modifications such as uridine thiolation. In *T. brucei*, thiolation at position 33 affects mitochondrial tRNATrp editing and gene expression. The tRNA thiolation pathway involves the proteins NFS, MTU1, and ISD11, using cysteine as a thiol donor. In this work, CRISPR-Cas9 was used to generate knockouts and c-myc-tagged strains to investigate their roles in *T. cruzi*. Immunofluorescence revealed both mitochondrial and cytosolic localization. Knockouts did not drastically impact epimastigote proliferation, but the Mtu1-Addback lineage had an increased cell doubling time. Nfs-/+ and Isd11-/+ mutants showed a 2.6-fold increase in differentiation into metacyclic. Nfs-/+ cells showed no defect in respiration. As tRNA thiolation remains poorly understood in *T. cruzi*, this study will reveal links between metabolism and gene regulation.

Poster 23 : Hunting for the protein kinases regulating autophagy in *T. brucei*

Dr Mathieu Cayla, *MRC CDA fellow, University of York*

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T. brucei is exquisitely sensitive to its environment, enabling the study of signaling pathways regulating autophagy. By tagging markers of autophagosomes, we demonstrated that autophagy is essential for quorum-sensing differentiation in bloodstream stages in vivo. We showed that this process is tightly regulated, occurring in short, successive waves rather than continuously. To identify the protein kinases controlling this pathway, we determined the proximity proteome of ATG16 (an early autophagy molecule) using APEX2. We are also developing a complementary high-throughput screen combining a kinome RNAi library (DRiF-Seq) with

FACS sorting to identify the specific kinases involved in other steps of the autophagy pathway. Our project and results represent the first kinome-wide analysis for the regulation of autophagy pathway in eukaryotes and will enhance our understanding of how trypanosome parasites adapt to, and persist, in vivo.

Poster 24* : Function of stealth proteins in Leishmania

Mr Ruben Moss-Keys, *PhD student, Oxford Brookes University*

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A major component of the Leishmania surface coat is the lipophosphoglycan (LPG), which is important for sand fly infectivity. LPG consists of a GPI anchor connected to a backbone of repeating phospho-sugar units, with the number of units increasing during parasite development in the sand fly. The protein machinery responsible for LPG backbone assembly is not fully defined. One possible candidate is the stealth proteins, whose orthologs in bacteria assemble the capsular polysaccharide, which is analogous to the LPG. Bioinformatic analysis of the stealth proteins in the parasitic kinetoplastids showed they were well conserved across these organisms but have been lost in African trypanosomes. In Leishmaniinae, we found two distinct groups of stealth proteins, with one having undergone a large expansion in this lineage. Deletion mutants of the stealth proteins will be created and assessed for phosphoglycan production to define this biosynthetic pathway.

Poster 25* : How is the unique trypanosome mRNA decapping enzyme regulated?

Mrs Alexia Achilles Amaral, *PhD Candidate, Würzburg University*

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mRNA decay is a crucial step in gene regulation. In most eukaryotes, this process follows a conserved 5´-3´ decay pathway, where decapping by the DCP1/DCP2 complex occurs after deadenylation and precedes degradation by exoribonucleases. Kinetoplastida lack the canonical DCP1/DCP2 complex and instead use the ApaH-like phosphatase ALPH1. ALPH1 is essential for parasite survival and partially localises to the posterior pole of the cell. ApaH-like phosphatases are absent in mammals, rendering ALPH1 a good drug target.

To investigate ALPH1's interaction network, we have identified interacting proteins with proximity labelling and immunoprecipitations. Now, we combine Alphafold predictions with proximity ligation assays to identify direct interactions and their intracellular localisations in more detail, including predicted mutually exclusive ones. The ultimate aim of this project is to understand how the unique trypanosome mRNA decapping complex is regulated.

Poster 26 : Transcript and Protein Isoforms of the RNA-Binding Protein 20 Gene

Mrs Natalia Kraeva, *PostDoc, Life Science Research Centre,University of Ostrava*

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One source of protein diversity in cells is the alternative splicing of intron-containing genes. Kinetoplastids are a group of flagellated protists that have lost most of their introns. Among the few genes that retain a single intron are those encoding poly(A) polymerase, RNA helicase, and RNA-binding protein 20 (RBP20). The latter two are universally conserved across trypanosomatids, suggesting their importance in parasite biology.

Recently, we demonstrated the presence of transcript isoforms for all three genes. This study aims to determine whether these transcript isoforms are translated. *Leishmania mexicana* was selected as the model organism, and RBP20 — an RNA-binding protein of unknown function — has been chosen for analysis. To track expression, the RBP20 was endogenously tagged at its C-terminus with a HaloTag. WB analysis revealed two distinct protein bands corresponding in size to the predicted products (exon1–exon2–Halo and exon2–Halo).

Poster 27* : No CENP-A? No problem! Kinetochore positioning in trypanosomes

Ms Aleksandra Ciszek, *Phd student , University of Edinburgh*

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Kinetochores assemble at centromeric DNA to interact with spindle microtubules. In many eukaryotes, a centromeric histone H3 variant CENP-A epigenetically specifies kinetochore assembly sites within centromeres. However, *Trypanosoma brucei* lacks CENP-A and conventional kinetochore proteins, instead relying on a unique set of proteins (KKTs). KKT2 and KKT3 form the base of trypanosome kinetochores and recruit other KKTs to assemble kinetochores. However it remains unknown how they specifically localise at centromeres. Here we show that the centromere localization (CL) domain of KKT2 and KKT3 interacts with the unmodified N-terminus of histone H3 *in vitro*. Using custom monoclonal antibody, we also show that the histone H3 N-terminus is unmodified specifically at centromeres in a subset of G1 cells. These results suggest that the N-terminus of histone H3 is unmodified at centromeres, which helps to determine kinetochores assembly sites in trypanosomes.

Poster 28* : Unraveling the biological role of base J in *Leishmania mexicana*

Miss Eva Duenas, *PhD student, Ostrava university*

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Leishmania are flagellated parasites causing leishmaniasis. These parasites, along with other trypanosomatids, contain a hypermodified residue called base J that constitutes up to 1% of total thymidine. In some trypanosomatids investigated in this respect, it is associated with transcription termination and implicated in metacyclogenesis. Dimethyloxalylglycine (DMOG) is an inhibitor of thymidine hydrolase activity, which affects J biosynthesis. We evaluated the DMOG effect during the *in vitro* differentiation of *L. mexicana*. Our data suggest that a decrease in base J level results in the arrest at the metacyclic stage and prevents *in vitro* differentiation into axenic amastigotes. This is corroborated by the reduction of base J level in the metacyclic promastigotes exposed to DMOG. Understanding the role of base J will contribute to the discovery of new targets.

Poster 29* : Ambiguous translation termination in Blastocrithidia nonstop

Mr Puranjaya Pancholi, *PhD Student, Biology Centre CAS*

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The universality of the genetic code is challenged by organisms that reassign stop codons. *B. nonstop*, a trypanosomatid, decodes all three canonical stop codons as sense codons: UAG and UAA as glutamate and UGA as tryptophan, respectively. Notably, UAA retains its function as a termination codon, indicating dual functionality. In addition, UAA codons are highly enriched in 3' UTRs, raising questions about translation termination fidelity. To investigate this phenomenon, we performed mass spectrometry on *B. nonstop* and identified over 25,000 peptides, including 300 C-terminal peptides. Using an in silico extended coding sequence database, we discovered that translation mostly terminates at the annotated stop codon. However, in a substantial number of cases, translation continues, producing extended proteins. Our results suggest that termination in *B. nonstop* via single UAA is insufficient and may be context-dependent, controlled by an alternative mechanism.

Poster 30 : Purification of recombinant Kinetoplastid kinetochore protein complexes.

Dr Samuel Taylor , *Postdoc, University of Edinburgh*

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Kinetochores consist of numerous proteins that together mediate chromosome-microtubule attachments throughout cell division. Interestingly, kinetoplastids lack homologs of any of the core kinetochore proteins that are conserved in the vast majority of eukaryotic organisms. Instead, kinetoplastid kinetochores consist of a unique set of proteins termed Kinetoplastid Kinetochore 1-20, 22-25 (KKT1-KKT20, KKT22-KKT25) and KKT-interacting proteins 1-12 (KKIP1-12). In the last decade, much progress has been made in characterising the hierarchy of trypanosome kinetochore proteins and their functions. However, a mechanistic understanding of how these proteins interact with one another remains to be elucidated. Here, I will share my progress in reconstituting recombinant trypanosome kinetochore protein complexes with the aim of future biochemical and structural investigation.

