British Society for Parasitology

Trypanosomiasis and Leishmaniasis Symposium

Advances in Basic and Applied Research

Granada, Spain, 8th- 11th March 2020

CSIC Consejo Superior de Investigaciones Científicas

CAMBRIDGE UNIVERSITY PRESS

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Venue Information

Address: Hotel Abades Nevada Palace,

Calle Sultana, 3, Granada GR 18008

Tel: +34 902 22 25 70

Taxi: There are 60 designated taxi ranks in Granada. They all have a square blue sign with a T. Taxis can be hailed on the street too. They have a green light on their roof when they are available.

You can book a taxi online using one of theses applications: pidetaxi Granada application https://www.granadataxi.com/pidetaxi-app or 1Taxi! application https://radiotaxigenil.com/taxi-online/

There are two taxi companies in Granada: Tele Radio Taxi Granada on **958 28 00 00** and Radio Taxi Genil on **958 13 23 23**. You can call them to book a taxi in advance or immediately, if they are available near you

British Society for Parasitology

Trypanosomiasis and Leishmaniasis Symposium

GRANADA 2020, SPAIN

Sunday, 8 to Wednesday, 11 March 2020

Advances in Basic and Applied Research

Dear Colleagues,

Nowadays many scientific meetings have grown very large and perhaps include too many broad fields and are rather impersonal and hard to navigate. The focused symposium in Granada and kindly provided by the British Society for Parasitology is centred on a small group of neglected diseases caused by related protozoa parasites and offers the perfect venue to meet together and with a suitable number of scientists to make the most of our time together. Furthermore, we chose a hotel as the symposium venue to increase considerably the potential for meeting, exchanging ideas and collaborating.

We hope the cultural backdrop of the city of Granada will enrich the scientific framework and the exchange and knowledge of the conference attendees in the late afternoon, after poster parties and at the Gala dinner in a restaurant located on the Alhambra hillside, overlooking the Albaicin old city centre.

The eight sessions included in the congress cover many of the major aspects of current research activity and all talks will be presented in the same auditorium in one concurrent session, avoiding the need to choose between talks. At the end of the auditorium room we have also located the poster panels towards the patio were coffee breaks take place to further encourage interactions. We anticipate that the meeting will facilitate a highly enjoyable and productive occasion, allowing delegates to present the most relevant advances of the year in the molecular and cellular biology of the kinetoplastids. This framework has also been designed to facilitate the participation and presentation of work by young researchers as well as established investigators. The BSP2020 Granada Symposium Committee will recognize best presentations and posters with awards to young researchers. In addition, a prominent member of the BSP will receive a whole career award in the opening plenary session of the Congress.

We sincerely hope that you are inspired by this meeting and will enjoy the cordiality of the ancient and beautiful city of Granada, forge new scientific friendships while learning and exchanging your latest findings, ideas and questions. I will close by thanking the British Society for Parasitology for allowing this great opportunity to exchange our research in Granada into this, sometimes forgotten, area of parasitology and neglected diseases.

Miguel Navarro PhD.

Professor of Research at the CSIC. Spanish National Council for Research

Local Organisers

Domingo Rojas; Diana Lopez-Farfan and Miguel Navarro with special thanks to Rosa Cruz for facilitating the meeting.

Contents

About Granada (Local Information)

Just a few years ago, in 2014, the Millennial of the founding of the first Kingdom of Granada, the Zirí, the predecessor of the Nasrid Kingdom, which led Granada to reach highest levels of cultural splendour, was celebrated. Since the beginning of its existence, the Kingdom of Granada has been a melting pot of cultures having collected and given the world important sages and writers. Throughout these thousand years, Granada has formed an attractive city structure with unique historical monuments such as the Alhambra or its unique cathedral and monastery of La Cartuja.

Today Granada is a modern, open city with a large University and several research Institutes of the Spanish National Research Council (CSIC) and the Andalusian Board of recognized prestige. An example of this is the Health Sciences Park where it focuses on the area of biomedicine, teaching, research, business and care work in a model of a future in Andalusia and Spain.

About Presentations

Information for Oral Presentations

Loading of oral presentations will take place in the hall and there is a desktop computer provided.

Please ensure that your presentation is loaded by the previous break or by 8:45 am of the day of your talk, at the latest if you are presenting in the morning sessions or by 15:00 pm if you are presenting in the afternoon sessions.

There will be a student volunteer who will help you load your presentation and assist you if any problems arise with the A/V equipment.

Speakers are respectfully requested to keep to their time slot.

All presentations should be as follows

50 mins – 45 mins oral with 5 mins for questions.

20 mins - 15-18 mins oral with 3-5 mins for questions .

Information for Poster Presentations

Posters will be displayed in the back of the main Hall

Posters should be A0 size (841 mm wide x 1189 mm high) in portrait format, and be no larger than 900 mm wide x 1200 mm high).

You will find Velcro coins on the board ready to attach your poster. Please put up your poster on before the first poster session and remove it after the last poster session.

General Meeting Information

Reception and Registration

Registration will be in the lobby outside the hall just to the left of the hotel reception. This will run through out the first 3 days of the meeting.

House keeping

Food and Refreshments

Coffee Breaks 11:10 -11:30
Lunch 14:00 - 15:00
Poster Sessions 17:30 – 19:30

Internet Access

Access codes are given on check-in at the Hotel. Wi-fi is available throughout the building.

About the British Society for Parasitology

About

Today many researchers and students are very passionate about the fascinating world of infectious diseases, parasites, their complex lifestyles and their associated impact on people and livelihoods.
To draw attention to the unique importance of parasitology as a distinct discipline within biology, The British Society for Parasitology was formed in 1962 from the Parasitological Section of the Institute of Biology. Today the Society is the central networking and meeting point for many professional and amateur parasitologists throughout the UK and across the world.

Did you know that the UK leads Europe – and Europe leads the world – in parasitology research? No European country publishes more parasitology research than the UK, and UK papers were cited more than those from any other country in the past 5 years (2011-2016, data from Elsevier SciVal, see News item for more details.)

As the leading academic society for a country preeminent in parasitology, the remit of the BSP is broad. It promotes and supports the academic study of parasitology in all its many guises. This can be from experimental to theoretical approaches as applied to infection biology and disease research, or from ecological to medical and veterinary studies in global health and international aid. Each year students are given financial support to attend BSP meetings and scholarship schemes are in place to support fieldwork and training events.

The membership of the BSP stands at around 1000 in number. Approximately a third of members are from overseas locations. Highlights of the annual BSP calendar include the annual residential meetings in spring and autumn which are focused upon general and specialist aspects of parasitology. The BSP has a close relationship with Cambridge University Press that prints a special issue on our autumn meeting.

The BSP Society is a Charitable Incorporated Organisation and managed by the BSP Council. This comprises a President, Honorary Officers and ordinary Council Members, who together act as Company Trustees. Co-opted

members of Council also include representation from the student, early career membership and other learned societies where clear synergies are apparent.

Membership

Joining the BSP gives parasitologists a number of important benefits:

Discounted registration fees at all BSP events
Networking and support from other parasitologists
Eligibility for travel grants and other awards
Regular e-communications highlighting parasitology news, events, job opportunities, etc.
Discounts on personal subscriptions to specific scientific journals and books

Website: http://www.bsp.uk.net

Membership: http://www.bsp.uk.net/membership/

Events: http://www.bsp.uk.net/events/

The BSP Council 2020

Professor Maria-Gloria Basáñez, Imperial College London - President (appointed April 2018 – April 2020)
Professor Colin Sutherland, London School of Hygien and Tropical Medicine - Vice President/President Elect (appointed April 2018)
Professor Kevin Tyler, University of East Anglia - Honorary General Secretary (appointed April 2019)
Dr Justin Pachebat, Aberystwyth University - Honorary Treasurer (appointed April 2019)
Dr Paul McVeigh, Queen's University Belfast - Honorary Meetings Secretary (appointed April 2019)
Dr Helen Price, Keele University -Honorary Communications Secretary (appointed April 2018)
Dr Robert Hirt, Newcastle University - Ordinary Member (appointed April 2018)
Dr Pegine Walrad, York University - Ordinary Member (appointed April 2018)
Dr James LaCourse, Liverpool University, Ordinary Member (appointed April 2018)
Professor Derrick Robinson, University of Bordeaux 2 - Ordinary Member (elected - tba April 2020)
Dr Joaquin Prada.University of Surrey - Ordinary Member (elected – tba April 2020)
Professor Joanne Cable, Cardiff University - British Ecological Society (co-opted)
Professors Stephen Phillips/John Ellis: Cambridge University Press Representatives (co-opted)
Dr Alison Mbekeani, Durham University - Early Career Representative (elected – tba April 2020)
Dr Juan Quintana Alcala, University of Edinburgh - Early Career Representative (elected – tba April 2020)
Mr Tom Pennance, Natural History Museum - Student Representative (appointed April 2016)
Ms Gala Garrod, Liverpool School of Tropical Medicine - Student Representative (appointed April 2018)
Ms Yasmine Kumordzi, Durham University - Student Representative (elected, tba April 2020)

Meeting and Secretariat Support : Fuller Solutions (Academic-events.com)

Timetable at a Glance

Day 1

Opening Plenary - at 19:00 to 20:00

Chair - Prof Miguel Navarro

19:00 (60 mins) - *Reflections on the evolutionary cell biology of kineplast parasite* (Prof Keith Gull)

Day 2

Host-Parasite Interactions - at 09:00 to 11:10

Chair - Prof Miguel Navarro

09:00 (50 mins) – (Invited Speaker) *Trypanosomes and their microenvironments* (Markus Engstler)

09:50 (20 mins) - *Crossing borders without a visa: Zinc-finger proteins and trypanosome migration in the tsetse* (Alvaro Acosta-Serrano)

10:10 (20 mins) - *Disruption of active trans-sialidase genes in Trypanosoma cruzi resulted in attenuated parasites that fully protect against challenge with a virulent strain* (Gabriela Burle-Caldas)

10:30 (20 mins) - *Life cycle progression analysis in Leishmania mexicana by single-cell RNA-sequencing reveals prospective mechanisms orchestrating promastigote to amastigote development* (Felix Warren)

10:50 (20 mins) - *Application of single-cell transcriptomics to resolve asynchronous differentiation of Trypanosoma brucei between slender and stumpy bloodstream forms* (Emma Briggs)

Coffee Break 11:10 to 11:40

Genomics, Evolution and Gene Expression -at 11:40 to 13:30

Chair - Prof Mark Field

11:40 (50 mins) - (Invited Speaker) *Leishmania genome: aneuploidy, mosaicism and collectivism* (Jean-Claude Dujardin)

12:30 (20 mins) - *Blastocrithidia, a trypanosomatid with all three stop codons reassigned* (Julius Lukes)

12:50 (20 mins) - *Expanded Genome-Wide Comparisons Give Novel Insights into Population Structure and Genetic Heterogeneity of Leishmania tropica Complex* (Tamara Salloum)

13:10 (20 mins) - *Comparative analysis of glycolytic metabolism in the livestock trypanosomes* (Pieter Steketee)

Lunch Break 13:30 to 15:00

Gene Expression of Surface Protiens -at 15:00 to 17:30

Chair - Prof Markus Engstler

15:00 (50 mins) - (Invited Speaker) *How does bloodstream form Trypanosoma brucei control vast levels of expression from a single copy Variant Surface Glycoprotein gene?* (Gloria Rudenko)

15:50 (20 mins) - *Antigenic variation in Trypanosoma brucei relies on dynamic inter-chromosomal interactions with the Spliced Leader RNA locus* (Joana Correia Faria)

16:10 (20 mins) - *Single-cell RNA-seq of salivary gland parasites reveals the shifting transcriptional landscape as parasites develop infectivity* (Sebastian Hutchinson)

16:30 (20 mins) - *Investigating trans-regulators in Leishmania: Evidence of post transcriptional epigenetics* (Pegine Walrad)

16:50 (20 mins) - *Post-transcriptional mRNA control by PBP1-containing mRNA-binding complexes* (Larissa Nascimento)

17:10 (20 mins) - *Discover and Characterisation of sequence-specific promoters driving RNA pol II polycistronic transcription in trypanosomes* (Miguel Navarro)

Poster Session A with Refreshments - at 17:30 to 19:30

Conference Dinner - at 18:30 till late

Day 3

Parasite Cell Biology - at 09:00 to 11:10

Chair - Prof Keith Gull FRS

09:00 (50 mins) - (Invited Speaker) *Using CRISPR-Cas9 knockout screens to dissect flagellar functions in Leishmania* (Eva Gluenz)

09:50 (20 mins) - *The trypanosome lysosomal membrane glycoprotein p67 is a hydrolase of the n-terminal nucleophile (ntn) superfamily* (James Bangs)

10:10 (20 mins) - *Genome-wide functional profiling of genes in T. congolense and pleomorphic T. brucei* (Catarina Gadelha)

10:30 (20 mins) - *A novel adenylate cyclase interacting protein is essential for swarming and tsetse fly colonization in trypanosomes* (Sabine Bachmaier)

10:50 (20 mins) - *The role of microtubule-organizing proteins in Trypanosoma brucei morphogenesis* (Amy Sinclair)

Coffee Break 11:10 to 11:30

Parasite Molecular and Cell Biology -at 11:30 to 14:00

Chair - Prof Juleus Lukes

11:30 (50 mins) - (Invited Speaker) *Meiotic sex in Chagas disease parasite Trypanosoma cruzi* (Martin Llewellyn)

12:20 (20 mins) - *Unconventional kinetochore kinases are important for mitotic progression in Trypanosoma brucei* (Midori Ishii)

12:40 (20 mins) - *XL-BioID identifies a new essential component of the inner Leishmania kinetochore* (Vincent Geoghegan)

13:00 (20 mins) - *Molecular and Functional Dissection of Distinct mRNA Export Pathways* (Samson Obado)

13:20 (20 mins) - *Suicidal Leishmania* (Vyacheslav Yurchenko)

13:40 (20 mins) - *The structure of T. brucei F-ATP synthase and the different bioenergetic consequences resulting from its los* (Alena Zikova)

Lunch Break 14:00 to 15:00

Immunology and Cell Biology -at 15:00 to 17:30

Chair - Prof Dolores González-Pacanowska

15:00 (50 mins) - (Invited Speaker) *How do trypanosome receptors for host macromolecules bind ligands and yet avoid the host immune response?* (Mark Carrington)

15:50 (20 mins) - *Identification of a mannose-binding lectin essential for full resistance of Trypanosoma brucei gambiense to the lytic action of the human serum*. (Jean-Mathieu Bart)

16:10 (20 mins) - *An invariant Trypanosoma vivax subunit vaccine antigen inducing protective immunity* (Gavin Wright)

16:30 (20 mins) - *Multimodal live imaging models for integrated analyses of Trypanosoma cruzi infections and digestive Chagas disease pathogenesis*. (Archie Khan)

16:50 (20 mins) - *Widespread roles of Trypanosoma brucei ATR in chromosomal segregation and nuclear genome transmission are linked to R-loops* (Jennifer Ann Black)

17:10 (20 mins) - *Trypanosoma brucei ISG65 binds complement C3 and increases virulence in the* bloodstream (Olivia Macleod)

Poster Session B with Refreshments - at 17:30 to 19:30

Day 4

Biochemistry and Gene Expression -at 09:00 to 10:50

Chair - Prof Gloria Rudenko

09:00 (50 mins) - (Invited Speaker) *Novel players in the control of gene expression in African trypanosomes* (Luisa Figueiredo)

09:50 (20 mins) - *Nucleoside-activated kinetoplastid Protein Kinase A (PKA) - evolution of a signaling nexus* (Michael Boshart)

10:10 (20 mins) - *A Trypanosoma brucei ORFeome-based Gain-of-Function Library reveals novel genes associated with melarsoprol resistance* (Galadriel Hovel-Miner)

10:30 (20 mins) - *Histidine Regulates pH Homeostasis in Acidocalcisomes of Trypanosoma cruzi* (Brian Suarez Mantilla)

Coffee Break 10:50 to 11:20

Novel Drug Therapies and New Targets -at 11:20 to 13:30

Chair - Dr Francisco Gamarro

11:20 (50 mins) - (Invited Speaker) *New insights into thymidylate biosynthesis in Trypanosoma brucei: a novel route for dUMP formation* (Dolores González-Pacanowska)

12:10 (20 mins) - *Picking and polishing the rough diamonds: Turning hits into first-in-class therapeutic molecules for visceral leishmaniasis and Chagas disease* (Julio Martin)

12:30 (20 mins) - *3'-Deoxyribofuranosyl 7-deazapurine nucleoside analogues: a new class of highly potent antitrypanosomal agents*. (Fabian Hulpia)

12:50 (20 mins) - *Target in Trypanosoma brucei for the novel and curative CT-series compound class identified as Topoisomerase II* (Matthew Gould)

Closing Plenary at 13:10 to 13:55

13:10 (45 mins) - (Invited Speaker) *Organising the trypanosome nucleus: Assembly of the nuclear lamina and connections to the nuclear pore complex*.(Prof Mark Field)

Closing Remarks at 13:55 to 14:10

13:20 (15 mins) - *Closing Remarks* (Miguel Navarro)

Buffet Lunch

Full Programme

Day 1 Talks

Opening Plenary - at 19:00 to 20:00

Chair - Prof Miguel Navarro

19:00 (60 mins) (Invited Speaker)

Reflections on the evolutionary cell biology of kineplast parasites

Presenter: **Prof Keith Gull FRS**, *Professor, Sir William Dunn School of Pathology*

Shape and form in kinetoplastid parasites are defined, in the main, by an internal microtubule-based cytoskeleton. However, a defining feature of these organisms is the existence of a flagellum whose nature varies somewhat between cell types. Epimastigotes, trypomastigotes and amastigotes are distinguished by the substructure of their flagella, the length of attachment to the cell body and other functional elaborations. In turn, the basal body complex – defining a "master organiser" region of the cell – orchestrates a variety of cellular microtubules and filament systems essential for inheritance of cell shape and completion of cytokinesis, in addition to regulating kinetoplast position and segregation. Critically, it also defines perhaps one of the most important pathogenicity attributes – the flagellar pocket. Microtubules are also critical for the functioning of the intranuclear mitotic spindle. Evolutionary cell biology and comparisons with other model organisms highlights conserved features and specialisations of kinetoplastid flagella, cytoskeleton and mitosis. All this has enabled a corpus of knowledge providing fascinating insights to the biology of movement, shape, sensing, proliferation and pathogenicity. This progress has also highlighted the current state of our ignorance.

Day 2 Talks

Host-Parasite Interactions - at 09:00 to 11:10

Chair - Prof Miguel Navarro

09:00 (50 mins) (Invited Speaker)

Trypanosomes and their microenvironments

Presenter: **Prof Markus Engstler**, *Professor and Chair, Julius-Maximilians-Universität Würzburg*

M Engstler[1];

[1] Julius-Maximilians-Universität Würzburg, Germany

Trypanosomes are amongst the most common parasites of vertebrates and are ubiquitous in arthropods. The majority occupy extracellular niches within their hosts, capable of thriving in a variety of tissue microenvironments and fluids. The ability to colonise a wide range of intra-organismal habitats may have contributed to their evolutionary success. There has, however, been limited consideration of the role this versatility plays in trypanosome adaptive radiation. This is partly due to a consistent focus on the biology of the parasites themselves, rather than their interaction with the varying microenvironments encountered within an infected host. Our work seeks to explore the ecology of trypanosomes in these habitats, namely the extracellular body fluids of the mammalian and insect hosts. In my lecture I will introduce concepts and technologies that explore trypanosome morphology and behaviour within select model microenvironments, principally the skin and circulatory system of mammals, and the tsetse alimentary tract.

09:50 (20 mins)

Crossing borders without a visa: Zinc-finger proteins and trypanosome migration in the tsetse

Presenter: **Dr Alvaro Acosta-Serrano**, *PI, Liverpool School of Tropical Medicine*

A F Acosta-Serrano[2]; A Casas-Sánchez[2]; L López-Escobar[2]; A Zardkoohi-Burgos[2]; C Cansado-Utrilla[2]; L R Haines[2]; S Wagstaff[2]; M Lehane[2]; J Shamsani[5]; S Dean[4]; A Darby[3]; N Hall[1]; L Wilson[5]; P Walrad[5]

[1] Earlham Institute, UK; [2] Liverpool School of Tropical Medicine, UK; [3] University of Liverpool, UK; [4] University of Warwick, UK; [5] University of York, UK

The journey of *Trypanosoma brucei* in the tsetse involves migration to several organs and tissues, including the salivary glands. This process occurs in a time manner and it is accompanied by a series of parasite developmental changes. To identify *T. brucei* genes involved with life cycle progression in the tsetse, we compared the transcriptome profiles of proventricular trypanosomes from a fly-transmissible strain and a mutant strain unable to infect salivary glands. We found >700 up-regulated transcripts in the fly-transmissible strain. The top hit was identified as a conserved hypothetical protein across kinetoplastid organisms, containing a predicted MYND (Myeloid, Nervy and DEAF-1) zinc finger domain in the C-terminus. While overexpression of TbMYND1 restored infectivity of salivary glands in the impaired strain, a CRISPR-Cas9 deletion of TbMYND1 in a fly-

transmissible strain resulted in a motility phenotype *in-vitro* and in a very poor infectivity of the fly's midgut and proventriculus. To investigate a possible interaction between the TbMYND1 and RBP6 pathways, we knocked out RBP6 using CRISPR-Cas9. RBP6 KO cells showed normal motility *in-vitro* and infected both the midgut and the proventriculus at comparable levels with the parental cells. However, they failed to produce epimastigotes in the proventriculus and consequently did not infect salivary glands. Pull-down assays showed that TbMYND1 interacts with a range of hypothetical proteins, most of them containing zinc finger domains, including another MYND protein (TbMYND2). Furthermore, TbMYND1 binds to >150 transcripts with products including proteins involved in flagellar attachment and cell cycle. We conclude that while RBP6 controls parasite differentiation at specific tsetse organs, TbMYND1 is a novel mRNA stabiliser that regulates trypanosome migration through the fly.