Poster 31 : Callunene and flagellum removal in trypanosomatids

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The group of Trypanosomatid species parasitising Hymenoptera has recently started to attract more research attention because of the severe negative effects some of these trypanosomatids have on the insect populations (honey bees). Substantial efforts are now being invested in the development of anti-trypanosomatid drugs that can be used in hymenopterans. One of such potential drugs was a volatile compound recently isolated from the heather (*Calluna vulgaris*) nectar and identified as 4-(3-oxobut-1-

enylidene)-3,5,5-trimethylcyclohex-2-en-1-one (callunene). This potent secondary metabolite was shown to affect infectivity of a bumblebee parasite, *Crithidia bombi,* which was explained by flagellum removal resulting in the inability of these parasites to attach to the ileum of the host (*Bombus terrestris*) and, thereby, preventing gut colonisation. This study aims to investigate the precise mechanism of action and its impact on the trypanosomatid flagellum.

Poster 32 : Characterization of phosphatidic acid phosphatase 2 in *Leishmania mexicana*

Miss Elora Kalita, *Phd student, ostrava university*

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Phosphatidic acid phosphatases (PAPs) are key regulators of lipid signaling, catalyzing the dephosphorylation of phosphatidic acid (PA) to diacylglycerol and, thereby, influencing membrane biogenesis and signal transduction. In *L. mexicana*, we identified three PAP2 orthologs: PAP2-1, PAP2-2, and PAP2-like. This study investigates PAP2-2 *via* CRISPR-Cas9-mediated knockout and phenotypic characterization. PAP2-2 loss reduced flagellum length, restored in the addback line. Surprisingly, while knockout and wild-type cells had similar growth, the addback line showed impaired proliferation. Knockouts also displayed altered oxygen consumption, suggesting mitochondrial involvement. Additionally, intracellular PA accumulated in knockouts, implicates PAP2-2's role in PA homeostasis. Together, these findings suggest PAP2-2 to be a critical modulator of lipid signaling, influencing flagellar structure, energy metabolism, and growth in *L. mexicana.*

Poster 33 : The *Trypanosoma brucei* DNA damage repairome

Dr Monica Zavala Martinez, *Postdoc, Institut Pasteur*

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In *Trypanosoma brucei*, the DNA damage response (DDR) is a coordinated process involving many DNA repair factors and post-translational modifications (PTMs). Antigenic variation via variant surface glycoproteins (VSGs), key for parasite survival, is linked to DDR, as DNA double strand breaks (DSBs) trigger VSG switching. While PTMs are vital for the accurate response to damage, only a few have been characterised. Using an unbiased single-locus biochemical screen that generates a DSB at a chromosomal internal region and at a bloodstream form expression site (BES), we performed a phosphoproteomic analysis over the course of 12 h post-DSB, to identify key DDR phosphorylations and their dynamics over time. Notably, the phosphorylation of S5 and S43 on RPA1 (Replication Protein A1), part of a complex that binds to single strand-DNA (ssDNA). Our study aims to map repair interactions in trypanosomes, defining the 'repairome' and providing insights into mechanisms driving VSG switching.

Poster 34* : The journey of tsetse-transmitted trypanosomes: from the skin invasion to systemic infection

Dr Sergio Araujo, *Postdoc, LMPH - University of Antwerp*

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Following tsetse-mediated transmission, *T. brucei* rapidly adapts to the skin, establishing local infection and progressing to systemic colonisation. Using fluorescent reporter lines, bioluminescent imaging, immune-deficient mouse models, and immunological profiling, we investigated the role of innate immunity in early infection dynamics. Despite robust recruitment of effector cells, parasites evade clearance and persist in tissues including adipose, spleen, and lungs. In the lungs, parasites localise to perivascular spaces of alveoli and bronchi, eliciting a strong local immune response marked by monocytes, macrophages, dendritic cells, γδ and activated αβ T cells, followed by neutrophils. Concurrent depletion of B cells, eosinophils, and NK cells increases susceptibility to secondary infections, as shown in RSV co-infection models. This newly identified asymptomatic lung reservoir complicates disease control but enables exploration of new diagnostics approaches.

Poster 35 : Breaking up with oxygen: Elucidating the bioenergetics of *Blastocrithidia* nonstop

Mrs Ingrid Škodová-Sveráková, *research assistant, Institute of Parasitology, Biology Centre CAS*

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Blastocrithidia nonstop, a unique trypanosomatid, possesses an altered genetic code and remarkable anaerobic ATP synthesis. Unlike most trypanosomatids, which struggle without oxygen, B. nonstop thrives in its absence, making anaerobiosis its default. This metabolic shift stems from the absence of respiratory complex I and endogenous quinone, both vital for efficient oxidative phosphorylation. To compensate, B. nonstop employs three alternative ATP-producing pathways: the succinate/fumarate/malate cycle, stepwise succinate reduction in glycosomes, and propionate synthesis in mitochondria. While it can use exogenous ubiquinone Q_{10} and complex V for oxygen-dependent ATP synthesis, this pathway is less efficient and slows cell division.

Poster 36* : Discovering the mechanism of how trypanosomes epigenetically assemble unconventional kinetochores at centromeres

Mr Sam Forsyth, *PhD student, University of Edinburgh*

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The kinetochore is the macromolecular protein complex that assembles specifically onto centromeres and binds spindle microtubules. In many eukaryotes, histone variant CENP-A plays a key role in determining kinetochore positions. However, evolutionarily divergent kinetoplastids do not have CENP-A but instead have an unconventional set of kinetochore proteins such as KKT1-25. Consequently, how centromere specificity is achieved in kinetoplastids remains unknown. Our current hypothesis is that KKT2/3 dock onto an unmethylated histone H3 N-terminus restricted to centromeres, implying that an unknown methyltransferase 'paints' the rest of the genome to prevent ectopic kinetochore assembly. By screening methyltransferases in *Trypanosoma brucei*, we have identified a putative methyltransferase, for which RNAi produces a loss of H3 N-terminus methylation. We are currently characterising the enzyme to deepen our understanding of kinetochore specification in these highly divergent parasites.

Poster 37 : Link between the early biogenesis of the small mitoribosomal subunit and RNA editing in *Trypanosoma brucei*

Mr Prashant Chauhan, *Participant, Biology Center CAS*

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Mitoribosomal small subunit (mtSSU) biogenesis in *Trypanosoma brucei* involves three precursors characterized by cryoEM. The earliest pre-mtSSU, which contains incomplete set of mtSSU proteins, immature rRNA, and 34 assembly factors (AFs), features a protrusion composed of an essential pentameric AF mtSAF24 capped with a disc-like structure of unknown composition. We removed the N-terminal domain of mtSAF24 to detach the cap from the complex. Comparison of complexes with and without the cap by IP-MS using an AF as a bait revealed that the cap is formed by a trimer of p22, a protein previously associated with U-indel RNA editing. Thus, p22 provides a direct link between mitoribosome biogenesis and RNA editing. Our IP-MS experiments also identified new mtSSU AFs, including homologs of three human AFs essential for mtSSU assembly and viability of *T. brucei*. We're currently isolating pre-mtSSU precursors containing the AFs for cryo-EM to elucidate early steps in mtSSU assembly

Poster 38* : Small heterocyclic compounds selectively targeting myo-inositol metabolism in *Trypanosoma cruzi*

Ms Veronica Harris, *PhD student, University of St Andrews North Haugh*

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In *T. cruzi, myo*-inositol is a precursor to phosphatidylinositol (PI), an essential membrane lipid. PI is required for inositol phosphorylceramide, phosphoinositides, and glycosylphosphatidylinositol-anchored molecules coating the parasite's cell-surface allowing parasite-host interactions. *T. cruzi de novo* synthesise *myo*-inositol. The first enzyme involved in *myo*-inositol *de novo* synthesis is *Tc*INO1 and is an essential *T. cruzi* gene. Thermal shift studies screened fragment libraries containing small heterocyclic compounds to find compounds interacting with recombinantly expressed and purified *Tc*INO1. Top compounds were tested against parental strain and genetically manipulated *TcINO1 T. cruzi.* Several compounds demonstrated EC_{50}<50 µM. Analogues were explored to understand the top compounds' activity against *T. cruzi* and effects on *Tc*INO1 enzyme activity.

Poster 39* : Characterising the Heat Shock Response in African Trypanosomes

Miss Hannah Pyle , *Masters by Research Student , Lancaster University*

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African trypanosomes, the causative agents of sleeping sickness (*Trypanosoma brucei*) and nagana (*Trypanosoma congolense*), experience host-derived increases in temperature as fever during infection. In response, these parasites activate a heat shock (HS) response that promotes survival under elevated temperatures. This work aimed to characterise the HS response in *T. brucei*, with preliminary comparative analysis in *T. congolense.*

Following a controlled HS, a reversible G2/M cell cycle arrest was observed via flow cytometry, accompanied by reduced motility and parasite viability. Using polysome profiling, it was found that HS induces a global

translational arrest through the collapse of polysomes into monosomes, enabling the reduction in active translation. This mechanism is thought to prioritise the selective translation of heat shock proteins and other mRNAs essential for survival over general housekeeping mRNAs.

While this polysome collapse using polysome gradient fractionation was first described in procyclic form *T. brucei* (Kramer et al., 2008), our findings confirm this and additionally its occurrence in the bloodstream form, indicating a conserved stress response mechanism across life cycle stages. Although polysome data for *T. congolense* are currently pending, its parallel physiological response is being investigated.

Understanding the HS response in trypanosomes is crucial for uncovering parasite survival strategies under host-induced stress and may highlight unique regulatory pathways absent in mammalian hosts. These insights offer potential avenues for future targeted therapeutic development.

Poster 40* : Kinetoplast associated proteins reliant on internal targeting signal for mitochondrial transport

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The kinetoplast of *Trypanosoma brucei* and its relatives represents one of the most complex organellar genome structures found in nature, composed of two classes of circular DNA molecule which form a chainmail-like network. This complex structure presents an extraordinary challenge for the faithful replication and inheritance of the network. Over 50 mitochondrial proteins are known to be involved in its replication, yet many gaps in our understanding of this process and the factors that mediate it remain. Here, we present the early characterization of four novel kinetoplast-associated proteins, all of which lack homologues outside kinetoplastids. Using expansion microscopy, we resolve previously unobservable differences in their spatial distribution in and around the kinetoplast, with multiple proteins displaying a unique localization pattern not reported in any previous kinetoplast protein. These unique localisations are further corroborated by transmission electron microscopy of cryosectioned cells. The newly identified kinetoplast proteins display a range of depletion phenotypes suggesting functional diversity, though they appear generally to affect cell cycle progression and minicircle replication. Interestingly, these proteins lack canonical presequence and instead appear to rely on a short internal signal near the C-terminus for mitochondrial transport.

Poster 41* : Distinct roles of three Oxa1 insertases in shaping the submitochondrial proteome of *Trypanosoma brucei*

Mr Jonathan Wong, *PhD researcher, Institute of Parasitology, Biology Centre ASCR*

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The mitochondrial insertase Oxa1 is essential for the translocation of nuclear- and mitochondrial-encoded transmembrane proteins and for respiratory chain (RC) complex biogenesis at the inner mitochondrial membrane (IMM). *Trypanosoma brucei* has three putative TbOxa1 paralogs with unknown roles in IMM biogenesis. We performed submitochondrial fractionation by carbonate extraction and label-free quantitative mass spectrometry to define the proteomes of mitochondrial subcompartments. To assess the impact of TbOxa1 proteins in shaping the submitoproteomes, we compared fractions from procyclic form TbOxa1KO and wildtype cells. Our TbOxa1 depletomes suggest overlapping and specific effects on IMM protein biogenesis, primarily RC complex components, supporting our biochemical data. Overall, our results enable systematic

analysis of submitoproteome landscapes and provide a reliable reference for future protein localization and function studies in the unique mitochondrion of trypanosomes.

Poster 42 : Modelling next-generation human colonic organoids to investigate neuro-immune crosstalk during enteric parasitic infections

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The enteric nervous system (ENS) and the immune system communicate through complex neuro-immune crosstalk, which is critical for maintaining gut health and responding to infections. However, our understanding of this interaction is limited by the lack of human-relevant models. We have established advanced human intestinal organoids that incorporate both the ENS and innate immune components to investigate how these systems coordinate during enteric parasitic infections. Our recent work has shed light on the involvement of inflammation and neuronal signaling in parasitic infection by *Trypanosoma cruzi* (a protozoan parasite that causes digestive Chagas disease), which was previously underappreciated. Using *Trypanosoma cruzi* as an infection model, this project explores host-pathogen interactions and identifies key neuro-immune pathways. The findings will provide insights into gut physiology and infection mechanisms, while also informing future therapeutic strategies.

Poster 43* : Heme limitation induces LHR2, an essential gene for Leishmania pathogenesis

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Leishmania spp. are intracellular parasites that cause leishmaniasis, a devastating disease with no effective treatment. These parasites are heme auxotrophs and must scavenge this essential cofactor from the host. Transcriptomic analysis of *Leishmania* major promastigotes cultured in the presence or absence of heme revealed numerous differentially expressed genes. Among those of unknown function, *LHR2* (*Leishmania* Heme Response-2) was the most upregulated gene in response to heme limitation. *LHR2* encodes a mitochondrial hemoprotein that is essential during the promastigote stage. Loss of a single *LHR2* allele severely compromises intracellular replication and prevents the development of cutaneous leishmaniasis in mice. This essential function depends on LHR2's ability to bind heme. Complementation studies in Saccharomyces cerevisiae revealed that LHR2 is a functional homologue of the yeast Dap1p, although it binds heme in a distinct ma

Poster 44* : Unveiling the Role of the CRL1 E3 Ubiquitin Ligase Complex in Leishmania: Implications for Promastigotes and Amastigotes Proliferation

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The ubiquitin-proteasome system (UPS) is the main regulator of intracellular proteolysis in eukaryotes and is essential for parasite host alternation. Cullin-1-RING ubiquitin ligases (CRL1) are the largest class of E3 ligases in mammals, regulating key processes like the cell cycle and proliferation. These multiprotein complexes include SKP1, CUL1, RBX1, and an F-box protein as a specificity factor by recruiting substrates. *Leishmania* UPS is poorly studied, and despite its relevance, CRL1 has yet to be described. Here, we demonstrate that LinfSkp1, LinfRbx1, and LinfCul1 form a LinfCRL1 complex in *L. infantum*, the causative agent of visceral leishmaniasis. LinfSkp1 and LinfCul1 interactomes revealed proteins involved in various intracellular processes, including six F-box-like proteins (Flp), described for the first time in *L. infantum*. The interaction of LinfFlp1–6 with LinfSkp1 was confirmed, and *in vitro* ubiquitination assays showed th

Poster 45* : Structure-function studies of the mRNA decapping enzyme of *Trypanosoma brucei*

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In Trypanosomatids, mRNA decapping is uniquely performed by ALPH1 - in stark contrast to all other eukaryotes, which use the Dcp2 Nudix hydrolase. This distinct cap cleavage mechanism offers promising opportunities for trypanocidal drug development and innovative biotechnology applications. In this project, we established a robust protein production protocol for T. brucei ALPH1 and its several truncated versions. Using SEC-MALS, we found that ALPH1 forms a homodimer, with oligomerization occurring via its C-terminal domain. To study its activity, we developed a one-step enzymatic assay using the direct fluorescent probe m^7GTP-pyrene. We are currently screening additional variants of the ALPH1 catalytic domain for crystallization trials. Solving the crystal structure of ALPH1 in complex with cap analogs and inhibitors will provide deeper insights into its reaction mechanism and substrate specificity, supporting our ongoing drug development efforts.

Poster 46 : Does ATM play a role in DNA repair and genome plasticity in Leishmania?

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Double Strand Breaks (DSBs) compromise genome stability. DSBs resolution can be orchestrated by the atypical kinase ATM which is recruited to and activated at DSB sites by the complex MRN. In *Leishmania*, this pathway is poorly understood however recent works suggest *Leishmania* ATM may possesses important,

parasite-specific functions. Here, we deleted ATM in *L. major* promastigotes. We found that ATM is non-essential though its loss modestly affects proliferation. However, we found under DNA damage conditions, ATM loss significantly sensitises parasites to a range of genotoxins, playing a role in modulating a damage induced G1/S phase checkpoint. Moreover, ATM is required for H2A phosphorylation (γH2A), a key signaller of DNA damage. Additionally, ATM loss enhanced genome instability suggesting this kinase plays a key role in maintaining the stability of the *Leishmania* genome.

Poster 47 : Genetic exchange and extant heterozygosity in natural isolates of "Old World" Leishmania isolates

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Leishmaniasis is a vector-borne disease caused by protozoa in the genus *Leishmania*. Experimental studies established that *Leishmania* can recombine, but the extent to which natural hybridization occurs in Old World populations is unclear. The current model for *Leishmania* evolution is a population of clones that expand predominantly asexually. We developed a pan-genus MLST scheme using 27 markers that we applied to ~200 previously typed strains, including *L. donovani*, *L. infantum*, *L. tropica*, and *L. major*. Phylogenetic and WGS analysis identified significant allelic diversity, heterozygosity, and both intra- and inter-specific hybrids in ~30% of isolates. Notably, *L. tropica* strains from the Middle East and India were predominantly heterozygous, whereas Eastern Mediterranean isolates were homozygous. Our results indicate that sexual reproduction is ongoing and plays a more significant role shaping the population structure within the Old World than previously envisaged.