10:10 (20 mins)

Disruption of active trans-sialidase genes in *Trypanosoma cruzi* resulted in attenuated parasites that fully protect against challenge with a virulent strain

Presenter: **Dr Gabriela Burle-Caldas**, *Post-Doctoral Research Associate, Durham University*

G A Burle-Caldas[1]; N S Aprigio dos Santos[1]; F L Mugge[1]; J T Castro[1]; V Grazielle-Silva11; M C Pereira[1]; J L Reis Cunha[1]; A E Oliveira[1]; N S Moretti[2]; A Coqueiro[1]; D Bartholomeu[1]; S Schenkman[2]; R T Gazzinelli[1]; S TEIXEIRA[1]

[1] Federal University of Minas Gerais, Brazil; [2] Federal University of São Paulo, Brazil

Trans-sialidases (TS) are unusual enzymes present on the surface of *Trypanosoma cruzi*, the causative agent of Chagas disease. Despite being codified by the largest gene family in the *T. cruzi* genome, only few TS members have catalytic activity. Active trans-sialidases (aTS) are responsible for the transfer of sialic acid from host glycoconjugates to mucins also present in the surface of the parasite. A major challenge in the study of these proteins is the presence of several copies of aTS genes dispersed in the genome, impairing the use of reverse genetics to disrupt aTS genes. Using CRISPR-Cas9, we generated TS knockout cell lines displaying very low levels of TS expression, as shown by quantitative PCR, western blot and immunofluorescence analyses with anti-TS antibodies, as well as TS activity, as shown by sialylation assays and labelling with antibodies that recognize sialic acid-containing epitopes. When tested in *in-vitro* infection assays, the TS mutants showed no differences in their invasion capacity or in their capacity to escape from the parasitophorous vacuole, but displayed impaired capacity to release trypomastigotes from infected fibroblasts. Two mutant cell lines were unable to establish an acute infection in BALB/c mice, whereas one of them, named TS KO7, which completely lost TS activity, showed almost undetectable levels of parasitaemia after infection of the highly susceptible IFN-gamma knockout mice. Importantly, BALB/c mice immunized with 5,000 trypomastigotes of the TS KO7 mutant were fully protected against a challenge infection with trypomastigotes from the virulent *T. cruzi* Y strain. Altogether, our results indicated that the *T. cruzi* TS have a role during the late time points of the intracellular development of the parasite and that TS knockout mutants can be used as live attenuated parasites in immunization protocols against Chagas disease.

10:30 (20 mins)

Life cycle progression analysis in *Leishmania mexicana* by single-cell RNA-sequencing reveals prospective mechanisms orchestrating promastigote to amastigote development

Presenter: **Mr Felix Warren**, *PhD Researcher, The University of Glasgow*

F Warren[1]; E Briggs[2]; R J Burchmore[1]; M S Llewellyn[1]; T Otto[1]; R McCulloch[1]

[1] The University of Glasgow, UK; [2] University of Edinburgh, UK

An essential step in the life cycle of *Leishmania mexicana* takes place in the vacuole of the mammalian macrophage, where amastigote forms arise after differentiation of metacyclics derived from promastigotes, both of which are found in the sandfly. The pathways and factors that drive the pronounced transcriptome changes seen as the parasite differentiates from promastigote to amastigote life cycle forms are poorly understood. To elucidate the timing and patterns of gene expression changes in the parasite life cycle, we employed single-cell RNA-sequencing (scRNA-Seq) at five time points as *L. mexicana* differentiated from promastigotes to axenic amastigotes in culture. scRNA-Seq revealed the transcriptomes of approximately 8,000 individual cells, and clustering analysis based on variable transcript abundance separated the cells into five discrete groupings. Investigation of known promastigote and amastigote marker genes allowed us to identify the trajectory of differentiation, including mapping of gene expression changes within the intermediate clusters, thereby revealing transitional life cycle stages. Amongst the transition stage genes we detected are chromatin-associated factors that have been found to be essential for mammal to tsetse life cycle progression in *Trypanosoma brucei*, but as yet undescribed in *L. mexicana*. From the scRNA-seq data we will describe the timing of differentiation events, factors that we predict to play roles in progression through this part of the life cycle, as well as genes potentially associated with survival strategies required during the switch from insect to mammalian host.

10:50 (20 mins)

Application of single-cell transcriptomics to resolve asynchronous differentiation of *Trypanosoma brucei* between slender and stumpy bloodstream forms

Presenter: **Dr Emma Briggs**, *Sir Henry Wellcome Fellow, University of Edinburgh*

E Briggs[1]; R McCulloch[2]; K Matthews[1]; T Otto[2]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

In the mammalian host, a quorum sensing (QS) process causes proliferative ‘slender’ form trypanosomes to differentiate into cell cycle arrested ‘stumpy’ forms in preparation for transmission. The transition between these developmental forms occurs asynchronously in the population and can be stimulated *in-vitro* using oligopeptides as the QS-signal. Although some molecules involved in the detection and transduction of the stumpy-induction signal have been identified, the hierarchy of events from QS signalling, through cell cycle exit, cellular

commitment, and molecular and morphological differentiation are ill defined. Using single-cell RNA-sequencing, the individual transcriptomes of 7000 trypanosomes undergoing differentiation to stumpy forms in response to the oligopeptide QS signal have been captured. Variable transcript abundance between parasites allowed the clustering of cell types, validated through known slender and stumpy markers, and a trajectory of differentiation to be deconvolved from the asynchronous developmental progression. Mining this reconstruction of gene expression change across differentiation now reveals the relative timing of events in differentiation as well novel genes and pathways, including at the key transition stage between the two cell types.

Coffee Break 11:10 to 11:40

Genomics, Evolution and Gene Expression -at 11:40 to 13:30

Chair - Prof Mark Field

11:40 (50 mins) (Invited Speaker)

Leishmania genome: aneuploidy, mosaicism and collectivism

Presenter: **Prof Jean-Claude Dujardin**, *Head of department, Institute of Tropical Medicine*

J C Dujardin[1]; G Negreira[1]; H Imamura[1]; I Maes[1]; P Monsieurs[1]; F Van den Broeck[1]; M A Domagalska[1]

[1] Institute of Tropical Medicine, Antwerp, Belgium

In *Leishmania*, aneuploidy appears to be the rule among cultivated parasites, it is observed in all species and it likely has an important functional role: (i) the 'average' aneuploidy of cell populations changes rapidly in response to new environments (drug pressure or host) and (ii) variation in aneuploidy is reflected in the level of the transcriptome, and to a great degree of the proteome, for genes located on polysomic chromosomes. Even in clonal populations, the copy number of specific chromosomes can vary between single cells, a phenomenon named mosaic aneuploidy (MA). Using 10X Single-Cell Genome Sequencing, we analyzed 1560 individual promastigotes in a *L. donovani* strain. Our data indicate that MA develops progressively but quickly and has an adaptive role, the different karyotypes representing individual solutions for the benefit of the cell population.

12:30 (20 mins)

Blastocrithidia, a trypanosomatid with all three stop codons reassigned

Presenter: **Prof Julius Lukes**, *P.I., Biology Centre, Czech Academy of Sciences*

J Lukes,[2]; A Nenarokova[1]; K Zahonova[1]; S Nenarokov[3]; A Kachale[1]; M Svobodova[1]; E Horakova[1]; V Yurchenko[4]; Z Paris[1]

[1] Biology Centre, Czech Academy of Sciences, Czech Republic; [2] Biology Centre, Institute of Parasitology, ASCR, Ceske Budejovice, Czech Republic; [3] Institute of Parasitology, Biology Centre, ASCR, Czech Republic; [4] University of Ostrava, Czech Republic

Blastocrithidia constitutes a small morphologically inconspicuous clade among the trypanosomatid flagellates. However, it distinguishes from all other kinetoplastids by an oddity in its genetic code, as it reassigned all three stop codons (UGA, UAA and UAG) into sense codons. We have sequenced, assembled and analyzed the genomes and transcriptomes of two *Blastocrithidia* species and another trypanosomatid from the sister *"jaculum"* clade, which has a canonical genetic code. The *Blastocrithidia* genome annotation has been performed with an in-house generated software, programmed to deal with a high number of the ambiguous stop codons. We have mapped the general trends in the across-genome distribution of the reassigned stop codons and confirmed by mass spectrometry the predicted amino acids specified by the in-frame stops. Moreover, we showed that only UAA has a double meaning, as it is also used as a genuine stop. We have also performed phylogenetic and experimental analysis of tRNAs that are responsible for decoding of the stop codons. We are attempting genetic modifications of *Blastocrithidia*, which would allow to experimentally address questions such as: How does its translation termination function? What is the impact of in-frame stops on translation? I will discuss possible mechanisms that triggered this massive reassignment.

12:50 (20 mins)

Expanded Genome-Wide Comparisons Give Novel Insights into Population Structure and Genetic Heterogeneity of *Leishmania tropica* Complex

Presenter: **Ms Tamara Salloum**, *PhD Candidate, Newcastle University*

T Salloum[1]

[1] Newcastle University, UK

Leishmania tropica is one of the main causative agents of cutaneous leishmaniasis (CL). Population structures of *L. tropica* appear to be genetically highly divers. However, the relationship between genomic heterogeneity and the diversity of *L. tropica* isolates pathogenicity and host range/preferences is still poorly known. In this study, we sequenced the genome of three new clinical *L. tropica* isolates, two derived from a recent outbreak of CL in camps hosting Syrian refugees in Lebanon and one isolate from Azerbaijan to further refine comparative genome analyses. Chromosome ploidy, single nucleotide polymorphism (SNPs), and gene copy number variations (CNVs) were investigated between 19 strains, one reference genome and 18 genomes, to further investigate the population genetics of *L. tropica* originating from various geographic locations. *In silico* multilocus microsatellite typing (MLMT) was performed to integrate the current diversity of genome sequence data in the wider genetic population structures available from microsatellite genotyping. Unique SNPs and CNVs profiles divided the 18 analysed strains into five populations based on principal component analysis. This suggests that various *L. tropica* lineages exposed to different environmental conditions experienced shifts in selective pressures, or important population bottle necks, or a combination of both processes, across their broad geographic distribution and host range. Gene ontology enrichment analysis of the genes with population specific SNPs and CNVs profiles revealed various biological processes, including iron acquisition, sterols synthesis and drug resistance. MLMT divided the strains in three populations that broadly correlated with their geographical distribution but not populations defined by SNPs. This study further highlights the complex links between *L. tropica* tremendous genomic heterogeneity and the parasite broad geographic distribution and insect and mammalian host range. Unique features identified in distinct populations reveal potential novel markers that could be exploited for the development of more accurate typing schemes to further improve our knowledge of the evolution and epidemiology of the parasite and ultimately also refine treatment regiments.

13:10 (20 mins)

Comparative analysis of glycolytic metabolism in the livestock trypanosomes

Presenter: **Dr Pieter Steketee**, *Postdoctoral Research Fellow, The Roslin Institute, University of Edinburgh*

P Steketee[1]; E A Dickie[2]; K Crouch[2]; S Jayaraman[1]; J Iremonger[1]; O Alfituri[1]; E Paxton[1]; M P Barrett[2]; L Morrison[1]

[1] The Roslin Institute, University of Edinburgh, UK; [2] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

The unicellular protozoan *Trypanosoma congolense* is a primary causative agent of animal African trypanosomiasis (AAT), a parasitic disease also caused to a lesser extent by the related pathogens *T. brucei* and *T. vivax*. There are limited chemotherapeutics to combat AAT, and drug resistance has emerged to the majority of compounds available. Whilst *T. brucei* has been the subject of much focus over the past century, *T. congolense* has been relatively ignored, even though it is responsible for significant livestock deaths and economic burden. Recent studies, including the sequencing of the *T. congolense* genome, have hinted at metabolic differences between mammalian-infective *T. congolense* and *T. brucei*. Using an omics-driven (in particular metabolomics and RNA-seq) approach, we have investigated and confirmed key differences in the metabolic usage and output of bloodstream forms of a laboratory-adapted *T. congolense* strain (IL3000), when compared to *T. brucei*. We have shown that the parasite consumes significantly less glucose than *T. brucei* and excretes large amounts of succinate and malate, in contrast to *T. brucei*, for which pyruvate is a primary output. In addition, we have investigated glycolytic gene essentiality by applying newly developed RNAi. Taken together, these data suggest bloodstream form *T. congolense* is not solely reliant upon glycolysis, but potentially utilises other metabolic pathways for central carbon and energy metabolism, highlighting significant metabolic differences between *T. congolense* and *T. brucei* that could have important implications in therapeutic design.

Lunch Break 13:30 to 15:00

Gene Expression of Surface Protiens -at 15:00 to 17:30

Chair - Prof Markus Engstler

15:00 (50 mins) (Invited Speaker)

How does bloodstream form *Trypanosoma brucei* control vast levels of expression from a single copy Variant Surface Glycoprotein gene?

Presenter: **Prof Gloria Rudenko**, *Professor of Molecular Microbiology, Dept. of Life Sciences, Imperial College London*

G Rudenko[2]; J Budzak[2]; R Jones[2]; C P Ooi[2]; A Thivolle[2]; R Lever[2]; C Tschudi[1]; N G Kolev[1]

[1] Department of Epidemiology of Microbial Diseases, Yale School of Public Health, United States; [2] Dept. of Life Sciences, Imperial College London, UK

African trypanosomes are protected by a dense layer of Variant Surface Glycoprotein (VSG) while multiplying in the bloodstream of the host. Prodigious amounts of VSG (10% total protein) are produced from a single VSG gene. The active VSG gene is located in one of 15 VSG Expression Sites (ES), which are controlled through highly stringent allelic exclusion. ES transcription is mediated by RNA polymerase I (Pol I), from a discrete nuclear structure referred to as an Expression Site body (ESB). If trypanosomes are forced to activate two ESs at the same time, these ESs dynamically share a single ESB. This argues that the ESB plays an essential role in monoallelic exclusion. We have discovered that there is an interplay between splicing and ES transcription, and inhibiting splicing in different ways inhibits ES transcription. In addition, we have discovered that a nuclear body conglomerate composed of four discrete nuclear bodies is associated with the active ES. These bodies include an ES Pol I transcription body (ESPB), a Spliced Leader Array Body (SLAB) and two different RNA processing bodies. In procyclic form *T. brucei* (which does not express VSG), three of these nuclear bodies are present, but dispersed throughout the nucleus. We propose that this novel nuclear body conglomerate forms a super-factory facilitating extraordinarily high levels of VSG synthesis.

15:50 (20 mins)

Antigenic variation in *Trypanosoma brucei* relies on dynamic inter-chromosomal interactions with the Spliced Leader RNA locus

Presenter: **Dr Joana Correia Faria**, *Postdoctoral Researcher, Wellcome Centre for Anti-infectives Research*

J Correia Faria[3]; V Luzak[1]; L S Müller[1]; B G Brink[1]; S Hutchinson[2]; L Glover[2]; T N Siegel[1]; D Horn[3]

[1] Biomedical Center Munich, Department of Physiological Chemistry, Ludwig-Maximilians-Universität München, Germany; [2] Institut Pasteur, Paris, France; [3] Wellcome Centre for Anti-infectives Research, University of Dundee, UK

T. brucei relies on monogenic antigen expression to evade the host immune response. Here, we demonstrate spatial integration of the active variant surface glycoprotein (VSG) gene with the spliced-leader (SL) array, a genomic locus involved in mRNA maturation. Chromosome conformation capture (Hi-C) revealed a strong inter-chromosomal interaction between the SL-array and the active-VSG that was reconfigured upon activation of another VSG. Super-resolution microscopy and ChIP-Seq analyses indicated that the VSG and SL compartments are occupied by the VSG exclusion proteins, VEX2 and VEX1, respectively, and interact in a heritable and splicing-dependent manner. Further, Hi-C analysis following VEX2-depletion, which permits multi-VSG expression, allowed multi-VSG interactions with the SL-array. Our results reveal a new mechanism to ensure monogenic VSG expression, requiring the spatial integration of transcription and RNA maturation in a single and exclusive, but switchable, organelle.

16:10 (20 mins)

Single-cell RNA-seq of salivary gland parasites reveals the shifting transcriptional landscape as parasites develop infectivity

Presenter: **Mr Sebastian Hutchinson**, *Post-doc, Institut Pasteur*

S Hutchinson[2]; S Foulon[1]; A Crouzols[2]; R Menfra[1]; B Rotureau[2]; A D Griffiths[1]; P Bastin[2]

[1] École supérieure de physique et de chimie industrielles, France; [2] Institut Pasteur, Paris, France

African trypanosomes must colonise the tsetse fly salivary gland for successful transmission. Epimastigote trypanosomes attach to and colonise the salivary gland epithelium where they undergo asymmetric cell division producing trypomastigote cells that differentiate into infectious metacyclic parasites. We applied inDrops, a microfluidic method for single cell encapsulation and mRNA barcoding to salivary gland parasites to obtain a detailed molecular understanding of these cells. Dimensionality reduction and clustering analysis produced discrete clusters of cells, corresponding to the epimastigote, pre-metacyclic and metacyclic stages. Our single cell resolution data revealed metabolic remodelling during this transition and allowed us to identify RNA binding proteins associated with specific clusters. Unexpectedly, in pre-metacyclic forms we detected up-to 6 *VSG* transcripts per cell, resolving into a single dominant transcript in metacyclic cells. We propose a model for the establishment of monoallelic expression in this system based on a competition for the active domain.

16:30 (20 mins)

Investigating trans-regulators in *Leishmania*: Evidence of post transcriptional epigenetics

Presenter: **Dr Pegine Walrad**, *Research Lecturer, University of York*

P B Walrad[3]; T R Ferreira[3]; L M De Pablos Torró[3]; A A Dowle[1]; E Parry[3]; S Forrester[3]; K Newling[1]; E A Ferreira[3]; K Hogg[1]; T R Larson[1]; M J Plevin[3]; A K Cruz[2]

[1] Biological Technology Facility, University of York, UK; [2] University of São Paulo, Brazil; [3] York Biomedical Research Institute, University of York, UK

RNA binding proteins (RBPs) are the primary gene regulators in kinetoplastids as transcriptional control is negligible. We recently conducted a comprehensive analysis of over 1,400 mRNA binding proteins (mRBPs) from the three main *Leishmania* lifecycle stages. We found a low correlation between transcript abundance and corresponding protein expression as well as stage-specific variation in protein expression versus RNA binding potential. Further to this, we found that RBPs select different RNA target pools in a stage-specific manner independent of target availability. Given the emphasis upon post-transcriptional regulation, *Leishmania* is an exceptional model for investigating post-translational modification (PTM) of RBPs. Arginine methylation is an evolutionarily conserved protein modification catalyzed by Protein aRginine Methyl Transferases (PRMTs). In *Leishmania major*, PRMT7 is a cytoplasmic protein implicit in pathogenesis with unknown substrates. Using comparative methyl-SILAC proteomics for the first time in protozoa, we identified 40 putative targets, including 17 RBPs hypomethylated upon PRMT7 knockout. PRMT7 can modify Alba3 and RBP16 *trans*-regulators as direct substrates *in-vitro*. Absence of PRMT7 *in-vivo* reduces Alba3 mRNA binding capacity to specific target transcripts

and impacts protein stability of multiple RBPs. RNA immunoprecipitation analyses demonstrate PRMT7-dependent methylation promotes Alba3 association with select target transcripts and indirectly stabilizes mRNA of *δ-amastin* surface antigen. These results highlight a novel role for PRMT7-mediated arginine methylation of RBP substrates, suggesting a regulatory pathway controlling gene expression and virulence in *Leishmania*. This work introduces *Leishmania* PRMTs as epigenetic regulators of mRNA metabolism with mechanistic insight into the functional manipulation of RBPs by PTM.

16:50 (20 mins)

Post-transcriptional mRNA control by PBP1-containing mRNA-binding complexes.

Presenter: **Dr Larissa Nascimento**, *PostDoc, ZMBH- Universität Heidelberg*

L Nascimento[2]; M Terrao[1]; F Egler[2]; B Liu[3]; K K Marucha[5]; E Erben[4]; C Clayton[2]

[1] CSL Behring - Marburg, Germany; [2] DKFZ-ZMBH Alliance, Germany; [3] Hebei Viroad Biotechnology Co.Ltd., China; [4] Instituto de Investigaciones Biotecnológicas, Argentina; [5] Kisii University, Kenya

We previously showed that the RNA-binding protein ZC3H11 recruits a complex of MKT1, PBP1 and LSM12 and poly(A) binding protein, resulting in mRNA stabilisation. We here demonstrate the existence of complexes that contain PBP1, LSM12, XAC1 (Tb927.7.2780), and either MKT1 or MKT1L (Tb927.10.1490). The C-terminus of MKT1L contains MKT1 domains and an inactive PIN domain, but also an N-terminal extension with regions of low-complexity. All of the proteins are present predominantly in the complexes. Although MKT1L depletion inhibited cell proliferation, we found no evidence for specific interactions with RNA-binding proteins or mRNA. MKT1, in contrast, appeared to be associated with many mRNAs, with the exception of those encoding ribosomal proteins. Evidence suggested that MKT1-containing complexes not only interact with several different RNA-binding proteins, but also specifically recruit one of the six translation initiation complexes, EIF4E6-EIF4G5. CFB2 is an unconventional mRNA-binding protein which interacts with MKT1 but also with SKP1, which is implicated in ubiquitination. RNAi targeting CFB2 causes loss of VSG mRNA and accumulation of cytosolic flagellar axonemes.

17:10 (20 mins)

Identification of elusive sequence-specific promoters of RNA polymerase II polycistronic transcription in African trypanosomes

Presenter: **Miguel Navarro***, Instituto de Parasitologia y Biomedicina (IPBLN)*

C Cordon-Obras[1]; C Gomez-Liñan[1]; S Torres-Rusillo[1]; I Vidal-Cobo[1]; D Lopez-Farfan[1]; A Barroso-del Jesus[1]; M Carrington[2]; **M Navarro[1]**

[1] Instituto de Parasitología y Biomedicina López-Neyra. Consejo Superior de Investigaciones Científicas, Spain
[2] Department of Biochemistry, University of Cambridge, UK,

Kinetoplastids have evolved in isolation for one billion years resulting in several divergent molecular and cellular processes. One example is protein-coding genes transcribed polycistronically by a typical RNA polymerase II (RNA pol II). Transcription most likely starts at divergent Strand Switch Regions (dSSRs), long sequences between divergently oriented polycistronic transcription units (PTUs). The lack of regulation in trypanosome transcription has become the paradigm in our field. Previous work suggests that changes in chromatin structure over broad SSR regions drives unregulated and dispersed transcription initiation.

We investigate such an exceptional feature in trypanosomes by first identifying RNA pol II-enriched regions using ChIP-Seq, as potential promoter sequences. The high resolution of this technique allowed us to accurately determine peaks of RNA pol II accumulation in the dSSRs. To functionally investigate pol II-enriched sequences unbiasedly, the peaks on chromosome VII were assayed for their ability to direct transcription using transient transfection. This analysis suggests that two unidirectional short sequence specific promoters within each dSSR make up the general structure. Primer extension analysis of nascent RNA allowed us to identify precise transcription start sites (TSS) of promoters inserted in a chromosome. Detailed analysis of one of these promoters defined 75bp as sufficient to fully drive transcription and identified essential nucleotides for precise initiation around the TSS. In addition, mutations to internal and downstream boxes led to dramatic decreased activity. In summary, we show that sequence-specific unidirectional RNA pol II promoters with proper TSS transcription initiation are present in the *T. brucei* genome. Our results challenge the currently accepted hypothesis that trypanosomes lack true promoters with transcription initiation control.

Poster Session A with Refreshments - at 17:30 to 19:30

Conference Dinner - at 18:30 till late

Day 3 Talks

Parasite Cell Biology - at 09:00 to 11:10

Chair - Prof Keith Gull FRS

09:00 (50 mins) (Invited Speaker)

Using CRISPR-Cas9 knockout screens to dissect flagellar functions in *Leishmania*

Presenter: **Dr Eva Gluenz**, *Royal Society University Research Fellow, Sir William Dunn School of Pathology*

E Gluenz[1]

[1] University of Oxford, Sir William Dunn School of Pathology, UK

Leishmania promastigote forms have a single motile flagellum with a canonical eukaryotic 9+2 microtubule axoneme and an extra-axonemal paraflagellar rod. When promastigotes differentiate to amastigotes inside macrophages, their flagellum is shortened and remodeled to a simpler 9+0 axoneme, which structurally resembles sensory cilia. Our aim is to study the role of the *Leishmania* flagellum in interactions with its insect vector and mammalian host cells, and to dissect mechanisms of flagellar motility more broadly. To this end, we generated a cell library of >500 *L. mexicana* deletion mutants lacking flagellar proteins. Mutants were generated using the LeishGEdit CRISPR-Cas9 method and we developed a streamlined pipeline to assess phenotypes individually. Each mutant also carries a unique 17-nt barcode, enabling a bar-seq strategy for measuring relative fitness of mutants in mixed pools. A screen for motility defects defined distinct mutant categories (faster swimmers, slower swimmers, slow uncoordinated swimmers and paralysed cells, including aflagellate promastigotes and cells with curled flagella and disruptions of the paraflagellar rod). In mixed infections of the permissive sand fly vector *Lutzomyia longipalpis*, paralysed *L. mexicana* promastigotes and uncoordinated swimmers were severely diminished in the fly after defecation of the bloodmeal; they did not reach anterior regions of the fly alimentary tract. These results show that *L. mexicana* need directional motility for successful colonisation of sand flies. We also tested the bar-seq method on mutant pools grown *in-vitro* (standard medium and under drug pressure), in different types of macrophages and in mouse models. These streamlined workflows for mutant production and bar-seq phenotyping can now be used to interrogate the fitness of diverse mutant cohorts exposed to a variety of assay conditions.

09:50 (20 mins)

The trypanosome lysosomal membrane glycoprotein p67 is a hydrolase of the n-terminal nucleophile (ntn) superfamily

Presenter: **Professor James Bangs**, *Professor and Chair, University at Buffalo*

C M Koeller[1]; T Smith[2]; A M Gulick[1]; **J D Bangs**[1]

[1] University at Buffalo (SUNY), United States; [2] University of St Andrews, UK

p67 is an essential transmembrane glycoprotein in *T. brucei*. It is synthesized as a gp100 glycoform that is cleaved in the lysosome to generate non-covalently associated N-terminal gp32 and C-terminal gp42 subunits. Cleavage is blocked by chemical inhibition of the lysosomal protease TbCatL. Other than death, the main phenotype of p67 knockdown is a grossly enlarged lysosome. Its exact function is unknown, but bioinformatics indicate p67 is the founding member of a phospholipase B-like (PLBL) subgroup of the NTN hydrolase superfamily, and p67 models precisely on the murine structure. NTNs autoactivate by internal cleavage at a nucleophilic residue to generate a (gp32) and b (gp42) subunits, which remain non-covalently associated. The N-terminal residue of the b subunit (Cys, Ser or Thr) then serves as the catalytic nucleophile for subsequent hydrolysis reactions. The conserved a/b cleavage site in p67 is C241/S242. Wildtype (CS) RNAiR p67, but not mutant (AA), is able to rescue growth under silencing of endogenous p67, confirming these residues as essential for function. The AA mutant is still cleaved by TbCatL to generate gp32/gp42 subunits, but gp42 is slightly longer at the N-terminus than WT. Thus, p67 activation involves upstream proteolytic cleavage within the gp32/gp42 linker region followed by downstream auto-activation at the CS junction. The hydrolytic activity(s) of the PLBL family is under debate. However, preliminary 'omic' data in knockdown cells suggest p67 has amidase activity, either ceramidase or peptidase. Multiple p67 orthologues are found in other parasitic protozoa including *Entameoba*, *Giardia* and *Trichomonas*, suggesting that these hydrolases contribute to pathogenesis in many parasitic diseases.

10:10 (20 mins)

Genome-wide functional profiling of genes in *T. congolense* and pleomorphic *T. brucei*

Presenter: **Dr Catarina Gadelha**, *Lecturer/Group Leader, University of Nottingham*

C Gadelha[2]; S D'Archivio[2]; G Awuah-Mensah[2]; S Whipple[2]; S Trindade[1]; L Figueiredo[1]; B Wickstead[2]

[1] Universidade de Lisboa, Portugal; [2] University of Nottingham, UK

High-throughput methods for gene function analysis such as RNA interference target-sequencing (RIT-seq) are powerful tools to understand parasite biology. To date, genome-scale screening has only been possible in culture-adapted *Trypanosoma brucei* (Lister 427), meaning that significant aspects of parasite biology cannot be directly addressed – nor the biology of the most important agents of the disease in cattle. To understand the biology of the parasite in contact with the host, we modified a novel genome-scale approach (DRiF-Seq) to create a library of ~250,000 RNAi mutants in pleomorphic bloodstream-form EATRO1125 *T. brucei* ("AnTat"). Robust quantitation of competitive growth rates shows that 60% of the non-VSG genes are associated with a significant loss-of-fitness in-vitro – suggesting that the proportion of the essential genome in trypanosomes is similar to that seen by barcode sequencing in Plasmodium. Further genes are necessary for survival in-vivo, and ~25% of mutants in non-VSG surface genes differ significantly in their behaviour in libraries in culture versus in mouse models of chronic infection. In addition, we have transferred the DRiF-Seq technology to the major causative agent of animal African trypanosomiasis *T. congolense*, generating 2 independent libraries of >300,000 mutants each in bloodstream-form IL3000 cells – covering 98% of all core genes. Parallel phenotyping of RNAi fragments over multiple timepoints reveals the characteristic timings and effect sizes associated with specific processes, and allows the functional dissection of essentiality within cellular structures/complexes. Moreover, intra- and inter-species DRiF-Seq comparisons reveal unexpected differences in specific biology between strains

of *T. brucei*, and also substantial differences between *T. brucei* and *T. congolense*. These data demonstrate the power of robust quantitation of mutant fitness at genome-scale, and point to aspects of parasite biology that are not well represented by common culture-adapted lines.

10:30 (20 mins)

A novel adenylate cyclase interacting protein is essential for swarming and tsetse fly colonization in trypanosomes

Presenter: **Dr Sabine Bachmaier**, *Postdoc, University of Munich (LMU), Biocenter*

S Bachmaier[3]; E Calvo Alvarez[2]; G Giacomelli[3]; L R Vieira[1]; A Aristodemou[3]; M K Gould[3]; I Forné[3]; A Imhof[3]; M Bramkamp[3]; D Salmon[1]; J V Abbeele[4]; B Rotureau[2]; M Boshart[3]

[1] Federal University of Rio de Janeiro, Brazil; [2] Institut Pasteur, Paris, France; [3] Ludwig-Maximillians-University of Munich, Germany; [4] The Institute of Tropical Medicine Antwerp, Belgium

Specificity in signal transduction depends on spatial and temporal aspects – components of a signal transduction pathway need to be at the right time at the right place in order to exert a specific response. *Trypanosoma brucei* encodes a large multigene family of transmembrane receptor-type adenylate cyclases. Specific subcellular localization has been reported for several isoforms, likely accounting for local cyclic AMP microdomains. Cyclic AMP signaling at the trypanosome flagellar tip has been implicated in the regulation of swarming behavior (social motility) *in-vitro* that correlates with the capacity for colonization of the tsetse fly insect vector. Here, we show colocalization data for known and novel flagellar tip proteins at single-molecule resolution and investigate their role in swarming. Deletion of a novel flagellar tip protein resulted in a complete block in swarming and a complete deficiency in tsetse salivary gland infection, consistent with the suggested role of swarming for insect colonization. Pull-down assays and interaction proteomics identify this flagellar tip protein as first physical interactor and regulator of trypanosome adenylate cyclases. We are investigating the mechanism of cyclase regulation by this novel interactor.

10:50 (20 mins)

The role of microtubule-organizing proteins in Trypanosoma brucei morphogenesis

Presenter: **Ms Amy Sinclair**, *Graduate Student, Brown University*

A N Sinclair[1]; T E Sladewski[1]; C L de Graffenried[1]

[1] Brown University, United States

The asymmetric cell shape of *Trypanosoma brucei* is required for its unique mode of motility and its pathogenicity. The polarized shape of the cell is mediated by an array of highly crosslinked subpellicular microtubules that underlie the plasma membrane. Despite the prominence of the array in *T. brucei* cellular architecture, little is known about how it is built and maintained during the lifetime of the cell. Using proteomic

methods, we have discovered new proteins that are a part of the inter-microtubule crosslinks of the array. Surprisingly, these proteins localize to specific array subdomains and appear to maintain and stabilize the microtubules at these regions. We have also identified a global regulator of the subpellicular array that is essential for confining the inter-microtubule crosslinking proteins to their subdomains. These results suggest that the array has defined domain identities that are composed of unique crosslinking complexes, which allows *T. brucei* to locally tune the biophysical properties of the array microtubules for motility and morphogenesis.

Coffee Break 11:10 to 11:30

Parasite Molecular and Cell Biology -at 11:30 to 14:00

Chair - Prof Juleus Lukes

11:30 (50 mins) (Invited Speaker)

Meiotic sex in Chagas disease parasite *Trypanosoma cruzi*

Presenter: **Dr Martin Llewellyn**, *University of Glasgow*

M Llewellyn[3]; P Schwabl[3]; H Imamura[5]; F Van den Broeck[5]; J A Costales[1]; J Maiguashca-Sánchez[1]; M A Miles[4]; B Andersson[4]; M J Grijalva[2]

[1] Center for Research on Health in Latin America, School of Biological Sciences, Pontifical Catholic University of Ecuador, Ecuador; [2] Infectious and Tropical Disease Institute, Biomedical Sciences Department, Heritage College of Osteopathic Medicine, United States; [3] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, UK; [4] London School of Hygiene and Tropical Medicine, UK; [5] Unit of Molecular Parasitology, Institute of Tropical Medicine Antwerp, Belgium

Genetic exchange enables parasites to rapidly transform disease phenotypes and exploit new host populations. *Trypanosoma cruzi*, the parasitic agent of Chagas disease and a public health concern throughout Latin America, has for decades been presumed to exchange genetic material rarely and without classic meiotic sex. Compelling evidence from 45 genomes sequenced from southern Ecuador demonstrates that *T. cruzi* in fact maintains truly sexual, panmictic groups that can occur alongside others that remain highly clonal after past hybridization events. These groups with divergent reproductive strategies appear genetically isolated despite possible co-occurrence in vectors and hosts. We propose biological explanations for the fine-scale disconnectivity we observe and discuss the epidemiological consequences of flexible reproductive modes. Our study reforms longstanding theory on clonality in trypanosomatid parasites and reinvigorates the hunt for the site of genetic exchange in the *T. cruzi* life cycle. Possible approaches for fully characterising genetic exchange in *T. cruzi* are discussed, as well as the potential power of forward genetic approaches to reveal the genetic basis of epidemiologically relevant traits.

12:20 (20 mins)

Unconventional kinetochore kinases are important for mitotic progression in *Trypanosoma brucei*

Presenter: **Dr Midori Ishii**, *Postdoc, Department of Biochemistry, University of Oxford*

M K Ishii[1]; B Akiyoshi[1]

[1] University of Oxford, UK

The kinetochore is a macromolecular protein complex that binds to spindle microtubules and the centromeric region of chromosomes and drives chromosome segregation in eukaryotes. Unlike most eukaryotes that have canonical kinetochore proteins, kinetoplastids including *Trypanosoma brucei* have unconventional kinetochore proteins, called KKT1–25 and KKIP1–12. Little is known about the function of these proteins. We used a procyclic form of *T. brucei* to characterize two paralogous kinetochore proteins with a CLK-like kinase domain, KKT10 and KKT19, which localize at kinetochores in metaphase but disappear at the onset of anaphase. We found that these proteins are functionally redundant. Double knockdown of KKT10/19 led to a significant delay in the metaphase to anaphase transition. A kinase-dead mutant of KKT10 failed to rescue the KKT10/19 depletion phenotype, suggesting that its kinase activity is essential. We also found that phosphorylation of two kinetochore proteins KKT4

12:40 (20 mins)

XL-BioID identifies a new essential component of the inner *Leishmania* kinetochore

Presenter: **Dr Vincent Geoghegan**, *Post-doc, University of York*

V Geoghegan[1]; N Jones[2]; A A Dowle[2]; T R Larson[2]; J Mottram[2]

[1] The University of York, UK; [2] University of York, UK

The recent discovery of the kinetoplastid kinetochore has revealed a unique set of proteins involved in chromosome segregation, most of which bear no resemblance to kinetochore proteins in other eukaryotes such as yeast or human. To date, 25 proteins have been discovered that form the kinetoplastid kinetochore, including 4 kinases, KKT2, KKT3, KKT10 and KKT19. This is a significant divergence from other kinetochores which do not contain kinases as core structural components and interestingly, KKT2 and KKT3 do not appear to belong to any known eukaryotic kinase family. The kinetochore has not been directly examined in *Leishmania,* therefore we investigated the proximal and interacting proteins of KKT2, KKT3 and KKT10 kinases by developing a novel proximity biotinylation method, XL-BioID. XL-BioID revealed that the interactomes of KKT2, KKT3 and KKT10 overlapped substantially, identifying the majority of the components of the *Leishmania* kinetochore, including a previously undescribed component, KKTA. Immunofluorescence confirmed this as a novel component of the kinetoplastid kinetochore which also appears essential as we were unable to generate a knock out. Applying XL-BioID to synchronised parasites, we investigated the dynamics of proteins proximal to KKT3 through the cell cycle to build an understanding of how the kinetochore is assembled. This analysis identified a small group of kinetochore proteins already present at the kinetochore in G1/S, which included KKTA. These inner kinetochore proteins may represent a constitutive chromatin associated network, upon which the rest of the kinetochore is built. Phosphorylation plays a crucial role in regulation of kinetochore assembly and function in other systems, however we have very little understanding of the role of phosphorylation in the kinetoplastid kinetochore. To identify potential substrates of KKT3, we combined XL-BioID with phosphopeptide enrichment to identify

phosphorylation sites proximal to KKT3 in the kinetochore complex. This approach is applicable to other kinases and should advance our understanding of phosphorylation based signalling pathways in kinetoplastids.

13:00 (20 mins)

Molecular and Functional Dissection of Distinct mRNA Export Pathways

Presenter: **Dr Samson Obado**, *Research Associate, The Rockefeller University*

S O Obado[2]; L Glover[1]; S Hutchinson[1]; B T Chait[2]; M C Field[3]; M P Rout[2]

[1] Institut Pasteur, Paris, France; [2] The Rockefeller University, United States; [3] University of Dundee, UK

The major cellular mRNA export factor Mex67/NXF1 (yeast/vertebrates), together with its partner Mtr2/NXT1, transport mRNAs through the nuclear pore complex (NPC) to the cytoplasm. In most eukaryotes, Mex67/NXF1 exists as a single protein, although in metazoa (including humans) additional tissue-specific isoforms of NXF1 exist. These paralogs may provide an additional level of tissue-specific gene control, yet their function has been difficult to separate from the many other levels of gene control exercised by metazoan cells. This mRNA export is dependent on an ATP-dependent remodeling machinery for its energy and directionality, in contrast to virtually all other nucleocytoplasmic transport pathways, which instead utilize the GTPase Ran. We have discovered that the unicellular protozoan, *Trypanosoma*, has two very distinct paralogs of Mex67 with differing roles in mRNA export. Control of mRNA levels in trypanosomes is almost exclusively post-transcriptional,
thus potentially positioning RNA export as a major mechanism for controlling gene expression. Indeed our results indicate that the two Mex67 paralogs, termed TbMex67 and TbMex67b, are life cycle specific, playing different roles in the mammalian bloodstream form (BF) of the organism versus the insect procyclic form (PF). In addition to extreme reliance on post-transcriptional gene regulation, we have previously shown that the trypanosomatid NPCs lacks the entire mRNA export platform and associating ATP-dependent machinery that is required to drive and provide directionality to mRNA export in yeast and metazoa. Instead, our results strongly indicate that mRNA export in these organisms is dependent for both directionality and energy on the Ran GTPase system, providing a new perspective on how Ran can be remodeled to mediate an alternate directional transport pathway across the NPC.

13:20 (20 mins)

Suicidal *Leishmania*

Presenter: **Dr Vyacheslav Yurchenko**, *Lab head, University of Ostrava*

V Yurchenko[3]; L Podešvová[3]; T Leštinová[1]; E Horakova[2]; J Lukeš[2]; P Volf[1]

[1] Charles University, Czech Republic; [2] Institute of Parasitology, Biology Centre, ASCR, Czech Republic;
[3] University of Ostrava, Czech Republic

Leishmania are obligate intracellular parasites known to have developed successful ways of efficient immunity evasion. Because of this, leishmaniasis, a disease caused by these flagellated protists, is ranked as one of the most serious tropical infections worldwide. Neither prophylactic medication, nor

vaccination has been developed thus far, even though the infection has usually led to strong and long-lasting immunity. Here, we describe a "suicidal" system established in *Leishmania mexicana*, a human pathogen causing cutaneous leishmaniasis. This system is based on the expression and (de)stabilization of a basic phospholipase A2 toxin from the *Bothrops pauloensis* snake venom, which leads to the inducible cell death of the parasites *in-vitro*. Furthermore, the suicidal strain was highly attenuated during macrophage infection, regardless of the toxin stabilization status. Such a deliberately weakened parasite could be used to vaccinate the host, as its viability is regulated by the toxin stabilization, causing a profoundly reduced pathogenesis.