Poster 48 : Mapping convergent trajectories of Leishmania adaptation reveals snoRNA clusters as major drivers of fitness gain.

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Leishmania exploits genome instability and gene dosage-dependent expression changes for adaptation. How these parasites filter toxic from beneficial gene dosage effects remains unknown. Our previously published studies propose the *Leishmania* ribosome as a possible biological filter that can harness genome instability for adaptation. Here we tested this hypothesis conducting three parallel evolutionary experiments (EEs) that allowed us to map genomic changes within and in-between adapting populations and distinguish signals driven by random genetic drift from those under natural selection.

Each EE comprised (i) isolation of an independent amastigote population from infected hamster spleens, (ii) in vitro promastigote conversion and short-term expansion for two passages (P2, non-adapted population), (iii) selection for in vitro fitness gain during 18 more in vitro passages (P20, adapted population), and (iv) establishment of 15 clonal populations from P20 to compare adaptive genomic trajectories.

Karyotypic analysis revealed convergent chromosome amplification across the three P20 populations and derived clones, all of which were showing strong haplotype selection. Aside these shared driver aneuploidies, each EE was characterized by distinct passenger amplifications, indicating complex, epistatic interactions between chromosomes. Gene copy number variation (CNV) analyses identified two main adaptive trajectories,

both involving large snoRNA gene clusters encoded on chr 26. Convergent CNV patterns confirmed that these clusters were under natural selection, suggesting they play a central role in parasite adaptation. Interestingly, the CNV change of these two clusters showed negative correlation: while in two EEs a cluster of 62 snoRNAs was amplified and a second cluster of 18 snoRNAs showed depletion, the third EE exhibited the opposite pattern. Overall, these genomic analyses demonstrate that, despite different evolutionary paths towards culture adaptation, there is clear evidence of convergence both within and between populations at the karyotypic and genotypic levels. Amplified chromosomes were enriched in snoRNA gene clusters, which further underwent significant gene copy number variation. Given the established role of snoRNAs in guiding methylation and pseudouridylation of ribosomal RNA, these small, non-coding RNAs emerge as key fitness factors, suggesting that the ribosome itself may act as a regulatory filter, modulating gene dosage effects through translational control. We now aim to assess the impact of snoRNA gene CNVs on translation efficiency using RiboProfiling and on ribosomal structure using Cryo-EM.

Poster 49 : Nutritional stress as a signal for *Trypanosoma brucei* differentiation to the mesocyclic-like form

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The developmental progression of *Trypanosoma brucei* within the tsetse fly involves tightly regulated transitions between distinct life cycle stages. After colonizing the midgut, procyclic form (PF) cells migrate to the proventriculus and differentiate into mesocyclic forms, marked by cell elongation, directional motility, and synchronized cell cycle arrest. However, the molecular signals driving this transition remain unclear. In vitro, stationary-phase PF cells exhibit phenotypes resembling mesocyclic-like features, including cell elongation and proliferation arrest. Transcriptomic and proteomic analyses revealed increased expression of motility- and vesicle transport-related proteins, alongside reduced levels of translation and chromosome segregation factors. Metabolomic profiling identified severe depletion of key metabolites such as glucose, glutamine, proline, tyrosine, and nucleotide derivatives. Notably, only tryptophan supplementation restored cell division. The mechanism by which tryptophan promotes cell cycle re-entry remains unclear, but it may involve its role as a nutritional signal, support for protein synthesis, or conversion into bioactive compounds. Possible mechanisms include activation of growth-related signaling pathways, restoration of NAD^+ levels, or production of indole derivatives influencing differentiation. These findings support a model in which metabolic stress and nutrient availability coordinate parasite development.

Poster 50 : Disruption of ecdysone signaling affects Leishmania infantum infection and microbiota homeostasis in *Phlebotomus perniciosus*.

Mrs Cecilia Stahl Vieira, *Researcher, Charles University*

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Introduction: *Phlebotomus perniciosus* is the natural vector of *Leishmania infantum*, the causative agent of visceral leishmaniasis in the Mediterranean basin. *Leishmania* development within the sand fly midgut relies on finely tuned modulation of vector immunity and gut microbiota. However, the mechanisms by which *L. infantum* activates immune responses and alters microbial communities to establish infection in *P. perniciosus* remain poorly understood. The steroid hormone ecdysone (Ecd) plays a central role in insect development and reproduction via its nuclear ecdysone receptor (EcR). In addition to its classical functions, ecdysone signaling has been implicated in regulating immunity and microbiota homeostasis, thereby

influencing pathogen susceptibility in insect vectors. Recently, we demonstrated that chemical disruption of ecdysone signaling using azadirachtin downregulates both ecdysone-related genes and antimicrobial peptides (AMPs), indicating a direct link between hormonal signaling and sand fly's immunity. Building upon these findings, we studied how *L. infantum* infection modulates both ecdysone signaling and immune-related gene expression in *P. perniciosus. We also* investigated how the disruption of ecdysone signaling in the vector, achieved through RNAi-mediated EcR gene silencing, affects immunity, gut microbiota composition, and susceptibility to *Leishmania* infection.

Methods: Double-stranded RNA targeting *EcR* (dsEcR) or a control sequence (dsLacZ) was synthesized and microinjected into the thorax of *P. perniciosus* females infected by *L. infantum*. The effects of *EcR* knockdown were evaluated by assessing the relative gene expression, by RT-qPCR, of ecdysone-related genes, Imd-related genes, and AMPs. Parasite load was quantified by measuring the expression of *Leishmania* actin. In addition, alterations on gut microbiota were quantified by RT-qPCR using 16S-rRNA universal primers for eubacteria, and 16S-rRNA primers for the following bacterial groups: Acetobacteraceae, Flavobacteriaceae, Enterobacteriaceae, and *Wolbachia*.

Results: *L. infantum* infection induces the expression of the ecdysone-related genes, along with upregulation of Imd-related genes *and AMPs*, coinciding with a significant reduction in gut microbiota population in *P. perniciosus***.** In contrast, RNAi-mediated silencing of *EcR* resulted in the transcriptional repression of downstream hormonal targets, confirming the disruption of ecdysone signaling, suppressed Imd-related genes, leading to a dysregulation of AMPs expression. *EcR* knockdown resulted in a significant reduction of parasite load. This effect was concomitant with a marked increase in total eubacterial abundance and shifts in the microbial composition in the vector gut. **Discussion:** Our results suggest that *Leishmania infantum* activates the ecdysone signaling pathway to shape the vector's immune response, inducing a controlled activation of the Imd pathway and suppressing gut microbiota, which may favor parasite establishment. When the ecdysone pathway is disrupted, this fine-tuned regulation is lost, leading to uncontrolled microbial proliferation and ultimately compromising parasite survival. Altogether, our findings highlight the pivotal role of ecdysone signaling in coordinating immune responses in sand flies and reveal its impact on *Leishmania* development. This work opens new perspectives for targeting hormonal pathways in vector control strategies.

Keywords: *Phlebotomus perniciosus*, ecdysone receptor, innate immunity, microbiota, *Leishmania infantum*, RNA interference

Poster 51 : Mitochondrial metabolic adaptations to mitochondrial genome loss in *Trypanosoma evansi*

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Trypanosoma evansi, a dyskinetoplastic parasite lacking mitochondrial DNA, presents a unique model for studying metabolic adaptations to mitochondrial genome loss. Unlike *T. brucei,* whose bloodstream form (BSF) mitochondrion maintains its membrane potential through ATP hydrolysis by the F_1F_o-ATP synthase*, T. evansi* cannot sustain proton pumping due to the absence of essential mitochondrially encoded component. Instead, it relies on an electrogenic membrane potential maintained by the ADP/ATP carrier (AAC), which exchanges glycolytic ATP^{4-} from the cytosol with matrix ADP^{3-}.