13:40 (20 mins)

The structure of *T. brucei* F-ATP synthase and the different bioenergetic consequences resulting from its loss

Presenter: **Alena Zikova**, *Group leader, Biology Centre, Institute of Parasitology*

A Zikova[3]; O Gahura[3]; C Hierro Yap[3]; B Panicucci[3]; M Slapnickova[3]; K Subrtova[1]; C E Dewar[2]; A Schnaufer[1]; A Muhleip[4]; A Amunts[4]

[1] Institute for Immunology and Infection Research, University of Edinburgh, UK; [2] Institute of Immunology and Infection Research, University of Edinburgh, UK; [3] Institute of Parasitology, Biology Centre, ASCR, Czech Republic; [4] Stockholm University, Sweden

Mitochondrial F-ATP synthases are key enzymes in energy conversion found in rows of dimers on the edge of the cristae. While the architecture of the catalytic sector is conserved across all eukaryotic phyla, the non-catalytic parts display surprising divergency in both structure and function. We have obtained high-resolution cryo-EM structural data of the *Trypanosoma brucei* F-ATP synthase dimer that map the subunit-rich peripheral stalk and a phylum-specific dimerization interface in atomic detail. In addition, we identify cardiolipin molecules in the cavity of the membrane-embedded domain, suggesting functional roles for lipids in complex stability. An RNAi screen of all accessory subunits identified a protein essential for the stability of dimers but not monomers. This leads to new studies that will specifically decipher the significance of the assembled dimer rows on the mitochondrial ultrastructure. Enzymatically, F-ATP synthases are reversible nanomotors that can synthesize or hydrolyze ATP depending on the energetic and physiological demands of the cell. In trypanosomes, the F-ATP synthase activity depends on the life cycle stage of the parasite as the procyclic stage (PCF) utilizes the ATP-producing direction of this complex while the bloodstream form (BSF) employes the reverse activity to generate the essential mitochondrial membrane potential. In PCF, a decrease in ATP synthase monomers leads to a growth phenotype, low ATP levels and elevated mitochondrial membrane potential. Interestingly, the plasticity of the parasite´s mitochondrion allows redirection of electrons from proton-pumping complexes III and IV to alternative oxidase, which lowers the mitochondrial ROS production. In contrast, the BSF mitochondrion can withstand a 90% loss of the ATPase monomers without an obvious effect on its physiology. However, when the F-ATPase complex is fully disrupted, the BSF cells experience a sudden drop in mitochondrial membrane potential that is followed by cell death. Our insights delve deeper into the connections between the F-ATP synthase structure and mitochondrial function.

Lunch Break 14:00 to 15:00

Immunology and Cell Biology -at 15:00 to 17:30

Chair - Prof Dolores González-Pacanowska

15:00 (50 mins) (Invited Speaker)

How do trypanosome receptors for host macromolecules bind ligands and yet avoid the host immune response?

Presenter: **Prof Mark Carrington**, *Professor, University of Cambridge*

M Carrington[1]

[1] University of Cambridge, UK

Trypanosoma brucei proliferates in the bloodstream and tissue spaces of mammalian hosts. This simple observation means that the trypanosomes have to acquire nutrients from the host and at the same time avoid both the adaptive and the various arms of the innate immune system.

The mechanism used to avoid the adaptive immune response has been well characterised and is based on antigenic variation of the variant surface glycoprotein (VSG) coat that covers the entire trypanosome surface. The host raises an antibody response to the VSG and when the titre is high enough the trypanosome is killed, however trypanosomes that have switched the identity of the expressed VSG escape. Iterations result in population survival. The VSG coat leaves the trypanosome with two problems. First, how does the trypanosome take up host macromolecular nutrients such as transferrin and haemoglobin-haptoglobin without any receptor attracting an antibody response. Second, how does it avoid the innate immune system and in particular the non-specific deposition of complement C3 on the cell surface?

We have determined the structure of receptor ligand pairs for transferrin and haptoglobin haemoglobin and these have revealed that both have N-linked glycosylation sites that are located in positions that would shield the parts of the receptors most accessible to antibodies. In addition, the members of the transferrin receptor gene family exhibit sequence variation concentrated in locations accessible to host antibodies and outside the transferrin binding site. However, we have shown that the haptoglobin haemoglobin receptor is readily accessible to antibodies and have shown that antibody drug conjugates are remarkably effective in curing infection in a mouse model.

Second, we have identified and characterised the structure of a receptor for host factor H, the most potent down regulator of the alternative complement pathway activated by the non-specific deposition of C3 on a cell surface. The receptor positions the inhibitory domains of factor H where they are able to inhibit events downstream of C3 deposition. The receptor has an N-linked oligosaccharide located to shield the receptor. In conclusion, receptors have evolved to work in the context of a VSG coat and to minimise antibody accessibility but they are accessible to antibodies and can be exploited in the development of antibody drug conjugates.

15:50 (20 mins)

Identification of a mannose-binding lectin essential for full resistance of *Trypanosoma brucei* gambiense to the lytic action of the human serum.

Presenter: **Dr Jean-Mathieu Bart**, *Investigator, Institut de Recherche pour le Développement (IRD)*

J M Bart[2]; C Cordon-Obras[3]; A Cooper[4]; C Clucas[4]; P Capewell[4]; A Benito[1]; A MacLeod[4]; M Navarro[3]

[1] Centro Nacional de Medicina Tropical, ISCIII, Madrid, Spain; [2] Institut de Recherche pour le Développement (IRD), France; [3] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain; [4] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

Among the three *T. brucei* subspecies, two (*T. b. gambiense* and *T. b. rhodesiense*) are lethal for humans, causing African sleeping sickness, while T. b. brucei is sensitive to the Apolipoprotein 1 (ApoL1), the major compound of the trypanolytic factor (TLF) present in the normal human serum (NHS). *T. b. rhodesiense* resists lysis by NHS by expressing the Serum Resistant Associated (SRA) protein that directly blocks the action of ApoL1. The resistance mechanism of *T. b. gambiense* appears more complex, involving multiple factors such as alterations in TLF endocytosis in the required receptor, expression of the *T. b. gambiense*-specific glycoprotein (TgsGP) and reduction of sensitivity to ApoL1 through altered cysteine protease activity. To discover additional factors involved in the resistance of *T. b. gambiense* to NHS, a gain-of-function strategy was conducted, consisting in the expression of a *T. b. gambiense* genomic library in an NHS sensitive *T. brucei* cell line. After 1% fresh NHS selection, clones able to grow in such conditions were isolated and sequenced to identify the inserted DNA fragment. We identified a gene that confers a 64-fold increase NHS-resistance compared to the T. b. brucei parental cell line when over-expressed. The N-terminal domain of this gene encodes a conserved protein that belongs to the mannose-binding lectin (MBL) family, while the C-terminal domain is specific to *T. brucei* spp but with unknown function. Involvement of TbMBL in NHS-resistance was further assessed by generating a TbMBL inducible knock-down cell line in *T. b. gambiense*. A 50% reduction in growth rate was observed when this induced cell line was challenged with 10% NHS. Data of colocalization analysis by immunofluorescence and proximity ligation assay (PLA) not only suggest that TbMBL traffics in the endo/exocytic pathway, but also that TbMBL partially colocalizes with TgsGP. To strengthen this data, co-immunoprecipitation (co-IP) was performed and TbMBL/TgsGP interaction was confirmed. Finally, by proteomic analysis, an interaction of MBL with the Haptoglobin-related protein (HPR), one of the components of the TLF, was revealed. Altogether, our data suggest an essential role of TbMBL in the full resistance of *T. b. gambiense* against the lytic NHS action, where the lectin could act as “molecular glue” to form a complex between TgsGP, HPR and putatively ApoL1.

16:10 (20 mins)

An invariant Trypanosoma vivax subunit vaccine antigen inducing protective immunity

Presenter: **Dr Gavin Wright**, *Senior Group Leader, Welcome Sanger Institute*

G Wright[2]; D Autheman[2]; C Crosnier[2]; S Clare[2]; D A Goulding[2]; C Brandt[2]; K Harcourt[2]; C Tolley[2]; M Khushu[2]; H B Ong[2]; A Romero-Ramirez[1]; C Duffy[1]; A Jackson[1]

[1] Liverpool School of Tropical Medicine, UK; [2] Wellcome Sanger Institute, UK

Trypanosomes are parasites that cause diseases including human African trypanosomiasis, and nagana in important livestock animals. A vaccine against trypanosomes would be an important control tool, but the parasite has evolved sophisticated immunoprotective mechanisms that present an apparently insurmountable barrier to vaccination. We show using a systematic vaccinology approach that protective invariant subunit antigens can be identified. Vaccination with a recombinant protein comprising the extracellular region of a conserved surface protein localised to the flagellum induced long-lasting protection. Immunity was passively transferred with immune serum, and monoclonal antibodies could induce sterile protection and revealed multiple mechanisms of antibody-mediated immunity, including a major role for complement. Our discovery identifies a vaccine candidate for an important parasitic disease that has constrained the socioeconomic development of sub-Saharan African countries.

16:30 (20 mins)

Multimodal live imaging models for integrated analyses of *Trypanosoma cruzi* infections and digestive Chagas disease pathogenesis.

Presenter: **Dr Archie Khan**, *Research Fellow, London School of Hygiene and Tropical Medicine*

A Khan[1]; H Langston[1]; C McCann[2]; M C Taylor[1]; J M Kelly[1]; M D Lewis[1]

[1] London School of Hygiene and Tropical Medicine, UK; [2] UCL Great Ormond Street Institute of Child Health, UK

Objectives: Gastrointestinal (GI) symptoms are found in a substantial subset of chronic Chagas disease (CD) patients but the mechanisms and pathogenesis of this form of the disease are poorly understood. The objective of this study is to establish robust animal models of digestive CD that will allow the pathological changes, including functional impairment of GI transit, to be analysed in the context of *T. cruzi* infection dynamics.
Results: We established C3H mice as a severe model of GI transit dysfunction. Using the carmine red dye tracer method, we determined total gut transit time in concert with real-time infection imaging of bioluminescent parasites. C3H mice infected with the TcI-JR strain showed a significant delay in total GI transit time at the acute and late chronic stages. We then used this model to identify the specific regions of the gut responsible for the delayed transit. Fluorescent tracers were administered at different times before sacrifice of the mice to focus on gastric emptying and intestinal transit using *ex vivo* imaging. There were no significant alterations in gastric emptying, but a proximal shift in the geometric centre of fluorescence in the intestine was observed in the infected mice compared to naïve controls. We found that the delay in gut motility localised to the colon, which is also a site of *T. cruzi* infection, as revealed by co-imaging of bioluminescence. To further understand the dysfunction in the colon, we used immunofluorescence assays to analyse the alterations in the enteric nervous system in the *T.cruzi*-infected mice, since this is the principal pathophysiological feature of digestive CD in humans. We found a reduction in the number of neurons and a loss of immunoreactivity of the nerve network in the colon.
Conclusion: The C3H:TcI-JR mouse:parasite combination provides an excellent experimental model of digestive CD, with symptoms closely resembling key clinical manifestations in humans. The experimental tractability of this model and the multimodal imaging technique, together with pathological aspects, represent an innovative platform to study digestive CD pathogenesis.

16:50 (20 mins)

Widespread roles of Trypanosoma brucei ATR in chromosomal segregation and nuclear genome transmission are linked to R-loops

Presenter: **Dr Jennifer Ann Black**, *Postdoc, University of Glasgow*

J Black[3]; K Crouch[3]; E Briggs[1]; L Lemgruber[3]; C Lapsley[3]; J C Mottram[2]; R McCulloch[3]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] University of York, Centre for Immunology and Infection, UK; [3] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

Timely error correction is critical for accurate genome transmission. When chromosomes fail to separate, or become fragmented or structurally aberrant, aneuploidy can arise, affecting gene dosage and potentially compromising cell fitness. In eukaryotes, cell cycle checkpoints act to safeguard the genome, with their activation enabling correction of DNA aberrations prior to transmission. S/G_2, G_2/M and the spindle assembly checkpoint (SAC) are three checkpoints that can be activated by the protein kinase ATR to promote faithful genome transmission and chromosome segregation. In *Trypanosoma brucei*, the role of ATR in cell cycle control is largely unknown, though our prior work has shown that loss of ATR rapidly compromises expression of VSG used for host immune evasion in mammalian infective cells. Here we show that prolonged depletion of ATR by RNAi results in pronounced accumulation of genotoxic stress markers, yet the cells continue to synthesise DNA and enter cytokinesis. Chromosome segregation defects, generation of 'micronuclei' and perturbation of the nuclear structure also occur. RNAseq reveals significantly altered expression of factors involved in chromosome segregation and genome maintenance, including kinetochore components, nuclear pore proteins, DNA repair activities and mitotic kinase aurora 1 (AUK1). ChIPseq of γH2A (to examine genotoxic stress distribution) and DRIPseq (to examine R-loop distribution) after ATR depletion revealed widespread overlapping accumulation, including at rRNA genes and in RNA Pol II intergenic regions. Conversely, increased accumulation of γH2A was associated with reduced R-loop levels within centromeres. Taken together, our data suggest ATR provides widespread roles, some of which involve tackling R-loops across the genome, as well as potentially acting on centromeric R-loops during chromosome segregation.

17:10 (20 mins)

Trypanosoma brucei ISG65 binds complement C3 and increases virulence in the bloodstream

Presenter: **Dr Olivia Macleod**, *Postdoc, University of Cambridge*

O Macleod[2]; M Carrington[1]; M C Taylor[3]

[1] Department of Biochemistry, University of Cambridge, UK; [2] Department of Biochemistry, University of Cambridge, UK; [3] London School of Hygiene and Tropical Medicine, UK

African trypanosomes interact with their hosts through proteins expressed on the external face of the plasma membrane. Invariant surface glycoproteins (ISGs) are abundant type 1 transmembrane surface proteins expressed in bloodstream forms. While aspects of ISG trafficking and immunogenicity have been studied, the

function of this protein family is unknown. *Trypanosoma brucei* ISG65 was expressed as recombinant protein and pulled down complement C3/C3b from serum. The interaction was confirmed with surface plasmon resonance. Complement C3 is a central component of the complement system, which leads to cell lysis. Crosslinking and homology modelling confirmed that ISG65 is a three helical bundle, similar to other trypanosome receptors. We generated ISG65-/- cell lines, which were highly attenuated in a mammalian model infection. Therefore, ISG65 may recruit complement C3 to perturb complement-mediated lysis and increase virulence in the bloodstream.

Poster Session B with Refreshments - at 17:30 to 19:30

Day 4 Talk

Biochemistry and Gene Expression -at 09:00 to 10:50

Chair - Prof Gloria Rudenko

09:00 (50 mins) (Invited Speaker)

Novel players in the control of gene expression in African trypanosomes

Presenter: **Luisa Figueiredo**, *Group Leader, Instituto de Medicina Molecular, Lisboa*

L Figueiredo[1]; I J Viegas[1]; J Pereira de Macedo[1]; M De Niz[1]; J A Rodrigues[1]; F Aresta-Branco[1]; S R Jaffrey[1]

[1] Instituto de Medicina Molecular – João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Portugal

Although first RNA modifications have been identified more than half a century ago, in both coding and non-coding RNAs, the roles these modifications play in regulating RNAs are incompletely understood. The most frequent mRNA modification in eukaryotes is N6-methyladenosine (m6A), which localizes near the stop codon. The work over the last decade has established that m6A mRNA modifications regulate a vast range of biology, from normal embryonic development and differentiation, to cancer. I will describe the surprising discovery that in *Trypanosoma brucei*, the parasite that causes sleeping sickness in humans and nagana in cattle, m6A modifications occur within the poly(A) tail of messenger RNAs (mRNAs). The m6A modification was found in the poly(A) of monoallelically expressed Variant Surface Glycoprotein (VSG) transcript, and protected VSG poly(A) tail from deadenylation, resulting in ultra-stable mRNA. Using genetic tools, we identified that a gr conserved 16-mer motif in the 3'UTR of VSG acts as a cis-acting motif required for inclusion of m6A in the poly(A) tail. Removal of this motif from the VSG 3' UTR results in poly(A) tails lacking m6A, rapid deadenylation and mRNA degradation. To our knowledge this is the first identification of an RNA modification in the poly(A) tail of any eukaryote, uncovering a novel post-transcriptional mechanism of gene regulation.

09:50 (20 mins)

Nucleoside-activated kinetoplastid Protein Kinase A (PKA) - evolution of a signaling nexus

Presenter: **Prof Michael Boshart**, *Professor of Genetics, University of Munich LMU*

M Boshart[1]; G Githure[1]; Y Volpato Santos[1]; V Ober[1]; S Bachmaier[1]; E Tromer[3]; S Becker[1]; J Basquin[2]; T Carell[1]; E Lorentzen[2]; R F Waller[3]

[1] LMU München, Germany; [2] Max-Planck-Institute for Biochemistry, Germany; [3] University of Cambridge, UK

Protein kinase A (PKA) is an essential ancient signaling protein, conserved throughout the eukaryotic kingdom. PKA is bound and regulated by the second messenger cyclic AMP (cAMP) in all unicellular and multicellular organisms reported to date and became the first paradigm of allosteric regulation of protein kinases. We showed that in *T. brucei, T. cruzi and Leishmania* PKA is not bound or activated by cAMP. A compound

screen, molecular modeling and medicinal chemistry provided membrane permeable 7-deazapurine derivatives as activators with nanomolar potency and exclusive specificity for parasite PKA that were used to explore the PKA-specific target phosphoproteome (Bachmaier et al. 2019, Nature Comm.). Here we report that nucleosides are physiological activators of kinetoplastid PKAs. This surprising evolutionary swap of kinase activator specificity in PKA has been investigated biochemically and biophysically, by several crystal and co-crystal structures and site-directed mutagenesis. The critical roles for nucleoside versus cAMP specificity and affinity of a Glu and an Arg in each binding pocket of the regulatory subunit and its extended C-terminal helix have been confirmed by synthetic conversion of *T. brucei* PKA to cAMP response. The evolutionary origin of nucleoside-regulated PKA has been addressed by structural and binding analysis of several euglenozoan PKA isoforms outside the *Kinetoplastida*. Nucleoside- and cAMP-dependent PKAs coexist in the ancestors and multiple gene losses of isoforms can be traced. The unique ligand specificity of trypanosomatid PKA together with druggability, essentiality and abundant structural information qualify PKA as promising target for drug development.

10:10 (20 mins)

A *Trypanosoma brucei* ORFeome-based Gain-of-Function Library reveals novel genes associated with melarsoprol resistance

Presenter: **Dr Galadriel Hovel-Miner**, *T. brucei Gain-of-Function Library, George Washington University*

G Hovel-Miner[1];M Carter[1]; D Schulz[2]

[1] George Washington University, United States; [2] Harvey Mudd College, United States

Forward genetics approaches are powerful tools for uncovering novel aspects of Trypanosomatid biology, pathogenesis, and therapeutic approaches against trypanosomiasis. We have generated a *T. brucei* ORFeome and used it to make an inducible Gain-of-Function library for broadly applicable forward genetic screening. Using a critical drug of last resort, melarsoprol, we conducted a proof of principle genetic screen. We identified 57 genes that were significantly overrepresented in melarsoprol survivor populations following Gain-of-Function library induction when compared with untreated library input. The hits arising from this screen support the significance of trypanothione, a key player in redox metabolism, as a target of melarsoprol. In addition, novel genes whose proteins localize to the mitochondria and flagellum were also identified. Thus, we present new genetic tools which are expected to promote major advances in Trypanosomatid biology.

10:30 (20 mins)

Histidine Regulates pH Homeostasis in Acidocalcisomes of *Trypanosoma cruzi*

Presenter: **Mr Brian Suarez Mantilla**, *Research fellow, Durham University*

R Docampo[1]; B S Mantilla[1]; L S Amaral[1]; S A Wella[1]

[1] Center for Tropical and Emerging Global Diseases, University of Georgia, United States

Trypanosoma cruzi, the etiologic agent of Chagas' disease, is well adapted to pH changes imposed by the insect vector (triatomine digestive tract pH: 5-6.0) and vertebrate host (blood pH: 7.4, lysosomes pH:4.5-5.5, host-cell cytosol pH:7-7.2) (Van der Heyden et al., *Biochem J*., 1996). Cytosolic pH in *T. cruzi* is mostly regulated by a H^+ATPase pump localized at the plasma membrane and is supported by K^+ and Cl^- channels (Van der Heyden et al., *Mol Biochem Parasitol*., 2000). However, little is known about intraorganellar pH homeostatic mechanisms. Amino acids play roles as osmolytes and also serve as carbon/nitrogen sources in *T. cruzi*. The amino acid histidine (pKa side chain: 6) can be deaminated into urocanate by the histidine ammonia-lyase (TcHAL) [EC 4.3.1.3] with concomitant production of ammonia (NH_3) (Barison et al., *J Bioenerg & Biomemb* 2016). This latter is converted into ammonium (NH_4^+, pKa 9.25) alkalinizing acidic compartments. Subcellular fractionation, western blot and immunofluorescence analyses showed that TcHAL is localized to the acidocalcisomes. To determine a possible function for this basic amino acid within these acidic compartments, we analyzed changes in pH driven after addition of L-histidine. Fluorometric measurements using acridine orange in isolated acidocalcisomes showed increases in pH triggered by histidine addition with concomitant production of ammonia. These results were confirmed by ratiometric recordings in parasites expressing a genetically-encoded pH sensor (ecliptic pHluorin-DsRed Express) fused to TcHAL. These cells displayed histidine-driven alkalinization after lowering pH with propionic acid. These data suggest that histidine deamination alkalinizes acidocalcisomes. This physiological phenomenon has been suggested as a trigger for polyphosphate (polyP) hydrolysis and Ca^{2+} release (Ruiz et al, *J. Biol. Chem*., 2001). Recent affinity purification studies from our lab (Negreiros et al., *Mol. Microbiol.*, 2018) identified TcHAL as a polyP-binding protein, and we aimed at validating such interaction. Kinetic studies of TcHAL from parasite lysates showed a 2-fold reduction in enzymatic activity in the presence of $polyP_{100}$. TcHAL amino acid deduced sequence predicted an intrinsically disordered region (IDR) at its C-terminus. Using CRISPR/Cas9-mediated endogenous C-tagging, we generated knock in parasites ($TcHAL^{K-L}$) harboring specific mutations (K525F, K526L, K531L and K533L) identified on this IDR. These $TcHAL^{K-L}$ cells displayed defects in enzyme activity, cellular localization, and failed to differentiate into metacyclic trypomastigotes when histidine was provided as main energy source. Our data suggest that histidine can be involved in pH regulation of acidocalcisomes through its interaction with polyphosphates.