We recently showed that the *T. brucei* BSF mitochondrion can produce ATP via substrate-level phosphorylation through succinyl-CoA synthetase (SCS) and acetate-succinate CoA-transferase (ASCT). We hypothesized that this pathway must be restricted in *T. evansi* to preserve reversed AAC activity. Indeed, whole-cell proteomic analysis revealed reduced expression of SCS and ASCT in *T. evansi* mitochondria compared to *T. brucei*. Functional studies demonstrated that ASCT overexpression in *T. evansi* is lethal, associated with a loss of

mitochondrial membrane potential likely caused by elevated intramitochondrial ATP interfering with AAC activity. In contrast, ASCT overexpression in *T. brucei* BSF had no effect under low tetracycline induction and only affected growth at high induction levels, possibly due to mislocalization of ASCT to the cytosol.These findings suggest that *T. evansi* has evolved specific regulatory mechanisms to limit mitochondrial ATP production and sustain AAC-dependent membrane potential. Ongoing work using BRET-based ATP biosensors, respiration assays, and membrane potential measurements will further clarify the metabolic strategies supporting *T. evansi* survival in the absence of mitochondrial DNA.

Poster 52* : A synchronicity toolkit: Counter-flow centrifugal elutriation for *L. mexicana* promastigotes

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Trypanosomatids, such as *L. mexicana,* have an atypical genome organisation, with genes arranged in polycistronic transcription units of functionally unrelated protein coding sequences. Gene expression is therefore primarily constitutive; regulated post-transcriptionally with an emphasis on post-transcriptional modifications. This could reveal new and interesting perspectives on mechanisms modulating critical cellular processes, such as the cell cycle. To progress our understanding of the cell cycle in these organisms it is desirable to have a synchronised population, allowing the study of specific cell cycle events. Current methods of cell cycle synchronisation (CCS) are limited. They are known to impact pathways included in, and beyond, the canonical cell cycle controls, frequently resulting in detrimental fitness effects. Counter-flow centrifugal elutriation (CCE) is a stringent CCS methodology. Here we present a novel CCE protocol optimised for synchronisation of *L. mexicana* (*Lm*CCE)promastigotes into stage-specific cell cycle fractions, with 7-fold enrichment. This methodology preserves cell fitness and maintains synchronicity across three cell cycles. CCE is a high-throughput, non-invasive approach, which can support down-stream experiments from a single synchronised population. *Lm*CCE has since been used to generate an RNA-seq dataset, temporally profiling mRNA abundance across a synchronised population. Moving forward, this will be used to elucidate the temporal landscape of prolonged, and acute, fluctuations in mRNA abundance across the cell cycle of *L. mexicana*. We also display preliminary bioimaging of *L. mexicana* promastigotes, utilising a newly optimised expansion microscopy protocol. These images demonstrate the potential to map specific changes in morphology and organelle replication temporally when used in combination with *Lm*CCE. We aim to profile the *L. mexicana* cell cycle with unparalleled synchronicity, demonstrating a foundational resource for the kinetoplastid community.

Poster 53 : Caught in the spotlight: Photoaffinity labelling for target identification in trypanosomatids

Dr Laurine Brouck, *Postdoctoral researcher, University of Dundee*

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Target identification and mode-of-action studies are critical to support rational drug design and accelerate drug discovery. While various genomic and proteomic approaches have been applied in trypanosomatids, photoaffinity labelling (PAL) has rarely been used for unbiased target identification in these parasites. PAL

relies on small-molecule probes bearing a photoactivatable crosslinker and a chemical handle to capture and enrich protein targets in live cells upon UV irradiation. As a proof of concept, we synthesised PAL probes based on a chromone core previously shown to inhibit *Trypanosoma cruzi* lysyl-tRNA synthetase 1 (KRS1). Affinity-based enrichment via streptavidin pulldown selectively retrieved both cytosolic KRS1 and its mitochondrial paralogue KRS2, supporting direct engagement. In parallel, we generated a fully functionalised fragment (FFF) library of >1,000 photoreactive compounds. These fragments encompass a broad range of chemical structures, each bearing a photoreactive group and an enrichment handle, enabling both phenotypic screening and downstream target identification. A representative subset of 90 FFFs yielded hit rates of 8.9% in *Trypanosoma brucei*, 4.4% in *Leishmania donovani*, and 3.3% in *Trypanosoma cruzi* and HepG2 cells. Ongoing target deconvolution aims to reveal novel druggable targets, thereby informing new strategies for anti-trypanosomatid drug discovery.

Poster 54 : Evolved for evolvability: Full assembly of the Sudanese *L.donovani* Ld1S reference genome uncovers a unique architecture promoting genomic adaptation and fitness gain through genome instability, haplotype diversity and asymmetric gene copy number variations

Dr Thomas Cokelaer, *Research Engineer, Institut Pasteur*

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In the absence of classical transcriptional control, *Leishmania* has evolved a highly plastic genome that allows for gene dosage-dependent expression changes. Using short- and long-read sequencing of the Sudanese *L. donovani* strain Ld1S, we uncovered a unique genome architecture evolved to promote genomic plasticity and evolutionary adaptation.

Nanopore sequencing data (110X read depth) was used to produce a complete telomere-to-telomere assembly of 36 chromosomes and the maxicircle. Closing 18 gaps in the previous reference finally yielded a fully assembled Ld1S genome of 34.7Mb in size. Identifying centromeres in *Leishmania* remains challenging due to the lack of conserved sequence motifs or defining signatures. Centromeres were detected by integrating Hi-C contact maps and confirmed by our in-house deep learning software. We revealed chromosome-specific motifs that were conserved across *Leishmania* species but diverged at the Trypanosomatidae taxonomic level (family), indicating lineage-specific evolution, which may underpin karyotypic plasticity and adaptive aneuploidies that we previously documented in *Leishmania*.

Analysing the new Ld1S assembly for the presence of alternative DNA conformations (non-B DNA) - which can promote genome instability by altering DNA topology - revealed a strikingly high density of G-quadruplex (G4) motifs compared to the related parasites *T. brucei* and *T. cruzi* as well as yeast. These motifs are enriched in centromeres and within tandem gene arrays, such as those encoding for gp63 and amastins, which are highly dynamic and known to undergo frequent gene CNV depletions or amplifications, potentially fine-tuning adaptive phenotypes as we have observed in parasite field isolates.

Finally, the high frequency of heterozygous SNPs and their patchy distribution are likely remnants of an ancient hybridization event, with subsequent gene conversion and recombination reducing linkage disequilibrium. By integrating heterozygous variants and allele-specific gene copy number changes (termed asymmetric gene CNVs) into our assembly, we produced a fully phased genome, revealing two distinct haplotypes. The presence of both quantitative (CNV) and qualitative (non-synonymous SNP) differences between these haplotypes further expands the adaptive potential of the *Leishmania* genome, explaining the strong haplotype selection we observed during fitness gain in culture.

In conclusion, our data reveal that the *L. donovani* genome architecture has evolved to enhance its evolvability by (i) promoting karyotypic instability via fragmentation of its genome into 36 chromosomes with divergent centromeres, (ii) increasing genomic instability through topological stress induced by highly abundant repetitive elements, includi

Poster 55* : Assembly of the *Trypanosoma brucei* F1-ATPase

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The mitochondrial F_oF_1-ATP synthase plays a key role in aerobic eukaryotes by generating ATP through the process of oxidative phosphorylation. While structural variations are observed in the membrane F_O domain throughout the eukaryotic tree of life, the matrix protruding F_1 domain is highly conserved. Exceptions are found within Euglenozoans, a diverse group of flagellate protists with unique features in their F_1-ATPase structure. A striking example is *Trypanosoma brucei*, an important model within this group. Its F_1 domain is elaborated with three copies of the novel subunit, p18, which interacts with the C-terminus of subunit α to form a distinctive pyramidal F_1 architecture. Moreover, subunit α undergoes proteolytic processing that removes an internal octapeptide and results in separate N-terminal and C-terminal polypeptides. This cleavage likely occurs before the assembly of the F_1 headpiece. These structural novelties raise questions about the assembly mechanism of this more intricate F_1 moiety, which may differ from the opisthokont models. Upon silencing the expression of canonical assembly factors ATP11 and ATP12 in *T. brucei*, we detect subcomplexes containing subunit p18 and β. The composition of these complexes will be determined by complexosome profiling. In addition, we are implementing proximity-labeling via TurboID tagging of the mature subunit α polypeptides and p18 to identify novel assembly factors. These approaches will help to unravel the unique assembly pathway of the trypanosomal F_1-ATPase.

Poster 56* : A robust high-throughput compatible screening approach for targeting the unique trypanosomatid mRNA decapping enzyme ALPH1

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Kinetoplastids lack homologues of canonical mRNA decapping enzymes. Instead, the ApaH-like phosphatase ALPH1 is fulfilling this role. ALPH1 meets drug target key criteria of essentiality, selectivity, tractability and draggability. With this target in hand, we developed a screening pipeline to identify inhibitors of ALPH1 activity. Our primary assay relies on the quantification of ADP liberated from a dinucleotide cap analog by a coupled enzymatic reaction. Specifically, liberated ADP is first converted into ATP by pyruvate kinase, fueling the generation of a luminescent signal by luciferin/luciferase. Identified inhibitors are tested for interference with ADP detection, to rule out false positives from inhibition of the coupled enzymatic reaction. We report

data on ALPH1 assay development and the preliminary outcome of our pilot screen. 12760 small molecules delivered 13 validated dose-dependent ALPH1 inhibitors, 8 out of which impact *T. brucei* viability.