Coffee Break 10:50 to 11:20

Novel Drug Therapies and New Targets -at 11:20 to 13:20

Chair - Dr Francisco Gamarro

11:20 (50 mins) (Invited Speaker)

New insights into thymidylate biosynthesis in *Trypanosoma brucei*: a novel route for dUMP formation

Presenter: **Prof Dolores González-Pacanowska**, *Professor, CSIC*

D González-Pacanowska[1]; M Yagüe-Capilla[1]; M Valente[1]; C Bosch-Navarrete[1]; L M Ruiz-Pérez[1]; V M Castillo-Acosta[1]

[1] Instituto de Parasitología y Biomedicina López-Neyra. Consejo Superior de Investigaciones Científicas, Spain

T. brucei is able to perform pyrimidine dNTP biosynthesis by making use of both the salvage and de novo pathways. Previous studies have shown that enzymes involved in dTMP biosynthesis, such as dihydrofolate

reductase-thymidylate synthase are essential. Interestingly, thymidine kinase and mitochondrial cytidine deaminase are also indispensable for viability. Studies in cytidine deaminase depleted cells clearly indicated the existence of nucleotidases that modulate intracellular deoxynucleoside pools and provide precursors necessary for dTMP biosynthesis. Indeed, dNTP break-down products can be reutilized in the salvage pathway or transported to other organelles in order to maintain intracellular homeostasis. In an effort to characterize nucleotidases in the *T. brucei* genome, we have identified the HD domain-containing nucleotidohydrolases TbHD52 which is strongly related to human sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1). SAMHD1 is a 3' exonuclease and dNTP triphosphohydrolase. It acts as a human immunodeficiency virus restriction factor by hydrolyzing dNTPs but also has a role in cell physiology regulating the intracellular dNTP pool during the cell cycle and facilitating DNA repair. The trypanosomal enzyme exhibits a conserved HD domain yet a variable N-terminus compared to the human counterpart thus lacking the canonical SAM domain. TbHD52 is essential for viability and knock-out cells are pyrimidine auxotrophs. The lack of TbHD52 can be counteracted by the over-expression of enzymes involved in dUMP formation and deficient cells show strong defects in cell cycle progression and nuclei and kinetoplast segregation. Furthermore, the enzyme presents a mitochondrial localization and cellular dNTP quantification and metabolomic analysis revealed a substantial accumulation of dCTP and cytosine derived metabolites in null mutants while thymidine derivatives are notably reduced. We propose that mitochondrial TbHD52 plays an essential role in the provision of deoxycytidine for cellular dNTP biosynthesis.

12:10 (20 mins)

Picking and polishing the rough diamonds: Turning hits into first-in-class therapeutic molecules for visceral leishmaniasis and Chagas disease

Presenter: **Dr Julio Martin**, *Biology Head, Kinetoplastids Unit, GSK*

J Martin[1]

[1] GSK, Spain

GSK is committed to global health and to discover innovative medicines that combat diseases of the developing world (DDW). No commercial return, but access to medicine is sought, and we pledge work hand-in-hand with public and private partners. GSK Open Innovation model lays on three pillars: Open Lab (providing access to our know-how and infrastructure), Open Source (sharing our data and assets with the worldwide research community) and Patent Pool (flexible IP protection).
We will describe how we are leveraging **Open Innovation** to build a portfolio of assets, as well as unveiling new targets, mechanisms of action and chemical biology knowledge. In this communication we will focus on two major kinetoplastid NTDs, i.e. visceral *Leishmania*sis (VL) and Chagas disease (CD). The GSK 1.8 million compounds collection has been screened phenotypically against their causative parasites, i.e. respectively *Leishmania donovani* and *Trypanosoma cruzi*, as well as *T. brucei* [1]. As a result, **three anti-*Kinetoplastida*l boxes** of 200 compounds each have been assembled representing the chemical and biological diversity identified. We envisage that the collaborative network which is emerging thanks to researchers and institutions will contribute to the research community with new therapeutic targets, chemical tools and further lead discovery programs. Furthermore, we will also review some of the two most **advanced preclinical NCEs discovered for VL** now entering First Time in Human studies, which have revealed novel targets unprecedented in the clinic, i.e. cyclin-dependent kinase [2] and proteasome [3].

[1] Peña, I. *et al.* (2015) *Scientific Reports* **5**, Article number: 8771.
[2] Wyllie, S. et al. (2018) *Nature* **560**,192–197.
[3] Wyllie, S. et al. (2019) *PNAS* **116** (19), 9318-9323.

12:30 (20 mins)

3'-Deoxyribofuranosyl 7-deazapurine nucleoside analogues: a new class of highly potent antitrypanosomal agents.

Presenter: **Dr Fabian Hulpia**, *postdoctoral fellow, Ghent University*

F Hulpia[1]; D Mabille[3]; G D Campagnaro[2]; G Schumann[5]; L Maes[3]; I Roditi[5]; A Hofer[4]; H P De Koning[2]; G Caljon[3]; S Van Calenbergh[1]

[1] Ghent University, Belgium; [2] Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [3] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium; [4] Umeå University, Sweden; [5] University of Bern, Switzerland

Trypanosoma brucei parasites, the causative agents of Human African Sleeping Sickness, are unable to synthesize the purine ring *de novo* from amino acid precursors. This biochemical difference with their host earmarks purine and purine nucleoside analogues as a potential source of antitrypanosomal agents. Two well-known trypanocidal nucleoside analogues, tubercidin and cordycepin, have serious limitations in their use as a therapeutic agent. In this presentation we will discuss the highly potent antitrypanosomal activity of hybrid analogues of tubercidin and cordycepin (3'-deoxyribofuranosyl-7-deazapurine nucleosides), of which 3'-deoxytubercidin cured an established CNS infection in mice. Additionally, data on transporter-mediated uptake and phosphorylation by the parasite will be presented.

12:50 (20 mins)

Target in *Trypanosoma brucei* for the novel and curative CT-series compound class identified as Topoisomerase II

Presenter: **Dr Matthew Gould**, *Postdoc, Wellcome Centre for Integrative Parasitology*

M Gould[3]; N Johnson[1]; R Palkar[1]; R Ritchie[3]; R S Schmidt[2]; M Kaiser[2]; P Maeser[2]; J Jiricek[1]; T Diagana[1]; S Rao[1]; M P Barrett[3]

[1] Novartis Institute for Tropical Diseases, United States; [2] Swiss Tropical and Public Health Institute, Switzerland; [3] Wellcome Centre for Integrative Parasitology, University of Glasgow, UK

Originally identified from an empirical screen of a 2.5 million compound library, the CT-series compound class has low nanomolar activity against BSF *Trypanosoma brucei*, and is curative against Stage I and Stage II rodent models of African Trypanosomiasis. Untargeted metabolomics uncovered significant increases in metabolites from the nucleotide pathway on CT exposure absent in CT resistant cells. Histone phosphorylation assays show CT treatment activates DNA repair mechanisms and mutants deficient in homologous recombination DNA repair pathways were hypersensitive to CTs. Combined, these data suggest CTs act by introducing double-stranded

breaks in DNA. Whole-genome sequencing of resistant mutants and genome-wide RNAi screens independently identified topoisomerase II alpha as playing an important role in the mode of action of CTs. Overexpression and CRISPR-mediated mutagenesis studies validate this protein as the likely target for CTs

Closing Plenary at 13:10 to 13:55

13:40 (45 mins) (Invited Speaker)

Organising the trypanosome nucleus: Assembly of the nuclear lamina and connections to the nuclear pore complex.

Presenter: **Prof Mark Field**, *Prof, University of Dundee*

M C Field[1]

1 School of Life Sciences, University of Dundee, UK

The nuclear lamina has multiple functions, including maintaining nuclear structural integrity and differential gene expression. Several lamina proteins are known in trypanosomes, two of which, NUP-1 and NUP-2, are essential, coiled-coil proteins of molecular mass 450 and 250kDa respectively. To uncover organisational principles of the trypanosome lamina we generated NUP-1 deletion mutants designed to identify domains of NUP-1 responsible for oligomerisation. We find that both N- and C-termini act as interaction domains and disruption of these interactions impacts additional components of the lamina, the nuclear envelope and nucleoporin TbNup98. By contrast there is remarkably little impact on transcription, crucially including silencing of telomeric variant surface glycoprotein genes. These data indicate that both terminal domains of NUP-1 have roles in assembling the trypanosome lamina and suggest an architecture based on a 'hub and spoke' configuration.

Closing Remarks at 13:00 to 13:15

13:20 (15 mins)

Presenter: **Prof Miguel Navarro**, *Prof. Head of the Lab., Instituto de Parasitologia y Biomedicina (IPBLN)*

Buffet Lunch

Poster Presentations

Poster 1 : Investigating the Kennedy Pathway: phosphatidylcholine and phosphatidylethanolamine synthesis in *Trypanosoma cruzi*.

Presenter: **Miss Leigh-Ann Booth**, *PhD student, University of St Andrews*

L Booth[1]; T K Smith[1]

[1] University of St Andrews, UK

The Kennedy pathway is the main biosynthetic pathway for phosphatidylcholine (PC) and phosphatidylethanolamine (PE), abundant eukaryotic structural lipids. The importance of PC/PE synthesis in parasitic protozoa is well demonstrated. Disruption of PC biosynthesis is fatal for *Plasmodium* and *Leishmania*; disruption of PE biosynthesis is similarly fatal for *Trypanosoma brucei*. However, information is lacking for phospholipid metabolism in *Trypanosoma cruzi*. Bioinformatic searches of the *T. cruzi* genome suggest that unusually there is a single choline/ethanolamine kinase at the start of the Kennedy pathway. Its inhibition could stop production of PE and PC. The putative choline kinase (TcCK) was recombinantly expressed and purified for characterisation. Kinetic studies revealed that TcCK is bifunctional, with a higher affinity for ethanolamine than choline. TcCK was renamed TcE/CK. Knockouts of TcE/CK are currently being generated and characterised.

Poster 2 : Exploiting and evaluating the fatty acid Δ-6 desaturase from *T. brucei* and C. fasciculata: understanding and bioengineering PUFAs biotransformation in trypanosomatids.

Presenter: **Miss Michela Cerone**, *PhD student, University of St Andrews*

M Cerone[1]; T Smith[1]

[1] University of St Andrews, UK

Trypanosomatids have a complex fatty acids synthase machinery, which includes a vast repertoire of elongases and desaturases enzymes. They can produce a wide variety of species of fatty acids (FAs) and polyunsaturated fatty acids (PUFAs), which are essential for their life cycle. Δ-6 desaturases from *Trypanosoma brucei* and Crithidia fasciculata were chosen for this study. Different approaches were applied to investigate their role in the overall mechanism of PUFA biotransformation: firstly, genetic manipulation, via heterologous expression of desaturases from kinetoplasts in E. coli and gene knock-down in *T. brucei* ; secondly chemical manipulation and supplementation of C. fasciculata culture media with long and short chain FAs from commercial and bio-waste sources. By using these strategies, we showed that it is possible to understand and tune the FAs biotransformation in trypanosomatids, and we highlighted the use of these enzymes as biocatalytic tools for production of valuable PUFAs

Poster 3 : One Health: Smashing silos during surveillance of climate-sensitive tsetse transmitted Trypanosomiasis in Kwale county,coastal Kenya

Presenter: **Mr Nicodemus Masila**, *Master of Environmental Health student , Flinders university*

N Masila[1]

[1] Flinders University, Australia

One Health is an emerging global concept aimed at enhancing health outcomes, with cognizance that human, animal and environmental health is inextricably linked. Trypanosomiasis, a neglected tropical disease transmitted by tsetse flies remains one of the debilitating vector-borne diseases negatively impacting livestock production, arable agriculture, public health, tourism and rural development sectors in Sub-Saharan Africa. The objective of this study was to establish tsetse species distribution, apparent density, infectivity in tsetse flies and cattle as well as the applicability of multidisciplinary collaboration using One Health approach in Kwale county, Kenya. The study applied both qualitative and quantitative research approaches. Both parasitological and entomological methods were used. Two species of trypanosomes and three tsetse species were found. Trypanosome infection rate in bovines was 2.72% (3/110) while in tsetse flies was 6.94%(22/317). Tsetse apparent density was 5.37.

Poster 4 : Cyclophilin 19 a new player in oxidative stress during *Trypanosoma cruzi* infection

Presenter: **Mr Gregory Pedroso dos Santos**, *PhD student, Universidade Federal de São Paulo*

G P Santos[3]; F M Abukawa[3]; N S Moretti[3]; N S Melo[3]; L M Alcântara[2]; B S Mcgwire[1]; S Schenkman[3]

[1] Ohio State University, Brazil; [2] Universidade de São Paulo, Brazil; [3] Universidade Federal de São Paulo, Brazil

Reactive oxygen species (ROS) produced by cells are described as important molecules against microorganisms infections. Despite that, several works have been shown that *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease, shows an opposite response when exposure to ROS, which increases its replication inside the host cell and the treatment with antioxidants present a reduction in the parasite burden. In this work, we investigate the conserved functions between cyclophilin 19 (TcCyP19) produced by the parasite and its ortholog in mammals, the cyclophilin A (CyPA), that it is a chaperone involved in the activation of the NOX enzymatic complex through the interaction with the regulatory subunit, the p47phox. Our data shows that TcCyP19 is secreted by the parasites into the cytosol of infected cells and demonstrated conserved functions described to CyPA as increasing the ROS production in the cells and consequently increase the parasite replication during the infection.

Poster 5 : Deazapurine nucleoside analogues as potent anti-*Trypanosoma cruzi* agents

Presenter: **Dr Fabian Hulpia**, *postdoctoral fellow, Ghent University*

F Hulpia[1]; C F Da Silva[2]; C Lin[1]; D da Gama Jean Batista[2]; L Maes[3]; G Caljon[3]; M de Nazaré Correia Soeiro[2]; S Van Calenbergh[1]

[1] Ghent University, Belgium; [2] Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; [3] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium

T. cruzi parasites are the causative agents of Chagas disease (CD). These parasites lack several enzymes of the purine de novo synthesis pathway, thus making them rely on the uptake and interconversion of purine nucleoside analogues. This constitutes an attractive rationale for the evaluation of modified nucleosides as antitrypanosomal agents. We have identified and optimized several series of purine nucleoside analogues[1,2] with potent activity against *T. cruzi*. In this presentation we will discuss three distinct series, their *in-vitro* evaluation and results from animal models of acute CD. (1) Hulpia, F. et al. *J. Med. Chem.* **2018**, *61*, 9287; (2) Lin, C. et al. *J. Med. Chem.* **2019**, *62*, 8847

Poster 6 : Identification of immunogenic non-Variant Surface Glycoprotein (VSGs) surface protein on wild Trypanosoma vivax

Presenter: **Mr Kwadwo Oworae**, *Mphil Student/Research Assistant , WACCBIP*

K O Oworae[1]; Y Aniweh[1]; T M Gwira[1]

[1] West African Center for Cell Biology of Infectious Diseases, Department of Biochemistry, Cell and Molecular Biology, University of Ghana., Ghana

The main challenge to vaccine and diagnostic development for trypanosomes, the causative agent for animal African Trypanosomiasis, is their potent antigen variation of their surface glycoprotein (VSGs) that densely covers their entire cell surface. Studies have shown that Trypanosoma vivax, second to *T. brucei* in prevalence, expresses less dense VSGs. However, preliminary data have shown the presence of non-VSG surface proteins. In this study, we identified a highly expressed non-VSG surface protein on *T. vivax* and determined its immunogenicity using synthetic peptides. Two herds of cattle from Adidome and Bolgatanga were selected for the study. Blood samples were collected at 8 weeks intervals for 4 timepoints. The infecting trypanosomes were PCR-typed targeting the trypanosome tubulin gene cluster. In silico analysis of Spliced Leader RNA-sequencing data identified TvY486_0029740, hypothetical protein, as a highly expressed *T. vivax* surface protein. Peptides of its extracellular domain (P1-12) were synthesized. The immunogenicity of the peptides was tested against the sera from selected cattle using ELISA and compared with a known *T. vivax* immunogenic recombinant protein (TvY486_0045500). Our data confirmed T. b. brucei as the most prevalent followed by *T. vivax* and *T. congolense*. Evidence of mixed infection with two or more trypanosome species were seen. Antibody response to the selected protein was significantly higher in infected cattle than naïve cattle. Although antibody response in Bolgatanga was significantly higher than that of Adidome, there was no significant difference in antibody response to the peptides across the various time points. P-11 was identified to be the most immunogenic with seropositivity of 100% to 89.1% of the recombinant protein. The magnitude of antibody response was also higher (p

Poster 7 : Trypanosomes struggling to make ends meet – identification of RNA editing ligase inhibitors by in silico screening

Presenter: **Miss Zandile Nare**, *PhD Student, University of Edinburgh*

Z Nare[1]; S Dang[1]; N Pham[2]; M Auer[2]; M Walkinshaw[4]; P Taylor[3]; A Schnaufer[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, UK; [3] School of Biological Sciences and Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, UK; [4] Wellcome Centre Cell Biology and Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, UK

Post-transcriptional uridylyl insertion/deletion mRNA editing is essential for mitochondrial gene expression in kinetoplastids, making it a potential drug target. Knock-down of REL1, a highly conserved key component of the editosome, is lethal in *Trypanosoma brucei*. The crystal structure of TbREL1 shows a well-defined ATP binding pocket with distinct differences to human DNA and RNA ligases, suggesting the feasibility of developing highly specific REL1 inhibitors. Using the in silico screening platform LIDAEUS, we screened ~300,000 compounds from the Asinex Gold library against TbREL1 and *L. donovani* REL1 (LdREL1). Of the top 14 in silico 'hits', four inhibited LdREL1 *in-vitro*, with IC50 values ranging from 0.75 to 10.47 µM. We are currently using differential scanning fluorimetry, X-ray crystallography, and on-bead confocal fluorescence nanoscanning to characterise the interactions of these inhibitors with REL1 to provide a rationale for hit prioritisation and hit-to-lead development.

Poster 8 : Atypical Human Trypanosomiasis: The skin, a potential reservoir?

Presenter: **Dr Gloria Chechet**, *University Academic staff , Ahmadu Bello University, Zaria*

G Chechet[1]; E D Akafyi[1];
[1] Ahmadu Bello University, Zaria, Nigeria

Here, we show case studies of the presence of animal Trypanosomal DNA detected in skin snips originally collected to detect Ivermectin resistance in Onchocerca volvulus in Taraba state, Nigeria; an area endemic for Onchocerciasis. PCR was carried out using the ITS-1 generic primers to detect and identify animal trypanosomes in the samples. Three of the samples tested produced a single strong band at about 300 bp while one sample produced a single strong band at about 240 bp which corresponds to Trypanosoma godfreyi. Three samples were cloned and sequenced to further confirm the species in comparison with those on the Tritrypdb database. The results from two out of the three samples of similar sizes corresponded to *Trypanosoma brucei* brucei and Trypanosoma evansi respectively. No match was found for the 240 bp sample. The skin could be a possible reservoir for the atypical Human Trypanosomiasis which is been overlooked since the parasites are known to dwell in the blood.

Poster 9 : DNA Damage and Antigenic Variation in African trypanosomes

Presenter: **Ms Emilia McLaughlin**, *PhD student, Institut Pasteur Paris*

E McLaughlin[1]; M D Urbaniak[2]; T Chaze[1]; M Matondo[1]; L Glover[1]

[1] Institut Pasteur, Paris, France; [2] Lancaster University, Biomedical and Life Sciences, UK

Trypanosoma brucei escapes detection by its mammalian host through antigenic variation of its Variant Surface Glycoprotein (VSG) coat. The VSG is expressed from a subtelomeric expression site (ES), where naturally occurring DNA double stranded breaks (DSB) are triggers for VSG switching. Interestingly, a DSB at the active ES results in 95% cell death (Glover et al., 2013). To understand how DNA repair and recombination contribute to antigenic variation we are characterising the DNA repair phosphoproteome, using an inducible meganuclease

system to introduce spatially constrained DSBs. We are currently validating specific DNA damage phosphophorylation sites to determine their role in antigenic variation. In parallel, we asked whether ES DSB lethality could be rescued by the expression of a second VSG. To our surprise, the second VSG gene is lost following an ES DSB, despite its spatial separation from the DSB. Combined, our work aims to identify the factors driving antigenic variation.