Poster 57* : Genomic and Proteomic Analyses Reveal a FYVE Zinc Finger Domain on Endosomes as a Target of Aminopyrazoles in Leishmania

Miss Rokaya Ahmad, *PhD student, University of Antwerpen*

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Aminopyrazoles have emerged as a promising class of antileishmanial compounds. To deconvolute their mode of action, chemical mutagenesis under aminopyrazole selective pressure coupled with whole-genome sequencing was performed. From the resulting panel of 28 resistant lines, we discovered an association between 10- to 49-fold resistance and multiple independent heterozygous mutations in the C-terminal FYVE zinc finger domain of LINF_180011100. Gene overexpression and CRISPR-Cas9 gene editing independently confirmed this association, conferring 10- to 30-fold aminopyrazole resistance. An N-terminal green fluorescent protein fusion demonstrated that the encoded protein localizes to endocytic vesicles. Proteomic analysis revealed its interaction with recycling endosomes linked to ribosomal translation machinery and mitochondria. This work validates LINF_180011100 as a drug target for aminopyrazoles and provides insights into the mode of action.

Poster 58* : Establishing a High-Content Screening Assay for Antileishmanial Compounds Targeting Nuclear Protein Import in Leishmania

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Compound screening in cell- or target-based assays represents a cornerstone of antileishmanial drug discovery. Nucleocytoplasmic transport of cargo molecules carrying a nuclear localization signal (NLS) is crucial for cell survival. We developed a cell-based assay for *in situ* nuclear protein import (NPI) to discover novel antileishmanial compounds and simultaneously elucidate their mode of action. To visualize nuclear transport, *L. infantum* was engineered to express an mCherry fluorescent protein with a C-terminal NLS. Confocal microscopy revealed nuclear localization of mCherry in both extra- and intracellular stages. Importazole, an importin-β inhibitor, exhibited dose-dependent inhibition of NPI and broad antiprotozoal activity. Lead compounds exerted antiparasitic activity independent of NPI impairment. Collectively, the leishmanial NPI assay provides an *in situ* readout for mechanistically informed drug discovery and enables investigating nucleocytoplasmic transport.

Poster 59 : Formol Gel Detection of Leishmanial Antibodies: A Pilot Study Of The Prevalence Of Visceral Leishmaniasis In Ekemkpon Community In Odukpani Local Government Area Of Cross River State, Nigeria

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Abstract

Visceral leishmaniasis is a form of parasitic infection transmitted to human beings by the bites of female sandflies of the genus Phlebotomus in African countries, including Nigeria. This study was carried out in Ekemkpon community of Cross River State in Southern Nigeria, as a survey for the presence of leishmanial antibodies in the area. Four millilitres of blood specimens were collected, by venepuncture, from 178 consenting residents of the study community. One millilitre of cell-free serum from each blood specimen was placed in a plain test tube, to which 2 drops of concentrated formalin (40%) were added. The preparation was allowed to stand on the bench for 20 minutes. A positive Formol Gel test was indicated by the whitening and gelling of the serum like the white of an egg within 5 minutes. Results showed an overall prevalence of 13.5% (24:178) for visceral leishmaniasis in the study area.

Poster 60 : Decoding efficacy and resistance space at a drug binding site

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Assessing the impacts of all possible mutations at a drug binding-site remains challenging. We used Multiplex Oligo-Targeting (MOT) for mutational profiling, and to decode efficacy and resistance space at an otherwise native binding-site; saturation-editing twenty codons in the T. brucei proteasome b5 subunit. MOT libraries were subjected to stepwise drug selection, amplicon-sequencing, and codon variant scoring, yielding dose-response profiles for >100 resistance-conferring edits. Codon variant scores were predictive of relative resistance observed using a bespoke set of mutants and the emerging resistance profile allowed us to readily predict spontaneous drug-resistance observed within 'accessible', single nucleotide mutational space. In silico analyses were closely aligned with, and predictive of, the empirical data. We conclude that MOT-library profiling facilitates assessment of all possible mutations at a drug binding-site and the design of more effective and durable drugs.

Poster 61* : Unveiling Glial Cells in the Nasal Mucosa as Host Cells for Leishmania with Potential Implications in Disease Outcomes

Dr Sergio Araujo, *Postdoc, LMPH - University of Antwerp*

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Neurological manifestations in leishmaniasis are often overlooked but may include meningoencephalitis, encephalopathy, and peripheral neuropathy. While innate phagocytes are the primary host cells, evidence indicates that *Leishmania* can exploit immune-privileged niches, including non-myeloid cells in perineural tissues. Here we demonstrate that olfactory ensheathing glia (OEGs) in the nasal mucosa represent a previously unrecognised host cell, offering a putative microenvironment that facilitates subclinical parasite persistence and may influence disease outcomes. Understanding the immunological role of OEGs in host-parasite interactions could have important implications for disease pathogenesis, diagnosis, and treatment. This may help explain relapses and treatment failures, particularly in mucocutaneous disease, and opens new avenues for research into neuroimmune interactions, highlighting the need to consider non-traditional host cells in chronic parasitic infections.

Poster 62* : Immunological mechanisms of respiratory co-infections: The impact of trypanosomes on secondary infections caused by major lower respiratory tract pathogens

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Cumulative evidence over the years has highlighted the diverse and complex effects of insect-borne protozoan parasites on the respiratory system. Our recent findings demonstrate that *Trypanosoma* infection alters the pulmonary immune landscape, potentially increasing susceptibility to co-infections. This study investigates the response of alveolar epithelial cells exposed to *T. brucei*, with a focus on secondary infections caused by major lower respiratory tract pathogens (RSV, *Streptococcus pneumoniae*, and *Mycobacterium abscessus*), aiming to characterize the immunopathology of these polymicrobial infections. Immunological analyses revealed the interplay between pathogens and immune modulation, offering insights into virulence, host responses, and potential markers. This multidisciplinary approach bridges parasitology, virology, and bacteriology, contributing to deeper understanding of the dynamics of co-infections involving parasitic and respiratory pathogens

Poster 63 : Uncovering Miltefosine Response in Leishmania Using Functional Genomics

Mr Jorge Arias del Angel, *Postdoctoral researcher, University of Wuerzburg*

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Miltefosine (MTF) remains a key anti-leishmanial, but rising relapse rates and emerging resistance highlight the need to better understand drug response. Using genome-wide CRISPR/Cas9 cytosine base editing in *Leishmania mexicana* and *L. major*, we identified genetic determinants of MTF resistance. We identified known components of the MTF transporter complex (LMT and LEM3/ROS3) and discovered a novel transmembrane protein, LMT17, essential for proper complex localization. Contrary to prior reports, we find the complex localizes to tubulovesicular structures likely in the Golgi and multivesicular tubule lysosome, suggesting MTF trafficking via endo/exocytic pathways. Additionally, parallel genome-wide fitness screening revealed that targeting an ABC transporter and the novel protein MRM354 did not confer MTF resistance but promoted rapid treatment recovery, indicating a persister-like phenotype. Together, our study reveals new players and mechanisms in MTF resistance.

Poster 64* : Deciphering the translation mechanism in *Blastocrithidia* nonstop: a trypanosomatid with all three stop codons reassigned

Miss Nathalia Ballesteros, *Translation in B.nonstop, Biology Centre CAS*

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The non-canonical genetic code of *B. nonstop* is decoded thanks to unique adaptations of its translation machinery. These include the evolution of cognate and near-cognate tRNAs that interpret all three standard stop codons as sense codons, with UAA serving dually as both a coding codon and a termination signal.

However, the dynamics of stop codon readthrough and the mechanism of translation termination in this protist remain poorly understood. Here, we employed a combination of ribosome profiling and proteomics to further elucidate the peculiarities of its translation apparatus. Interestingly, while most proteins terminate at the annotated UAA stop codon, a substantial number contain C-terminal extensions. Notably, UAA is overrepresented in 3' UTRs, and its frequency and proximity correlate with reduced readthrough. Overall, this suggests that multiple UAAs promote termination in a context-dependent manner, potentially involving additional regulatory components.

Poster 65 :

Trypanosoma brucei reprograms its transcriptome and surface proteome as it traverses the tsetse fly

Mr Fernando Batista, *Postdoc, Institut Pasteur*

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Trypanosoma brucei reprograms its transcriptome and surface proteome as it traverses the tsetse fly, yet mRNA levels poorly predict protein abundance. We therefore adapted CITE-seq for *T. brucei*: methanol-fixed cells were stained with DNA-barcoded, stage-specific antibodies, and subjected to compatible the Chromium 10x pipeline yielding paired RNA–protein profiles for 1,526 single cells collected 8- and 21-days post-infection. Four developmental clusters reconstructed the progression from midgut procyclics to late proventricular forms. GPEET protein appeared only in a few early midgut cells, whereas EP procyclin peaked in early proventricular cells alongside higher EP2 mRNA and lower serine/threonine kinases and dual-specificity phosphatases. This first single-cell multi-omic map of *T. brucei* differentiation exposes regulatory nodes unseen in bulk or transcript-only assays and offers a blueprint for high-resolution kinetoplastid atlases.

Poster 66 : Distinct Localization of Leishmania tarentolae and Trypanosoma platydactyli in the Natural Vector Sergentomyia minuta: Could This Reflect Co-evolution?

Dr Tomas Becvar, *Postdoc, Faculty of Science, Charles University*

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Leishmania tarentolae and *Trypanosoma platydactyli* often co-circulate in geckos in Southern Europe, likely transmitted by *Sergentomyia* sand flies. Their life cycles are poorly understood. We studied their development in *S. minuta* using xenodiagnosis on wild-caught *Tarentola mauritanica* with confirmed single or co-infections. We assessed parasite localization, intensity, and morphology at multiple time points post-blood meal (PBM). *L. tarentolae* localized in Malpighian tubules, sometimes spreading anteriorly. *T. platydactyli* heavily colonized the midgut, often reaching the stomodeal valve (90%). In co-infections, parasites occurred throughout the gut: Malpighian tubules (100%), hindgut (94%), anterior midgut (89%). Parasite morphotypes remained consistent comparing single and co-infections. This lack of co-influence inside of the vector suggests long co-evolution and mutual adaptation of these parasites.

Poster 67 : Thermo-sensing and heat-stress responses in trypanosomes

Dr Gustavo Bravo Ruiz, *Post Doc, University of Dundee*

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T. brucei experience temperature fluctuations due to fever, during transfer between hosts, or due to diurnal shift. We assessed transcriptomic, proteomic, and lipidomic responses after growth at 34, 37 or 40°C for 6h. Analysis of post-transcriptional responses using machine learning models suggested a thermo-sensory 'zipper' hypothesis involving unmasking of positive-regulatory sequences in hundreds of mRNA untranslated regions. Among heat-inducible proteins, we identified phospholipase A1 (PLA1), and a putative homolog of human Hikeshi, which impacts the nuclear pool of HSP70. Both *pla1* and *hikeshi* null trypanosomes were hyper-sensitive to heat-shock. Further analysis of the mutants indicated roles in lipid remodelling and the nuclear transport of chaperones, respectively. Our findings suggest RNA-based thermo-sensors in trypanosomes, as well as specific responses impacting homeoviscous membrane adaptation and the subcellular accumulation of HSPs.

Poster 69 : Three paralogs of Oxa1 insertases in *Trypanosoma brucei* show specific functions in biogenesis of mitochondrial membranes

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Oxa1 is an insertase of proteins into the inner mitochondrial membrane (IMM) essential for the biogenesis of OXPHOS complexes. *Trypanosoma brucei* encodes three putative Oxa1 paralogs, TbOxa1-1, 1-2 and 1-3. In the bloodstream form, which relies on ATP synthase to maintain IMM potential, knock-out of TbOxa1-2 caused reduced membrane potential and a decreased ATP synthase levels, due to defects in assembly of the nuclear encoded c-ring. In procyclic *T. brucei*, KO of TbOxa-1 and 1-2 resulted in growth arrest and loss of cI and cIV, and cIII and ATP synthase, respectively. Proteomic analysis of submitochondrial fractions showed distinct alterations across mitochondrial compartments, together indicating specific roles of TbOxa1 paralogs in the IMM biogenesis. The ablation of TbOxa1-2 led to accumulation of IMM proteins in matrix, suggesting its role in conservative sorting. We identified a rhomboid peptidase-like protein that facilitates ATP synthase assembly together with TbOxa1-2.

Poster 70* : From FROG to FASTQ: Isolating an African amphibian trypanosome genome directly from natural infections in the peripheral blood with single cell sequencing

Mr Bernie Jordaan, *PhD student, University of the Free State*

BJ Jordaan[2]; M Castelli[1]; L Gammuto[1]; EC Netherlands[2];
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Species of *Trypanosoma* infecting African amphibian and reptile hosts are among the least understood taxa within the genus. Due to the diverse lifestyles and ecology of these hosts, they show considerable potential to broaden our understanding of the largest evolutionary split within the genus, between the aquatic and terrestrial clades. Recent studies have indicated that the diversity of trypanosomes infecting herpetofauna, appears more complex than previously understood. Traditional morphological systematic classification has been aided by the availability of new techniques, such as Next-Generation Sequencing and single cell isolation, allowing for improved diagnoses, species characterisation, and resolution of mixed infections.
In light of recent work characterising amphibian and reptile trypanosomes across Southern Africa, a single species emerged as particularly notable from an evolutionary and phylogenetic perspective, *Trypanosoma*

grandicolor. This species, infecting the aquatic frog host *Xenopus laevis*, was found to occupy a basal position within the aquatic trypanosome clade, specifically at the primary divergence between the amphibian and fish/turtle groups. Therefore, by building upon the recent molecular and morphological redescription of *T. grandicolor*, the current study aimed to utilise single cell isolation and advanced molecular techniques and to characterise this species' genome and shed light on the unique genetic adaptations, evolutionary history, and development of pathogenic traits within the genus.
In this study, live single trypanosome cells were successfully isolated from the peripheral blood of naturally infected, wild-caught frog hosts. After a whole genome amplification, Illumina short-read sequencing was performed on these samples, from which the preliminary results indicate a partial trypanosome genome was obtained. Currently, the researchers are expanding upon the current dataset with increased sequencing depth and the combination of long-read sequencing to obtain the whole genome sequence of this species. Continued and future work is aimed at gaining a better understanding of host-parasite relationships, pathogenicity, vectors, and other ecological factors; using life cycle studies, haemocultures, and transmission experiments.

Poster 71* : Zinc, Copper and Manganese Treatment Influence on *Trypanosoma congolense* Infection Dynamics and Trans-sialidase Expression in Albino Mice

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Trypanosoma congolense, a causative agent of animal African trypanosomiasis affects human and agricultural development in Africa. The survival of this parasite within the host bloodstream relies on enzymes such as trans-sialidase, which facilitate its evasion of host immune responses by desialylating sialic acids from the host cell surface. Our study aimed to investigate the expression patterns of *T. congolense* trans-sialidase (TconTS) genes, the immunoglobulin G (IgG) response, and the parasitological effects of Zinc, Copper and Manganese in *T. congolense*-infected mice. Our findings indicate that prophylactic treatment administered 5-days pre-infection resulted in a prepatent period of 6-8 days and a survival period of 21-40 days for Zinc, Copper, and Manganese, with combination groups exhibiting prolonged survival. Conversely, therapeutic treatment administered 10-days post-infection yielded a prepatent period of 6-9 days and a sur

Poster 72 : Cross-subgenus hybridization between Leishmania and Sauroleishmania informs on parasite genomic compatibility and transcriptomic adaptation

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Leishmania can enter a cryptic sexual cycle generating hybrids, but the mechanisms involved - including the impact of parental genetic distance - are poorly understood. We report the *in vitro* generation of a hybrid between *L. infantum* and *L. (Sauroleishmania) tarentolae*, two species from sister phylogenetic clades circulating in South Italy, providing evidence of genomic compatibility. Whole-Genome Sequencing indicates that the hybrid is tetraploid but not a simple sum of parental genomes. RNA-seq analysis of the hybrid uncovers significant differences in the abundance of orthologous transcripts expressed from both parental genomes, driven by either parent-specific gene copy number variations or differential mRNA turnover. These results demonstrate that, beyond genomic restructuring, post-transcriptional regulation may serve as an additional mechanism shaping viable hybrid phenotypes, potentially enhancing parasite adaptability and fitness.