Poster 10 : Novel insights into trypanosome mRNA biology with correlative single molecule FISH on LR-white-embedded sections

Presenter: **Dr Susanne Kramer**, *Group leader, Biozentrum der Universität Würzburg*

S Kramer[1]; E Meyer-Natus[1]; A Schnaufer[2]; M Engstler[1]

[1] Biozentrum, Lehrstuhl für Zell-und Entwicklungsbiologie, Universität Würzburg, Germany; [2] Institute of Immunology and Infection Research, University of Edinburgh, UK

Single mRNA molecules are frequently detected by fluorescence in situ hybridisation (FISH) using branched DNA technology. While providing strong and background-reduced signals, the method is inefficient in detecting mRNAs within protein dense structures, in particularly in organisms with plasma membrane associated structures such as yeast and many protozoa. Moreover, abundant mRNAs cannot be detected as single molecules. To establish a quantitative, correlative single-molecule FISH method applicable to all mRNA targets, irrespective of the cellular compartment, we have hybridized slices of shock-frozen, LR-white embedded trypanosomes. Since mRNA detection is restricted to the surface of the matrix, even highly abundant mRNAs can be detected as single molecules, and detection is independent on the mRNA environment. Importantly, FISH can be combined with immunofluorescence, electron microscopy and electron tomography. Several applications of the method will be presented.

Poster 11 : Ubiquitin Conjugating Enzymes in *Leishmania*: When do they work, with whom, and can they be targeted?

Presenter: **Mr Daniel Harris**, *PhD student, University of Glasgow*

D Harris[4]; M Gabrielsen[1]; B Rodenko[2]; F El Oualid[2]; J Mottram[3]; D Huang[1]; R Burchmore[5]

[1] Beatson Institute for Cancer Research, UK; [2] UbiQ Bio BV, UK; [3] University of York, UK; [4] Wellcome Centre for Integrative Parasitology, Institute of Biodiversity, Animal Health, and Comparative Medicine, University of Glasgow, UK; [5] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

Leishmania parasites modulate protein levels in a developmentally coordinated pattern as they move through their digenetic life cycle. Protein ubiquitination regulates protein turnover in many organisms, by targeting proteins for degradation. We aim to identify the ubiquitin conjugation system in *Leishmania mexicana*, and characterise key enzymes for future drug discovery efforts. Ubiquitin activating (E1), and ubiquitin conjugating enzymes (E2) have been identified from promastigote forms through bioinformatic and mass spectrometric methods. Additionally, a ubiquitinome has been catalogued using immunoprecipitation, and enzymes involved in SUMOylation have been discerned. An abundantly expressed E1 enzyme has been recombinantly generated in a functional form, which is currently undergoing protein crystallography, so a structure may aid in drug design.

Poster 12 : Instability of aquaglyceroporin (AQP) 2 contributes to drug resistance in *Trypanosoma brucei*

Presenter: **Dr Juan Fernando Quintana Alcala**, *Postdoctoral Research Associate, University of Glasgow*

J Quintana Alcala[2]; J Bueren-Calabuig[1]; F Zuccotto[1]; H P De Koning[3]; D Horn[1]; M C Field[1]

[1] School of Life Sciences, University of Dundee, UK; [2] Wellcome Centre for Integrative Parasitology, Institute of Biodiversity, Animal Health, and Comparative Medicine, University of Glasgow, UK; [3] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

Defining mode of action is vital for both developing new drugs and predicting potential resistance mechanisms. African trypanosome pentamidine and melarsoprol sensitivity is predominantly mediated by aquaglyceroporin 2 (TbAQP2), a channel associated with water/glycerol transport. TbAQP2 is expressed at the flagellar pocket membrane and chimerisation with TbAQP3 renders parasites resistant to both drugs. Two models for how TbAQP2 mediates pentamidine sensitivity have emerged; that TbAQP2 mediates pentamidine translocation or via binding to TbAQP2, with subsequent endocytosis, but trafficking and regulation of TbAQPs is uncharacterised. We demonstrate that TbAQP2 is organised as a high order complex, is ubiquitylated and transported to the lysosome. Unexpectedly, mutation of potential ubiquitin conjugation sites, i.e. cytoplasmic lysine residues, reduced folding and tetramerization efficiency and triggered ER retention. Moreover, TbAQP2/TbAQP3 chimerisation also leads to impaired oligomerisation, mislocalisation, and increased turnover. These data suggest that TbAQP2 stability is highly sensitive to mutation and contributes towards emergence of drug resistance.

Poster 13 : Role of mRNA processing in regulating mRNA levels in *Trypanosoma brucei*

Presenter: **Miss Albina Waithaka**, *PhD student , Heidelberg University*

A Waithaka[1]; L Nascimento[1]; C Clayton[1]

[1] DKFZ-ZMBH Alliance, Germany

Suboptimal splicing signals may delay *T. brucei* trans-splicing and predispose precursors to degradation. The length and sequence of the polypyrimidine tract (PPT), a key splicing signal, affects reporter protein expression in transient assays. To test the effect of PPTs on mRNA processing, we constructed a pol-II-transcribed dual reporter plasmid encoding blasticidin–S deaminase (BSD gene)and puromycin N-acetyl-transferase (PAC gene) in which trans-splicing of PAC directs polyadenylation of BSD. PPT deletion causes alternative trans-splicing and polyadenylation, leading to cells that are blasticidin-resistant but susceptible to puromycin. A PPT with only 3Us can drive splicing at the correct position, although the efficiency is very low and the cells grow poorly in puromycin. Alternative PPT and 5'-UTR sequences are being tested. We have inserted two boxB sequences upstream of the PPT and are now tethering splicing factors and potential regulators to assess their functions.

Poster 14 : Drug target deconvolution in Kinetoplastids using thermal proteomics profiling

Presenter: **Dr Victoriano Corpas Lopez**, *Postdoc, University of Dundee*

V Corpas Lopez[1]; R Milne[2]; R Wall[2]; I Gilbert[2]; M De Rycker[2]; P Wyatt[2]; S Wyllie[2]

[1] School of Life Sciences, University of Dundee, UK; [2] University of Dundee, UK

Early identification of a compound's mode of action can greatly benefit the discovery process. Thermal proteomics profiling (TPP) is a powerful, unbiased tool that can be used to identify potential ligands of protein targets. TPP takes advantage of the fact that a ligand binding to its target protein can significantly stabilise that protein, increasing its melting temperature (T_m). Briefly, parasite lysates are treated with vehicle (DMSO) or test compound and aliquots subjected to a range of temperatures. The resulting soluble, non-denatured proteins were then derivatised with tandem mass tags (TMTs) prior to LC/MS-MS analysis. Tm's were calculated for all identified proteins in the presence and absence of test compound. Thermal proteome profiling (TPP) was used to confirm on-target engagement of a validated N-myristoyltransferase inhibitor in *Leishmania donovani* and a lysyl-tRNA synthetase inhibitor in *Trypanosoma cruzi*.

Poster 15 : Identification of *Leishmania donovani* cell surface and secreted proteins important for host infection

Presenter: **Dr Adam Roberts**, *Postdoctoral Fellow, Sanger Institute*

A Roberts[1]; C Brandt[1]; S Clare[1]; H B Ong[1]; G J Wright[1]

[1] Wellcome Sanger Institute, UK

The cell surface of *Leishmania donovani* plays an important role in its interaction with the host. To systematically identify important extracellular proteins required for visceral leishmaniasis, we have utilised a CRISPR/Cas9 approach to create a library of parasites lacking secreted or type 1 transmembrane proteins. Using a bioluminescent murine infection model, we have individually assessed >30 mutants for their ability to establish an infection *in-vivo*. We have found that as many as 30% of the mutants have altered pathology of infection. These *in-vivo* phenotypes broadly fall into two categories: mutants unable to develop an initial sustained infection; and mutants able to infect the liver, but without dissemination to the spleen. Together, these data suggest that the surface and secreted proteins of *L. donovani*, may provide an untapped resource for identifying important parasite effectors required for visceral leishmaniasis and new targets for vaccine design.

Poster 16 : Conserved, yet dispensable: structure-function analysis of the C-terminal domain of the *Trypanosoma brucei* variant surface glycoprotein

Presenter: **Dr Nicola Gail Jones**, *staff scientist, Biozentrum der Universität Würzburg, Lehrstuhl für Zell- und Entwicklungsbiologie*

N G Jones[1]; A Borges[1]; M Engstler[1]

[1] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany

The bloodstream form of *Trypanosoma brucei* is covered by a highly dynamic, homogenous, dense protein layer which consists predominantly of only one member of a large family of GPI-anchored variant surface glycoproteins (VSGs). Its functions include acting as a protective shield and antibody clearance. In addition, antigenic variation allows the parasite to persist in its host by repeatedly side-stepping the host's immune attack. Though much progress has been made in understanding the mechanisms guiding VSG expression and switching, fairly little is known about the diversity of VSG structures and the ensemble architecture of the coats they form. Recent findings suggest much more diversity in the *T. brucei* VSG family than previously thought, and this might play a role in modulating the parasite/host interface and therefore the parasite's interactions with its host. In addition, recent structural analyses combined with diffusion measurements of VSGs have suggested conformational flexibility in the VSG dimer resulting in two main conformations being observed. *T. brucei* VSGs consist of a larger N-terminal domain, and a smaller C-terminal domain which harbours the GPI-anchor attachment site. The C-terminal domain is composed of both structured and linker regions and conformational changes in the protein as a whole appeared due to changes in the orientation of its C-terminal domain. To test which parts of the C-terminal domain are essential for this proposed VSG flexibility we conducted a systematic mutagenesis study. We found that the structured regions of the C-terminal domain were not required for coat formation. A minimal C-terminal domain consisting of a linker region and a flexible or structured GPI-anchoring region were sufficient for allowing coat formation and maintaining cell growth.

Poster 17 : FoF1-ATP synthase subunit α – a tale of two fragments

Presenter: **Mr Karolina Subrtova**, *Postdoc, University of Edinburgh*

K Subrtova[1]; M Jain[2]; B Panicucci[2]; A Zikova[2]; A Schnaufer[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Institute of Parasitology, Biology Centre, ASCR, Czech Republic

F_oF_1-ATP synthase produces ATP in bacteria, mitochondria and chloroplasts. Its function is reversible, as an ATPase it generates the mitochondrial membrane potential in bloodstream form *Trypanosoma brucei*. The F_1 moiety is formed by a hexamer of alternating subunits α and β, which form the active sites for ATP synthesis/hydrolysis and sit on a central stalk consisting of subunits γ, δ and ε. The composition and structure of F_1 were considered to be conserved across all domains of life, until analysis of the Euglenozoa F_1 revealed an additional subunit, p18, and a processing event that cleaves subunit α into two fragments and removes an octapeptide. We are investigating the functional significance of the cleavage event and the nature of the peptidase responsible. Preliminary data suggest that RNAi-mediated ablation of α in *T. brucei* can be efficiently rescued by a recoded full-length version, but not by the C-terminal fragment alone or a version with a mutated octapeptide sequence.

Poster 18 : RNA editing ligase as a drug target: inhibitor discovery by high throughput screening

Presenter: **Ms Laurine Brouck**, *PhD student, University of Edinburgh*

L Brouck[3]; Z Nare[3]; M F Sardis[3]; J Koszela[4]; N Pham[4]; S Zimmermann[3]; M Speake[2]; S McElroy[2]; C Smith[5]; V Feher[1]; M Greaney[5]; R E Amaro[1]; M Auer[4]; A Schnaufer[3]

[1] Department of Chemistry and Biochemistry, University of California San Diego, United States; [2] European Screening Centre Newhouse, UK; [3] Institute of Immunology and Infection Research, University of Edinburgh, UK; [4] Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, UK; [5] School of Chemistry, University of Manchester, UK

Uridylyl insertion/deletion mRNA editing is critical for mitochondrial gene expression in kinetoplastids. RNA editing ligase 1 (REL1) and its paralog, REL2, are both key components of the RNA Editing Catalytic Complex (20S editosome), but genetic studies suggest that only REL1 is essential for the survival of *Trypanosoma brucei*. We developed a high-throughput *Tb*REL1 activity screen and identified several promising series of inhibitors in large libraries of small molecules. Several compounds inhibit REL1 from different trypanosomatid species, including *Leishmania donovani* and *Trypanosoma cruzi*, but are much less potent against a related bacteriophage RNA ligase, suggesting good specificity. We are now studying the target-ligand interactions of these inhibitors in more detail and will present results from differential scanning fluorimetry and on-bead confocal fluorescence nanoscanning assays that guide our hit-to-lead development efforts.

Poster 19 : Investigating RNA-binding proteins as post-transcriptional regulators of differentiation, virulence and infectivity in *Leishmania mexicana* parasites.

Presenter: **Mr Ewan Parry**, *PhD Student, University of York*

E Parry[1]; P B Walrad[1]; J Mottram[1]

[1] York Biomedical Research Institute, University of York, UK

The fundamental dependence of kinetoplastid parasites on post-transcriptional control makes *Leishmania* an ideal model for the study of RNA-binding protein function, providing valuable insight to the broader field of trans-regulators. Constitutive transcription of polycistrons in *Leishmania* means that post-transcriptional regulators like mRNA-binding proteins (mRBPs) play a prominent role in gene regulation. The Walrad lab recently isolated the mRNA-bound proteome of the three main lifecycle stages in *Leishmania* (Pablos et al.,2019). This dataset has been analysed for candidate mRBPs with a putative involvement in parasite differentiation or stage specificity to the human infective stages. Through the barcoded removal of prioritised mRBPs, we are screening for key regulators with essential roles in lifecycle progression or virulence using a range of bespoke techniques. A pooled, barcoded knock out library of mRBPs has been produced and challenged with *in-vitro* and *in-vivo* conditions to assess differentiation capacity, infectivity and parasite survival. Further characterisation of targets identified in this screen will include immunoprecipitation (IP) to identify and visualise target mRNAs and protein cofactors that form dynamic mRNP complexes. Visualisation of mRNP complexes will provide further functional insight. Isolation of essential gene regulatory pathways could isolate new targets for antile*ishmania*l therapeutics.

Poster 20 : Dissecting the ICL REPAIRtoire of *Trypanosoma cruzi*

Presenter: **Miss Monica Zavala Martinez**, *PhD Student, Queen Mary University of London*

M Zavala Martinez[3]; F Olmo[1]; F Caudron[2]; M C Taylor[1]; S R Wilkinson[2]

[1] London School of Hygiene and Tropical Medicine, UK; [2] Queen Mary University London, UK; [3] Queen Mary University of London, UK

Genomes are constantly challenged by agents that promote DNA damage, with interstrand crosslinks (ICLs) representing a particularly dangerous lesion. Formed when the complementary strands within the DNA double helix become covalently linked, ICLs block essential cellular processes that require strand separation and, if left unchecked, can lead to chromosomal breakage, rearrangements, or cell death. Here we show that *Trypanosoma cruzi*, the causative agent of Chagas disease, is highly susceptible to azirindyl- and nitrogen mustard-containing ICL-inducing agents, including towards several novel prodrugs developed at QMUL. In most eukaryotes the nucleases MRE11 and SNM1, and the helicase CSB function in complementary and overlapping mechanisms to repair ICL damage. To assess the role of these enzymes in *T. cruzi* we ectopically expressed GFP tagged versions of each protein and demonstrated that TcSNM1, TcMRE11 and TcCSB were all found in the nucleus throughout the parasite cell and life cycles, and that treatment with the archetypal ICL-inducing agent mechlorethamine resulted in a time-dependent increase in GFP fluorescence signal. Further, parasite lines null for TcSNM1, TcCSB, and/or TcMRE11 were generated and phenotyped with regard their susceptibility to mechlorethamine. This revealed that all three enzymes help fix ICLs and indicated that TcSNM1 functions epistatically with TcMRE11 and TcCSB to repair such damage: working is on-going to determine the interaction between TcMRE11 and TcCSB. By unravelling how *T. cruzi* repairs ICL damage specific inhibitors targeting key components of these pathways could be developed and used to increase the potency of the trypanocidal prodrugs previously identified at QMUL.

Poster 21 : The trypanocidal prodrugs benznidazole and nifurtimox promote DNA damage in Trypanosoma brucei

Presenter: **Mrs Isatou Drammeh, PhD Student**, *Queen Mary University of London*

I Drammeh[2]; S R Wilkinson[1]

[1] Queen Mary University London, UK; [2] Queen Mary University of London, UK

Benznidazole and nifurtimox represent front line treatments for treating diseases caused by trypanosomal parasites. These nitroheterocyclic compounds function as prodrugs and are converted into toxic by-products by type I nitroreductases. How the resultant metabolites then mediate their trypanocidal effects is unclear but may involve formation of DNA damage. To determine whether this is the case in *Trypanosoma brucei*, the causative agent of African trypanosomiasis, and evaluate how treatment affects protein expression, CRISPR-Cas9 was used to endogenously tag 14 genes that encode DNA repair enzymes from the homologous recombination (HR), nucleotide excision repair, base excision repair or ICL repair pathways, respectively with sequences coding for mNeonGreen. Recombinant lines were validated by PCR, the continuity of gene fusions evaluated by DNA sequencing and the localization of fusion proteins assessed by fluorescence microscopy. The change in expression of mNeonGreen tagged proteins was monitored over a 6-hour period in cells derived from benznidazole or nifurtimox-treated cultures. Our results revealed the involvement of HR factors in response to benznidazole treatment with the initial recruitment of MRE11 to the DNA damage site followed by γH2A, a DNA damage epigenetic marker analysed in parallel, and then TbRAD51. In response to nifurtimox, preliminary data shows that TbRAD51 levels do increase in response to treatment although MRE11 and/or γH2A involvement is

less clear. Our data shows that the mechanism of action benznidazole does involve formation of DNA damage in the *T. brucei* nuclear genome with the resultant lesions repaired by components belonging to the HR pathway.

Poster 22 : Long-term *Trypanosoma brucei* live-cell imaging using agarose micro-chambers identifies novel RNAi phenotypes and protein localization during cell division

Presenter: **Prof Christopher de Graffenried**, *Assistant Professor, Brown University*

C L de Graffenried[1]; R S Muniz[1]; T E Sladewski[1]; L Renner[2]

[1] Brown University, United States; [2] Leibniz Institute of Polymer Research, Germany

Long-term live cell imaging of *Trypanosoma brucei* would provide unique insights into how the parasite divides and allow the establishment of direct causal relationships between RNAi phenotypes as they emerge over time. However, *T. brucei* must remain motile to divide, which has made it difficult to observe individual cells for entire cell cycles at high spatial and temporal resolution. We have developed a live-cell imaging platform using agarose micro-chambers that allows procyclic cells to be confined in small volumes and imaged continuously for up to 36 h. We have used the chambers with a microscope that employs focal plane detection and simultaneous DIC/fluorescence imaging to observe cells dividing twice at magnifications ranging from 20X to 100X. To test this system, we have triggered RNAi against important cytokinetic regulators and identified novel phenotypic stages that were not evident in our previous fixed-cell analyses. We have also monitored duplication of cellular structures such as the hook complex and the cleavage furrow using fluorescently tagged marker proteins. Our imaging approach has the potential to address many questions about the organization and timing of cellular events in *T. brucei* that have never been studied before.

Poster 23 : Cytidine deaminase-like is an essential zinc finger protein in *Trypanosoma brucei* with RNA binding activity

Presenter: **Miss Ana Moro Bulnes**, *PhD student, Instituto Parasitología y Biomedicina López-Neyra*

A Moro-Bulnes[1]; G Pérez-Moreno[1]; S Castanys[1]; L M Ruiz-Perez[1]; D González-Pacanowska[1]

[1] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain

Enzymes of the deaminase superfamily catalyse deamination of bases in nucleotides and nucleic acids and exert different biological roles. Members that act on free nucleotides or bases, such as the cytidine deaminases (CDD/CDA) are mainly involved in pyrimidine salvage. However, other representatives of the deaminase superfamily accomplish the deamination of bases in RNA and DNA. Using an integrated sequence and structure analysis, a group of deaminases has been reported that represents a divergent branch of the CDD/CDA-like clade. This group of proteins appears to be widely distributed across numerous microbial eukaryotes namely kinetoplastids, stramenopiles, chlorophyte algae and the alveolate Perkinsus while there is no evident orthologue in mammals. The kinetoplastid versions exhibit two N-terminal CCCH Zn-finger domains and additionally present an insert of a distinct Zn-chelating domain within the deaminase domain. Since CCCH Zn fingers bind to nucleic acids, it is conceivable that these proteins perform RNA editing or DNA modifying functions. With the aim of establishing the function of this CDA-like protein in *Trypanosoma brucei*, several studies have been

accomplished. Immunofluorescence analysis revealed that the protein (TbCDA-like) is mainly located in cytosolic granules in the procyclic form of the parasite which excludes its role as a DNA-binding protein. Moreover orthogonal organic phase separation revealed that TbCDA-like is an RNA binding protein. RNAi-mediated depletion of the enzyme in procyclic forms resulted in a defective growth phenotype and impairment of the cell cycle. Likewise over-expression of the enzyme was cytotoxic thus supporting the need for a fine-tuning of the levels of the enzyme. Attempts to obtain knock-out mutants in either bloodstream or procyclic forms were unsuccessful, suggesting that TbCDA performs an important function in RNA biology.