Poster 73 : Decoding chromosome segregation in African trypanosomes: A closer look at the role of KIN-A motor domain

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The kinetoplastid parasite *Trypanosoma brucei* possesses unique kinetochore proteins (KKT1-25 and KKIP1-12) that facilitate chromosome segregation by providing linkage between DNA and spindle microtubules. The chromosomal passenger complex (CPC), a key regulator of mitosis in eukaryotes, is conserved in trypanosomes. However, unlike the conventional CPC, trypanosomes lack Borealin and Survivin homologs and instead have a pentameric complex consisting of Aurora B homolog AUK1, INCENP^{CPC1}, CPC2 and two orphan kinesins KIN-A and KIN-B. Our recent studies have shown that KIN-A and KIN-B ensure kinetochore localization of the other CPC subunits. Interestingly, the N-terminus of KIN-A harbours a conserved motor domain that might contribute to microtubule association. Here, we examine the effect of loss of function mutations in the microtubule-binding site of KIN-A on localization of other CPC components and its effect on chromosome segregation in *T. brucei*.

Poster 74 : Expanding the *Trypanosoma cruzi* toolbox: new cellular and molecular techniques that that aid dissection of fundamental biology.

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Trypanosoma cruzi has a complex life cycle, complicating the use of classic molecular and cellular biology methods to study its biology. Advances in the past decade, including the use of bioluminescent and fluorescent parasites, have revealed key aspects of *T. cruzi* infection related to pathology, drug discovery, and host immune response. However, the mechanisms underlying treatment failure and parasite persistence remain unclear.

Here, we present recently developed techniques that we have used to dissect the intracellular life of this protozoan. These approaches enable: (i) isolation of intact, transcriptome- and proteome-preserved amastigotes for omics analyses; and (ii) application of epifluorescence microscopy with AI software to monitor infection dynamics at the subcellular level in real time. These methodologies provide valuable tools to elucidate amastigote biology and can be readily applied to other intracellular protozoa.

Poster 75 : Comparative proteomic analyses of the related EIF4G3 and EIF4G4 initiation factors from Leishmania reveal novel and unique protein partners with likely roles during translation regulation

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Initiation of eukaryotic translation requires binding to the mRNAs of the eIF4F initiation complex, formed by joining of the eIF4G and eIF4E subunits. Five eIF4G homologues form multiple eIF4F-like complexes in *Leishmania* and *Trypanosoma* species, with those based on the related EIF4G3 and EIF4G4 active during translation, but with likely non-redundant roles. Here we opted to investigate these two eIF4Gs further in *Leishmania infantum*, aiming to better define functional differences between them and contribute to the understanding of mechanisms regulating gene expression in this pathogen. To accomplish this, we first generated transgenic cell lines expressing either of these two eIF4Gs tagged with a C-terminal HA epitope. Both HA-tagged proteins were first found capable of replacing the corresponding native proteins after deletion of the endogenous genes, confirming their functionality. Expression analysis then showed the two HA-tagged proteins expressed as multiple isoforms, with EIF4G4 isoforms differing during promastigote growth. Cytoplasmic extracts from both sets of cell lines were then used in a large-scale investigation of proteins associated with each of the two eIF4Gs, from different growth phases: early exponential, late exponential and stationary. EIF4G3-HA and EIF4G4-HA co-precipitated throughout with their known eIF4E partners, respectively EIF4E4 and EIF4E3. EIF4G3 also more consistently co-precipitated with PABP1, RBP23 and EIF4AI, with EIF4G4 having greater association with PABP3 and the HEL67 helicase. A variable number of translation factors and ribosomal proteins were found with both baits, reflecting roles in translation. Our extensive analyses, investigating also proteins with possible moonlighting roles and uncharacterized polypeptides, not only revealed new proteins bound to both baits, likely reflecting novel roles associated with the mRNA metabolism, but also identified new specific partners for EIF4G3 and possibly EIF4G4. These include potential regulators restricted to selected growth phases. Overall, EIF4G3 had new and more defined binding partners, with EIF4G4 having an enhanced interaction with other translation initiation factors. Further studies on newly identified partners and likely regulators, for both eIF4Gs, might reveal unique and conserved aspects of the *Leishmania* and *Trypanosoma* translation and further contribute to the overall understanding of eukatyotic translation, while also helping to define targets for novel translation inhibitors with therapeutic potential.

Poster 76 : ECLIPSE: an intervention programme to improve patient journey and reduce stigma for people with cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka

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Poster 77 : Exploring potential vectors and reservoir hosts of Leishmania (Mundinia) martiniquensis in Central Europe

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Mundinia subgenus includes six *Leishmania* species with poorly understood transmission. Reports of *L. martiniquensis* in livestock in Central Europe prompted investigation into local vectors and reservoirs. We experimentally infected sand flies, biting midges, and rodents (*Mus musculus*, *Rattus rattus*, *Clethrionomys glareolus*) with two strains (Cu2, Aig), and screened wild midges and rodents from affected areas. The parasite did not develop in sand flies but established mature infections in midges. Both strains persisted in all rodent species and were transmissible to vectors. Cu2 caused skin lesions and mortality in *C. glareolus*; Aig was asymptomatic. No *Leishmania* DNA was found in field samples. Our findings suggest *M. musculus* and *R. rattus* may act as reservoirs, and *Culicoides* as a vector; however, further studies are needed to confirm the parasit

Poster 78 : Improving the diagnosis of visceral leishmaniasis (VL) in Mekelle, a highly endemic region in Ethiopia.

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Accurate and feasible diagnostics tests for VL are still sought for. Diagnostic performance of 5 different VL diagnostics, i.e. rk39 RDT, direct agglutination test (DAT), microscopy, loop-mediated isothermal amplification (LAMP), and miniature direct-on-blood PCR–nucleic acid lateral flow immunoassay (dbPCR-NALFIA) was assessed with qPCR as reference test in Mekelle. In total, 235 suspected VL cases and 104 non-endemic healthy controls were recruited. Among the suspected VL cases, 144 (61.3%) tested positive with qPCR. Sensitivities for rk39 RDT, DAT, microscopy, LAMP assay, and dbPCR-NALFIA were 88.1%, 96.5%, 76.6%, 94.3%, and 95.8%, respectively and specificities were 83.3% (RDT), 98.0% (DAT), 100% (microscopy), 97.4% (LAMP), and 98.9% (dbPCR-NALFIA). In conclusion, rk39 RDT and microscopy exhibited low sensitivity. DAT had good diagnostic performance. LAMP and dbPCR-NALFIA performed very well, with feasibility for implementation of molecular diagnostics in endemic areas.

Poster 79* : Probiotics vs. Parasites: The effects of in vivo probiotic treatment on ex vivo Leishmania amazonensis-infected mouse macrophages

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The microbiome is fundamental in the host's immunobiology and dysbiosis leads to pathological conditions, potentially affecting parasitic diseases. To investigate how oral probiotics affect infection and antiparasitic

treatment of *Leishmania* in macrophages, Swiss mice were orally treated with 109 CFU multi- or single-strain probiotic formulations (PB8, Bifilac), their peritoneal mouse macrophages (PMMs) were obtained and infected *ex vivo* with *L. amazonensis* amastigotes. The effects of prior probiotic administration on ex vivo infection and treatment responses to 1 µM miltefosine and N6-methyltubercidin were evaluated. Flow cytometry measured the inflammatory mediator release in the supernatant of the PMMs. PB8 or Bifilac administration significantly reduced ($p < 0.05$) ex vivo infection of PMMs from male mice by 27% and 12%, respectively. No gender-dependent effect or improved antiparasitic activity of 1 µM miltefosine or N6-methyltubercidin was observed in probiotic-treated PMMs. *Ex vivo Leishmania* infection stimulated TNF, MCP-1, and IL-6 production by PMMs ($p < 0.05$). A trend of increase was recorded elevated levels of TNF and IL-6 in PB8-treated male groups (around 43 and 52%, respectively) were not statistically significant. Collectively, probiotic treatment of mice influences Leishmania infection in PMMs. Clinical applications in *leishmaniasis* warrant further studies.

Poster 80 : Codon biased translation mediated by Queuosine tRNA modification is essential for the virulence of *Leishmania mexicana*

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The complex life cycle of the human parasite *Leishmania mexicana* requires rapid translational adaptation for survival in different hosts. We show that Q-tRNA modification at the anticodon position 34 provides a novel mechanism for regulation of gene expression. Q-tRNA levels increase substantially during Leishmania differentiation from the insect stage to the mammalian-infective stage. Mutant cells lacking the enzyme responsible for Q incorporation exhibit substantial changes in the proteome. Downregulated proteins were enriched in Q-tRNA-dependent NAU codons, whereas upregulated proteins contained more NAC. Subsequently, the parasite virulence is decreased, as demonstrated by independent infections of macrophages and mice. Taken together, these results highlight the critical role of Q-tRNA modification in maintaining translational balance and reveal a novel layer of gene expression regulation, which facilitates parasite adaptation to changing environments

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