Poster 24 : CRISPR-Cas9 gene editing in sand flies and triatomine bugs to induce inheritable refractoriness (*Leishmania* spp. and *Trypanosoma cruzi* respectively) facilitated by gene drives

Presenter: **Mr Luke Brandner-Garrod**, *PhD student, London School of Hygiene and Tropical Medicine*

L Brandner-Garrod[1]; R Edwards[1]; F Olmo[1]; L Thompson[1]; R Atherton[1]; L Bell-Sakyi[2]; M Rogers[1]; T Walker[1]; M Yeo[1]

[1] London School of Hygiene and Tropical Medicine, UK; [2] University of Liverpool/ Institute of Infection and Global Health/ Department of Infection Biology, UK

Chagas disease and leishmaniasis currently infect up to 17 million people worldwide, causing an enormous burden of mortality and morbidity. Vector control is difficult, drug regimes are imperfect, and currently, there are no human vaccines. New approaches to control are needed. CRISPR-Cas9 gene editing allows for precise genomic knockouts and also insertions of exogenous DNA. Modified traits can be introduced in a manner that targets the germline to facilitate rapid spread through the target insect population (gene drive). Two main approaches have been previously applied to anopheline mosquitoes. Firstly, the expression of antimalarial peptides, and secondly, the suppression of populations below that required for parasite transmission by reducing reproductive capacity. Both approaches have achieved remarkable results in laboratory and semi-field settings. Here we aim to develop a platform to assess, introduce, and express anti-parasitic peptides mediated through CRISPR-Cas9 gene drives applicable to sand flies and triatomine bugs. Introduced traits will spread rapidly through populations leading to a new approach for interrupting transmission in both Chagas disease and leishmaniasis. We initially developed a suite of CRISPR-Cas9 knockout constructs targeting non-lethal endogenous genes associated with phenotypic markers or refractoriness to the associated parasites. The constructs utilise a range of promoters to deliver Cas9 and gRNA directly to pre-blastoderm embryos. *In-vitro* assessment of CRISPR system components was initially performed using a range of assays and applied to embryo-derived cell lines. Additionally, we have developed a novel non-microinjection transfection approach to deliver CRISPR-Cas9 components directly to insect embryos as well as applying conventional microinjection techniques.
Transgenic homology-directed repair constructs, incorporating candidate anti-parasitic peptides and full gene drive components, will target the germline. This approach has the potential to deliver a powerful and novel platform to interrupt disease transmission applied to both *Leishmania spp.* and *Trypanosoma cruzi*.

Poster 25 : X-ray microtomography (Micro-CT) imaging as a tool for studying leishmaniasis and Chagas disease vectors.

Presenter: **Dr Luis Miguel de Pablos Torró**, *Assistant Professor, University of Granada*

L M de Pablos Torró[2]; F Morillas[3]; C I Espino[1]; A Osuna[2]

[1] Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Panamá, República de Panamá, Spain; [2] Departamento de Parasitología, Grupo de Bioquímica y Parasitología Molecular CTS-183, Universidad de Granada, Spain; [3] Department of Parasitology, Facultad de Farmacia, Universidad de Granada, Campus de Cartuja, s/n, 18011, Granada, Spain

X-ray microtomography or "micro-CT" consists in an x-ray imaging in 3D to resolve structures at small scale (micrometers) with massively increased resolution. This methodology is non-destructive and provides in-depth morphological, physiological, functional and/or evolutionary information of the biological sample. Therefore, the objectives were the development of new protocols for tissue and whole-body 3D reconstruction of parasite vectors. To create 3D reconstructions, a Xradia 5110 VERSA ZEISS X-ray microtomograph was used with the following parameters: 4x objective, 60 kV voltage an exposure time of 40 seg. 3D volumetric reconstructions and segmentation of different tissues were produced using Dragonfly (ORS) software. Examples of full body and deep tissues of *Rhodnius prolixus* and *Phlebotomus ariasi* are showed in this work. We believe that Micro-CT could be a useful tool for researchers working in either fundamental or applied biology of parasitic diseases.

Poster 26 : The TDR Targets 6 database: intensive chemogenomic data integration for drug discovery

Presenter: **Dr Fernán Agüero**, *Assistant Professor, Universidad de San Martin -- CONICET*

F Agüero[3]; L Urán Landaburu[3]; P Maru[1]; D Shanmugam[1]; A Chernomoretz[2]

[1] Biochemical Sciences Division, CSIR- National Chemical Laboratory, India; [2] Fundación Instituto Leloir, Argentina; [3] Universidad de San Martin -- CONICET, Argentina

The volume of biological, chemical and functional data deposited in the public domain is growing rapidly, thanks to next generation sequencing and highly-automated screening technologies. These datasets represent invaluable resources for drug discovery, particularly for less studied neglected disease pathogens. To leverage these datasets, smart and intensive data integration is required to guide computational inferences across diverse organisms. The TDR Targets chemogenomics resource integrates genomic data from human pathogens and model organisms along with information on bioactive compounds and their annotated activities. The new TDR Targets 6 release is based on chemogenomic network models providing links between inhibitors and targets, network-driven target prioritizations, and novel visualizations of network subgraphs displaying chemical- and target-similarity neighborhoods along with associated target-compound bioactivity links. Available data can be browsed and queried through a new user interface, that allow users to perform prioritizations of protein targets and chemical inhibitors. As such these novel features facilitate the investigation of drug repurposing against pathogen targets, which can potentially help in identifying candidate targets for bioactive compounds with previously unknown targets. In this poster presentation we will provide a walk of the new features and data in TDR Targets 6. The database is available at https://tdrtargets.org.

Poster 27 : Elucidating host and parasite factors involved in *Leishmania* quiescence

Presenter: **Miss Victoria Bolton**, *PhD, University of Glasgow*

V Bolton[1]; K Hargrave[2]; D Paape[2]; M P Barrett[3]; C Goodyear[2]; M K Macleod[2]; R Burchmore[3]

[1] University of Glasgow, UK; [2] University of Glasgow, Institute of Infection, Immunity and Inflammation, UK; [3] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

Leishmania persistence is influenced by both host and parasite factors that are incompletely understood. We wish to explore the role of quiescent, persistent parasites in subversion of a protective immune response. Estrogen-regulated Hoxb8 myeloid progenitors were differentiated to macrophages *in-vitro* and infected with *L. mexicana* over a prolonged time to model a state of persistence. To separate quiescent from actively proliferating parasites, a list of target genes associated with growth arrest in *L. mexicana* was identified. Knock out mutants were generated using CRISPR-Cas9 technology to determine their role in quiescence of *Leishmania* in our macrophage model of persistence. Using this optimised system, the activation state, cytokine profile and metabolic profile associated with *L. mexicana* quiescence in infected macrophages will be determined.

Poster 28 : The role(s) of conserved motifs within the 3' untranslated region of the variant surface glycoprotein of African trypanosomes

Presenter: **Mr Majeed Bakari Soale**, *Doctoral Student, Universität Würzburg*

M Bakari Soale[1]; H Zimmerman[1]; C Batram[1]; N Jones[1]; M Engstler[1]

[1] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany

Trypanosoma brucei utilizes a dense variant surface glycoprotein (VSG) to protect itself from host immune factors. The parasite undergoes an elaborate system of antigenic variation where it expresses only a single variant of the VSG out of a repertoire of ~2000 genes. The molecular processes and the order of events governing the expression and regulation of the VSG are not very well understood. The regulation of gene expression in trypanosomes is largely post-transcriptional. *Cis*-elements within the 3' untranslated regions (3'UTR) of most eukaryotic genes are involved in post-transcriptionally regulating genes and gene products. In *T. brucei*, a highly conserved 16mer motif within the 3'UTR of VSGs has been shown to modulate stability of VSG transcripts and hence its expression. This 16mer motif is however 100% conserved in all *T. brucei* VSGs. This unusually high conservation of a 'stability motif' led us to hypothesize that the motif is involved in other essential roles/processes besides stability of the VSG transcripts. We have demonstrated that an intact 16mer is not required for the expression of wildtype VSG levels. We have further shown that the intact motif is not required for silencing of the VSG during switching and also during differentiation from bloodstream forms to procyclic forms. Crosstalk between the VSG and procyclin during differentiation to the insect vector stage is also unaffected in 16mer mutant parasites. This study further seeks to identify and characterize interacting partners of the motif to enable us to discern the functional significance driving the surprising 100% conservation of an RNA-motif.

Poster 29 : ROS molecules as drivers of *Trypanosoma brucei* differentiation

Presenter: **Miss Michaela Kunzová**, *PhD student, Biology Centre CAS, Czech Academy of Science*

M Kunzová[1]; E Doleželová[3]; F Butter[2]; L Kortus[3]; A Zíková[3]

[1] Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic; [2] Institute of Molecular Biology, Germany; [3] Institute of Parasitology, Biology Centre, ASCR, Czech Republic

Mitochondria are dynamic organelles able to shape their metabolism and release signal molecules, e.g., radical oxygen species (ROS), to regulate cell fate decisions. During cellular differentiation of *Trypanosoma brucei* procyclic stage to the bloodstream form, the parasite undergoes a dramatic metabolic rewiring from oxidative phosphorylation to aerobic glycolysis. To shed light on the molecular mechanisms behind this metabolic switch we utilized the *in-vitro* differentiating cell line overexpressing RNA binding protein 6 (RBP6). We compared RBP6 differentiating cells with a cell line that exogenously expresses a natural ROS scavenger – catalase (RBP6_catalase). This cell line has low levels of cytosolic ROS and does not undergo differentiation. Detailed analyses of RBP6_catalase cellular proteome together with cell-based assay and morphological characteristics suggest that differentiation is blocked at early stages, i.e., before the transition from epimastigotes to metacyclic forms.

Poster 30 : Exploiting bioluminescence: fluorescence imaging to assess the replicative and differentiation status of intracellular *Trypanosoma cruzi in-vivo*

Presenter: **Dr Fernanda Costa**, *Research Fellow, London School of Hygiene and Tropical Medicine*

F C Costa[1]; F O Olmo[1]; M C Taylor[1]; J M Kelly[1]

[1] London School of Hygiene and Tropical Medicine, UK

Chagas disease is the most important parasitic infection in Latin America, affecting 6 - 8 million people. The infection is considered to be life-long and there are major limitations to current treatment options. The front-line drugs, benznidazole and nifurtimox, are toxic and their efficacy against chronic infections is unsatisfactory. The development of more effective treatment for Chagas disease has been hampered by limitations in our understanding of parasite biology. Recently, we generated a *Trypanosoma cruzi* strain expressing a bioluminescent/fluorescent fusion protein. This reporter strain enables *in-vivo* bioluminescent imaging in whole animals and facilitates detection of individual fluorescent parasites in tissue sections at a cellular level. In addition, we developed methodology for rapid CRISPR-Cas9-mediated manipulation in *T. cruzi* that enables the rapid generation of null mutant and fluorescently tagged parasites in a background where the *in-vivo* phenotype can be rapidly assessed. Using these approaches, we demonstrated that parasite replication and differentiation within individual infected host cells is asynchronous, both *in-vitro* and *in-vivo*. To explore this further, we have fluorescently tagged two developmentally-regulated genes in the same parasite: DNA topoisomerase 1A and the paralyzed flagella 16 (PF16). DNA topoisomerase 1A is a mitochondrial enzyme associated with kDNA replication, and in trypanosomes is localised at the antipodal replicative sites. PF16 gene encodes an axoneme central apparatus protein. Using highly sensitive imaging procedures, this allows us to more definitively assess parasite replication and differentiation status, and monitor flagellum morphogenesis within host cells, during both the acute and chronic stages of murine infections. This manipulated parasite line will be a valuable tool for investigating tissue- and host cell specific influences that impact on parasite replication and development.

Poster 32 : Regulating the master regulator RBP10 in *Trypanosoma brucei*

Presenter: **Ms Tania Bishola**, *PhD student, DKFZ-ZMBH Alliance*

T Bishola[2]; M Terrao[1]; C Clayton[2];
[1] CSL Behring - Marburg, Germany; [2] DKFZ-ZMBH Alliance, Germany

T. brucei RBP10 is expressed in growing bloodstream forms but not in stumpy or procyclic forms. It binds to the 3'-untranslated regions (UTRs) of procyclic-specific mRNAs and targets them for destruction. RBP10 is required for bloodstream form gene survival, and its expression in procyclic forms enables the trypanosomes to grow as bloodstream forms. Using differentiation-competent trypanosomes, we found that the developmental regulation of RBP10 expression is mediated by the 6.7 Kb 3'-UTR of the mRNA. By testing progressively shorter segments of the 3'-UTR for regulatory properties in comparison with the control actin 3'-UTR, we found that the regulatory sequences are scattered throughout the RBP10 3'-UTR. An (AU)10 motif was found to enhance translation in bloodstream forms. We are now investigating the role of different RNA-binding proteins in regulating the expression of RBP10.

Poster 33 : Identification of SAMHD1 orthologues in *Trypanosoma brucei* reveals novel features of pyrimidine dNTP homeostasis

Presenter: **Ms Miriam Yague Capilla**, *PhD , Instituto Parasitologia y Biomedicina Lopez-Ne*

M Yagüe-Capilla[1]; V M Castillo-Acosta[1]; M Valente[1]; C Bosch-Navarrete[1]; L M Ruiz-Perez[1]; D González-Pacanowska[1]

[1] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain

The preservation of genomic integrity is essential to ensure viability and proliferation of all organisms. In this context, the correct maintenance of dNTP pools plays a key role, as alterations in the nucleotide levels give rise to processes that seriously compromise cell integrity, such as genotoxicity and mutagenesis. Hence, both synthesis and degradation of nucleotides are strictly regulated by several enzymes and this area has been of great interest for the discovery of novel targets against different diseases. Whereas ribonucleotide reductase is the main enzyme involved in the *de novo* biosynthesis of deoxyribonucleotides, dNTP catabolism relies on the activity of 5'-nucleotidases, nucleoside phosphorylases and deaminases. To date, dNTPases involved in deoxynucleoside homeostasis in *T. brucei* have been poorly characterized. For this reason, and since in humans the sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1) appears to be the most relevant nucleotidase controlling dNTP pools, in this work we have aimed at characterizing trypanosomal orthologues of this enzyme. Interestingly, we have identified two HD domain containing nucleotidases in *T. brucei*. The paralogues, named TbHD82 and TbHD52, conserve all the residues involved in catalysis and substrate binding present in human SAMHD1. We present data with regard to their distinct intracellular localization and role in cell viability, as well as an analysis of the contribution of TbHD52 to the maintenance of dNTP pools and genomic integrity. Thus, we propose this class of enzymes as relevant players in nucleotide homeostasis in trypanosomes.

Poster 34 : How does the *Leishmania* Origin Recognition Complex contribute to genome replication?

Presenter: **Dr Catarina de Almeida Marques**, *Post-Doc Research Associate, University of Glasgow*

C A Marques[2]; J Damasceno[2]; M S Da Silva[1]; J M Batista[2]; R McCulloch[2]

[1] Instituto Butantan, Cell Cycle Special Lab, São Paulo, Brazil; [2] Wellcome Centre for Integrative Parasitology, University of Glasgow, UK

DNA replication is initiated at genomic loci called origins. In eukaryotes, each chromosome is replicated from multiple origins of replication to ensure their complete duplication within the S phase of the cell cycle. However, our knowledge of DNA replication is largely derived from limited sampling of the eukaryotic tree, meaning it is unclear how conserved or diverse this process is. Genome-wide mapping of origins in *Leishmania* suggest three areas of divergence: a single predominant origin in each chromosome, subtelomeric replication outside S phase, and an unusual Origin Recognition Complex (ORC). Here we describe a newly identified member of the kinetoplastid ORC that we suggest is a highly diverged ORC3 subunit. To explore how DNA replication is executed in *Leishmania*, we have generated conditional knockout mutants of the putative LmORC2 and LmORC3 subunits in L. major and analysed the resulting effects of their depletion in cell survival and DNA replication dynamics.

Poster 35 : Non-invasive monitoring of drug action: Employing high-content live cell imaging for Chagas' disease *in-vitro* drug discovery

Presenter: **Mrs Anna Fesser**, *AnnaFesser, Swiss Tropical and Public Health Institute*

A F Fesser[3]; O Braissant[4]; F Olmo[1]; J M Kelly[1]; P Mäser[3]; M Kaiser[2]

[1] London School of Hygiene and Tropical Medicine, UK; [2] Swiss Tropical and Public Health Institute, Switzerland; [3] Swiss Tropical and Public Health Institute, University of Basel, UK; [4] University of Basel, Switzerland

Trypanosoma cruzi is the causative agent of Chagas disease, the most important parasitic infection in South America. Current therapies are characterized by toxicity and limited efficacy and improved treatment regimens are urgently required. The pharmacodynamics of drug action are difficult to assess *in-vitro.* To address this problem, we have employed a green fluorescent *T. cruzi* line to develop a new approach applicable to high-content live cell imaging. Our novel assay design allows us to monitor parasite numbers for five days after drug addition, at four-hourly intervals. Exponential growth models reveal large variability in the starting number of parasites extrapolated from those models, but repeatable growth rates in untreated controls. For treated parasites, the growth rates depend on both the drug concentration and the time of drug exposure, in a drug-specific manner. Employing these exponential models, we can determine the tipping point of drug action, i.e. the time point, when the net growth rate becomes negative, for each drug concentration. This is a novel approach for determing the pharmacodynamics of drug action *in-vitro*.

Poster 36 : TriTrypDB: an advanced data mining resource for *Kinetoplastida*

Presenter: **Dr Kathryn Crouch**, *Bioinformatician, University of Glasgow*

K Crouch[1]

[1] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

TriTrypDB (https://tritrypdb.org) is a free online data mining resource supporting *Kinetoplastida*, including the human pathogens *T. brucei*, *T. cruzi* and *Leishmania* spp., as well as a wide variety of related organisms including pathogens of livestock and insects. TriTrypDB is part of the Vector and Eukaryotic Pathogen Database (VEuPathDB https://veupathdb.org) bioinformatics resource centre that integrates a diverse array of data types and provides sophisticated data mining tools for protozoan parasites, pathogenic and non-pathogenic fungi, and invertebrate vectors of pathogenic organisms. TriTrypDB offers a single portal for:

> ***Browsing***: Genomes can be browsed using an integrated JBrowse platform, where tracks showing coverage of publicly available sequencing data (DNAseq, RNAseq, ChIPseq) and locations of other genome features can be viewed in parallel. Our gene pages act as an encyclopedia of all the data we hold for each gene.
> ***Searching***: Our powerful strategies system uses an intuitive graphical platform to facilitate data mining across diverse data types including functional data (InterPro domains, GO terms, EC numbers), transcript or protein abundance, SNV profiles, orthology, associations with metabolic pathways, and much more.
> ***Annotating***: Expert community knowledge about gene function, phenotypes, cellular localisation, links to papers and much more can be added through our user comments system. Comments are immediately visible to other users and available to search. Our forthcoming Apollo service (currently in beta) is a web-based platform for editing genome annotations, supporting community annotation efforts.
> ***Data analysis***: Our Galaxy server provides a private workspace with pre-loaded genomes and sample workflows for RNAseq analysis and variant calling, as well as an array of common tools for users to create their own workflows. Results from some workflows can be transferred to the private My Data Sets area, where data can be explored using JBrowse and the strategies system and integrated with other information available in TriTrypDB.

Visit the help desk during any poster session to find out more. We have stickers! VEuPathDB is funded by NIH contract 75N93019C00077. TriTrypDB receives additional funding from the Wellcome Trust WT108443MA and Wellcome Biomedical Resources 218288/Z/19/Z. Presented on behalf of the VEuPathDB team.

Poster 37 : Minor groove binders as antitrypanosomal agents for animal African trypanosomiasis

Presenter: **Dr Federica Giordani**, *Research Assistant, University of Glasgow*

F Giordani[6]; A I Khalaf[5]; K Gillingwater[3]; F J Scott[5]; C J Suckling[5]; L J Morrison[4]; H de Koning[2]; M K Gould[6]; D McGuinness[6]; R Peter[1]; M Witty[1]; M P Barrett[6]

[1] Global Alliance for Livestock Veterinary Medicine, UK; [2] Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [3] Swiss Tropical and Public Health Institute, Switzerland; [4] The Roslin Institute,

University of Edinburgh, UK; [5] University of Strathclyde, UK; [6] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK

Control of the livestock disease nagana (animal African trypanosomiasis, or AAT) relies on the use of a handful of trypanocides, whose efficacy is jeopardized by the recurrent problem of drug resistance. In the search for new compounds, we have developed a class of minor groove binders (Strathclyde minor groove binders, S-MGBs) active *in-vivo* against the major veterinary trypanosomes causing AAT: *Trypanosoma congolense* and *T. vivax*. Selection of resistance to these compounds *in-vitro* proved unsuccessful. However, a RIT-seq analysis in *T. brucei* allowed the identification of nuclear genes potentially associated with increased resistance to a compound belonging to this class. Disruption by CRISPR-Cas9 of one of these genes – a class I transcription factor subunit, allowed generation of S-MGB resistant trypanosomes, albeit levels of resistance remained low, substantiating RNAi data. Development of resistance to the S-MGBs might be hampered by their targeting multiple DNA sites, hence making parasite adaptation unlikely.

Poster 38 : Study of mesenchymal stromal cells as an alternative therapy for cutaneous leishmaniasis

Presenter: **Mr Tadeu Diniz Ramos**, *PhD student, Federal University of Rio de Janeiro*

T Diniz Ramos[1]; J D Silva[1]; P M Rocco[1]; H L de Matos Guedes[1]

[1] Federal University of Rio de Janeiro, Brazil

Cell therapy using stem cells can have several goals, among them, to re-establish the homeostasis of the immune system by taking advantage of the immune properties of these cells. One of the most promising cell types are the mesenchymal stromal cells (MSC), which are multipotent adult stem cells present in almost all tissues. *Leishmania*sis is a disease caused by protozoa of the genus *Leishmania*. One of the characteristics of the disease is an imbalance of host immune responses to foster parasite survival. In a previous work, was observed that use of bone marrow-derived mesenchymal stromal cells (BM-MSCs) in a *L. amazonensis*-infected BALB/c mouse model worsens the pathology by enabling increased parasitic loads. In this work, we evaluated BM-MSCs as a treatment in the C57BL/6 (B6) mouse model, which presents a partially resistant profile, which resembles the human profile, whereas BALB/c mice have a profile of susceptibility to this disease. And in addition, we have also evaluated the treatment with adipose tissue-derived mesenchymal stromal cells (AD-MSCs), because despite similar phenotypic characteristics, MSCs derived from different tissues express and stimulate factors in different proportions. Co-culturing *L. amazonensis*-infected B6 peritoneal macrophages with BM-MSCs led to a higher parasitic load and lower production of NO. However this was not observed when cells were co-cultured with AD-MSCs. Treating infected-B6 mice with BM-MSCs had no significant differences on the parasite control, however, the use of AD-MSCs showed partial control of lesion development, without differences in the parasitic load. Furthermore, fibroblasts cultivated with conditioned medium from a culture of infected macrophages with conditioned medium from AD- MSCs promoted faster wound healing. Despite a non-significant difference in the production of VEGF, we observed a higher production of TNF- and IL-10 in the co-culture of macrophages and AD-MSCs. Based on this data, we combined cellular therapy with the conventional pentavalent antimonial chemotherapy to treat infected B6 mice. We observed that the mice that received the combined treatment showed a reduced lesion size and at 52 days of infection, a lower parasitic load was found, when compared to the other groups. At the site of infection we detected a small production of IL-10, but we were not able to detect the production of either IL-4 or IFN-γ indicating a high resolution of the infection without effect

in the percentage of regulatory T cells. These results suggest the combined treatment using AD-MSCs and pentavalent antimonials as an alternative treatment to cutaneous leishmaniasis.

Poster 39 : Reductive evolution of dual-localised polynucleotide kinase phosphatase function within trypanosomatid parasites

Presenter: **Dr Sarah Allinson**, *Senior Lecturer, Lancaster University*

D M Kania[1]; M L Ginger[2]; **S L Allinson**[1]

[1] Lancaster University, Biomedical and Life Sciences, UK; [2] University of Huddersfield, UK

A critical function for any organism is the ability to maintain genetic integrity in the face of the inherent chemical instability of DNA. Breaks in the phosphodiester backbone can have particularly devastating consequences for cells if they remain unrepaired and for this reason, and the chemical diversity of break ends, all organisms characterised to date possess a suite of enzymes dedicated to processing this type of damage. Polynucleotide kinase phosphatase (PNKP) is one such enzyme that has been extensively characterised in mammals. PNKP is bifunctional, with both phosphatase and kinase domains that enable it to process both 5'-hydroxyl and 3'-phosphates to yield ends suitable for the subsequent completion of repair. Here we report our characterisation of TbPNKP, a homologue of PNKP found in *Trypanosoma brucei*. We demonstrate that TbPNKP possesses DNA repair activities and that genetic knockouts of the *TbPNKP* gene are hypersensitive to DNA damaging agents. Using a combination of bioinformatic analysis and cell extract assays we have further characterised the 3'-phosphate and 5'-hydroxyl processing capabilities of a range of trypanosomatids. We find that PNKP functionality has been lost in a step-wise manner in the *Leishmania* lineage, with evidence of the presence of monofunctional 3'-phosphatases in some species and complete loss of PNKP in others. We speculate how the loss of PNKP activity could be compensated for in these organisms.

Poster 40 : mRNA decapping by an ApaH-like phosphatase in trypanosomes

Presenter: **Miss Paula Castaneda**, *PhD student, Wurzburg University*

P A Castañeda-Londoño[2]; N Banholzer[2]; C M do Nascimento Moreira[2]; N Zhang[1]; M Zoltner[5]; M C Field[1]; F B Holetz[3]; B Dallagiovanna[3]; N Karolak[4]; M W Górna[4]; **S Kramer**[2];

[1] School of Life Sciences, University of Dundee, UK; [2] Biozentrum, Lehrstuhl für Zell-und Entwicklungsbiologie, Universität Würzburg, Germany; [3] Instituto Carlos Chagas, Fundação Oswaldo Cruz-Fiocruz, Curitiba, Brazil; [4] Biological and Chemical Research Centre, University of Warsaw, Poland; [5] Faculty of Science, Charles University, BIOCEV, Prague, UK

5' to 3' decay is the major mRNA degradation pathway in many organisms, including trypanosomes. First, the m^7G cap is removed by the nudix domain hydrolase Dcp2, which is part of a larger decapping complex. Trypanosomes lack homologs to all decapping complex proteins and we have recently identified an ApaH-like phosphatase (TbALPH1) as the major mRNA decapping enzyme of trypanosomes. In vitro, TbALPH1 has mRNA decapping activity in a wide range of conditions without cap-type preference and surprisingly, largely independent on its C and N terminal domains that embed the catalytic domain. In vivo, these C- and N-terminal extensions determine enzyme localisation and protein interactions, likely regulating enzyme specificity. Even though ApaH-

like phosphatases are present in all eukaryotic super-groups, our phylogenetic studies strongly suggest that their usage in mRNA decapping is unique to kinetoplastida.

Poster 41 : Assessing the *Leishmania* and *Trypanosoma cruzi* sphingolipid biosynthetic enzymes as novel drug targets

Presenter: **Dr Gabriela Burle-Caldas**, *Post-Doctoral Research Associate, Durham University*

G A Burle-Caldas[1]; S N Smith[1]; E A Alpizar-Sosa[1]; L N Filipe[1]; C C Briggs[1]; N Jones[4]; C F Estevez Castro[2]; D F Chame[2]; J Mottram[4]; E Gluenz[3]; P Denny[1]

[1] Durham University, UK; [2] Federal University of Minas Gerais, Brazil; [3] University of Oxford, UK; [4] University of York, UK

*Leishmania*sis and Chagas Disease are Neglected Tropical Diseases that affects millions of the world's poorest. Vaccines are absent and drug therapy is limited and often ineffective. Sphingolipids (SphLs) are an integral part of all eukaryotic cellular membranes and have indispensable functions as signalling molecules. In addition, they have important roles in controlling a myriad of cellular events. Disruption of either the *de novo* synthesis or the degradation pathways has been shown to have detrimental effects on cell viability. The identification of selective inhibitors of fungal SphL biosynthesis promised potent broad-spectrum anti-fungal agents, which later encouraged testing some of those agents against protozoan parasites. In this work we seek to analyze and validate SphL biosynthesis as a potential drug target in *Leishmania mexicana* and *Trypanosoma cruzi,* the causative agents of the leishmaniasis and Chagas disease respectively. To accelerate this work, we performed a high throughput CRISPR/Cas9 screen in an attempt to knockout a total of 46 genes involved in SphL, as well as glycerophospholipid and sterol, biosynthesis. Twenty-one of these were indicated to be essential, including inositol phosphorylceramide synthase (IPCS) which catalyses the production of the primary yeast and kinetoplastid complex SphL - IPC. However, deletion of IPCS and ceramide synthase ([CerS] - key in the production of the core SphL - ceramide) from *L. mexicana* was achieved in repeat experiments. Subsequent results indicated that knockout of these enzymes did not affect promastigote replication. However, compared to the parental cell line, both, displayed rounded morphology and an impaired capacity to differentiate into amastigotes. Knockout of *T. cruzi* IPCS similarly impaired the capacity of epimastigotes to differentiate into trypomastigotes. To enable further analyses of these potential targets, the production of active enzyme is required. However, expression and analyses of enzymes involved in the SphL biosynthetic pathway is challenging due to their hydrophobic transmembrane nature. Therefore, in this work, we are using a cell free system designed for *in-vitro* expression of active polytopic membrane proteins, to produce recombinant IPCS and CerS from *L. mexicana* and *T. cruzi.* We have optimized the production of *L. mexicana* IPCS and we are currently optimizing expression of IPCS and CerS from *T. cruzi* and CerS from *L. mexicana.* These will form the basis of assays to study function and inhibition of these novel potential drug targets.

Poster 42 : Screening microbial extracts for novel antimalarial and anti-Chagas natural compounds

Presenter: **Mrs. Cristina Bosch**, *Researcher, Instituto de Parasitología y Biomedicina López-Neyra (CSIC)*

C Bosch-Navarrete[2]; G Pérez-Moreno[2]; F ANNANG[1]; J Martín[1]; I Pérez-Victoria[1]; L M Ruiz-Perez[2]; O Genilloud[1]; F Reyes[1]; F Vicente[1]; D González-Pacanowska[2]

[1] Fundación MEDINA, Spain; [2] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain

The combined risk population of Malaria and Chagas disease is about 4 billion people. In 2018, there were 228 million cases of malaria worldwide while for Chagas disease more than 6 million were estimated to be infected. Estimated deaths for malaria were approximately 405,000 in 2018. Despite the huge resources available, parasite drug resistance is a recurring problem in the fight against these diseases. For treatment of malaria the delivery of new, effective and affordable drugs is a long-standing priority especially for use in small children and during pregnancy. In the case of Chagas' disease there is an urgent need for effective chronic stage drugs with uncomplicated administration. To address these challenges, the identification of novel first-in-class compounds is needed. Microbial extracts present a very rich source of novel chemistry for excellent first-in-class drugs or templates for the synthesis of novel, biologically active molecules. We have established a malaria/Chagas disease drug discovery platform at MEDINA/IPBLN-CSIC. Using this facility we have recently screened a subset (14,400) of Fundación MEDINA´s over 200,000 microbial extracts collection. We report here our robust screening strategy and its huge potential for the discovery of novel drugs against these two diseases.

Poster 43 : Towards CRISPR-Cas9 mediated control strategies for *Lutzomyia longipalpis* through genetic modification of olfaction.

Presenter: **Mr Rhodri Edwards**, *PhD student, London School of Hygiene and Tropical Medicine*

R Edwards[3]; L Brandner-Garrod[3]; L Thompson[3]; C McMeniman[2]; F Olmo[3]; L Bell-Sakyi[1]; M Rogers[3]; T Walker[3]; M Yeo[3]

[1] Infection Biology, Institute of Infection and Global Health, University of Liverpool, UK; [2] John Hopkins Bloomberg School of Public Health, United States; [3] London School of Hygiene and Tropical Medicine, UK

Current control strategies for leishmaniasis do not provide a panacea, and the development of new approaches for disease control are required. CRISPR-Cas9 is a new and highly targeted method to manipulate genomes in a manner not previously possible. The CRISPR-Cas9 system for genetic modification of insects, in an inheritable manner, through the development of gene drives has been achieved in Anopheline and Culicine vectors using two main approaches; either the manipulation of reproductive capacities resulting in population suppression, or the expression of anti-pathogen effector molecules peptides in vectors of malaria parasites, yellow fever virus, and West Nile virus with the aim of interrupting transmission. Here we apply a novel approach through CRISPR-Cas9 genetic modification, to disrupt olfaction and affect host seeking behaviours. Induced modifications will target the germline in an inheritable manner through development of CRISPR-Cas9 gene drive to enable rapid spread of the disrupted olfactory traits through sand fly populations. For this approach a suite of guide RNAs and plasmid constructs selectively target (via knockout) non-lethal phenotypic and olfactory targets in *Lutzomyia longipalpis*. Initial *in-vitro* verification of CRIPSR-Cas9 components and constructs is undertaken and validated within novel sand fly cell lines. Selected CRISPR-Cas9 components are subsequently introduced into sand fly embryos using an inhouse microinjection platform, and a novel non-microinjection plasmid delivery system. Behavioural bioassays on emergent transgenic insects will be applied to determine the effects of the resultant targeted knockouts. Lastly, more refined homology directed repair CRISPR-Cas9 constructs containing full gene drive components will induce expression of endogenous DNA and modified traits through the germline, and

facilitate super-Mendelian inheritance. The work described here has the potential to contribute towards new control strategies for the vectors of leishmaniasis by interrupting transmission and reducing the burden of human suffering.

Poster 44 : Kinetoplastid pantothenate kinase is an essential multi-domain protein required for Coenzyme A synthesis

Presenter: **Miss Rebecca Roscoe**, *Postgraduate student, London School of Hygiene and Tropical Medicine*

R Roscoe[1]; F C Costa[1]; F Olmo[1]; J M Kelly[1]; M Taylor[1]

[1] London School of Hygiene and Tropical Medicine, UK

Chagas disease, caused by the parasite *Trypanosoma cruzi* continues to pose a major public health problem in South and Central America and with migration is becoming a global concern. Thousands of deaths occur each year as a result of cardiac and digestive pathology induced by the parasite.
In order to develop new, safer therapies, a better understanding of the biochemical pathways essential to parasite survival is necessary. One such critical molecular pathway is the synthesis of the cofactor Coenzyme A from vitamin B5 or pantothenate. This occurs in five steps, the first of which involves the phosphorylation of pantothenate by pantothenate kinase (PanK), forming 4'-phosphopantothenate. In kinetoplastids, this enzyme is structurally unique in comparison to mammals and other eukaryotes, as it contains two N-terminally fused domains, putatively identified as phosphatase and fatty acyl-CoA synthetase domains by orthology. We have used gene-editing techniques to investigate the role of this protein in growth and survival of *T. cruzi.* CRISPR-Cas9 mediated tagging of the N- or C- terminal showed that the protein was expressed as a single polypeptide comprised of all three domains. Gene deletion using this technology was unsuccessful, suggesting that at least one activity of the protein was essential in epimastigotes. Depletion of PanK by RNA interference (RNAi) in *T. brucei* indicated that the protein is essential in these parasites. The RNAi phenotype could be complemented by expression of the *T. cruzi* orthologue. Further analyses combining inducible knockdown with site-directed mutagenesis suggested that the conserved Arg-1270 in the pantothenate kinase domain of TcPANK is critical to protein function implying that the PanK domain was essential. However, the conserved Ser-1254 was found to be non-essential for complementation. Future work will determine the biological and enzymatic role of kinetoplastid-specific domain fusions within the enzyme and inform on its suitability as a drug target.

Poster 45 : TARBRAINFEC: Nanosystems conjugated with antibody fragments to target/treat brain infections

Presenter: **Dr Jose A García-Salcedo**, *Group leader, FIBAO*

J A García-Salcedo[4]; L Peng[1]; S Czarnocka-Śniadała[5]; A Alcami[2]; M Bhide[3]; A Tsatsakis[6]; M Ortiz-González[4]

[1] Centre Interdisciplinaire de Nanoscience de Marseille, (CNRS), France; [2] Centro de Biologia Molecular Severo Ochoa, (CSIC), Spain; [3] Institute of Neuroimmunology, (SAS), Slovakia; [4] Instituto de Investigación Biosanitaria de Granada, Ibs. Granada, Spain; [5] Nanosanguis, Poland; [6] University of Crete, Greece

Infectious diseases affecting the central nervous system (CNS) remain an important source of morbidity and mortality. A major obstacle for curing brain diseases is the blood-brain barrier (BBB), which impedes therapeutic

agents to reach the brain and target the related pathogens. In this project, we want to develop a drug delivery nanosystem coated with antibody fragments, called nanobodies (Nbs) as a proof of concept for targeting brain infections caused by bacteria, virus and parasite. These Nbs against neurotropic pathogens also carry the transferrin receptor ligand which can overcome BBB via the receptor-mediated transcytosis. To do this, we will first generate nanobodies (Nbs) against neurotropic pathogens using two bacterial (Neisseria meningitides and Borrelia burgdorferi), herpes simplex virus (HSV) and the protozoan parasite *Trypanosoma brucei* as the model systems. These Nbs will be further engineered to bear the transferrin receptor ligand for crossing BBB. The so-created Nbs will be then conjugated to the drug-loaded nanoparticles constructed using polymer or dendrimer nanovectors. The obtained nanodrug candidates will be characterized for their size, morphology, surface charge and stability as well as drug loading and release profile etc. The ability of these nanosystems to across BBB will be assessed using an *in-vitro* model of BBB, and their preclinical safety and biological activity against the neurotropic pathogens N. meningitides, B. burgdorferi, herpes simplex virus (HSV) and *T. brucei* will be assessed *in-vitro* using cell based experiments and *in-vivo* using animal models. The success of this project will validate the proof-of-concept study to combine the nanobody technology with the nanotechnology based drug delivery for effectively overcoming BBB and targeting pathogens in brain infections. We expect to generate, in this project, clinical useful pilot results for the best performing candidates for future translation, and at the same time, research data of general scientific interest useful to the broad scientific community.

Poster 46 : Global binding profiles of *Leishmania major* RPA1, HUS1 and yH2A in response to replication stress

Presenter: **Dr Stela Virgilio**, *Postdoctoral researcher, Universidade de São Paulo - USP*

S Virgilio[1]; J A Black[2]; M S Bastos[1]; G Almeida da Silva[1]; K Crouch[2]; J D Damasceno[2]; R McCulloch[2]; L R Tosi[1]

[1] University of São Paulo, Brazil; [2] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

The eukaryotic ATR signaling pathway orchestrates the cellular response to a wide variety of DNA injuries, including double strand breaks and replicative stress. Accumulation of single-stranded DNA causes ATR recruitment and activation, leading to the stalling of cell cycle progression, protection of DNA replication forks and recruitment of DNA repair activities through phosphorylation of downstream factors, such as Chk1 and H2A(X). The tripartite complexes RPA (RPA1-RPA2-RPA3) and 9-1-1 (RAD9-RAD1-HUS1) have central roles in the ATR pathway. To understand the interplay of these factors, and uncover the ATR pathway, in *Leishmania*, we have mapped RPA1, HUS1 and yH2A enrichment in response to replication stress using chromatin immunoprecipitation followed by sequencing (ChIP-seq). RPA1 and HUS1 were most clearly enriched at intergenic sequences within transcription units, at transcription start and termination sites, and at subtelomeres, suggesting these are locations of replication stress or instability in wild-type cells. Addition of high levels of hydroxyurea, which stalls DNA replication, dramatically altered RPA1 localisation, with highly elongated accumulation around the single mapped origin in each chromosome and at the telomeres. Lower levels of hydroxyurea primarily resulted in increased RPA1 signal around transcription start and termination sites. Together, these data suggest these loci to be the major locations of interaction between the DNA replication and transcription machineries. Localisation of yH2A largely recapitulated the enrichment of RPA1 and HUS1, perhaps indicating repair activities may be recruited to these potentially fragile genomic regions. An important question is how ATR contributes to the orchestration of these processes. Supported by: FAPESP. Keywords: RPA, ChIP-seq, replication, transcription

Poster 47 : Histones and their acetylation in African trypanosomes

Presenter: **Miss Marketa Novotna**, *PhD student, University of Dundee*

M Novotna[2]; J Kovarova[1]; C L Wallace[2]; M Zoltner[1]; M Field[1]; J Faria[1]; D Horn[1]

[1] School of Life Sciences, University of Dundee, UK; [2] University of Dundee, UK

Chromatin modification is important for virtually all aspects of DNA metabolism, but relatively little is known about these connections in trypanosomatids, which have highly divergent histone tail sequences. *T. brucei* HAT2 is an essential MYST-family histone acetyltransferase (a writer) responsible for H4K10 acetylation at transcription initiation sites, while DAC3 is an essential histone deacetylase (an eraser). We aim to understand how such factors control gene expression and other chromatin-based functions. We used Cas9-based editing to precisely GFP-tag each gene. Microscopy revealed a punctate nuclear distribution in each case, while affinity purification followed by mass-spectrometry revealed associations with specific histone variants and bromodomain factors (readers). We now aim to develop strains with just one copy of selected histone genes to facilitate histone-tail mutagenesis, such that the roles of specific histone modifications and modifier complexes can be understood.

Poster 48 : Same parasite - different coats: A case of surface functionality transfer between trypanosome species

Presenter: **Mr. Erick Aroko**, *Doctoral student, Universität Würzburg*

E Aroko[1]; N Jones[1]; M Engstler[1]

[1] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany

Despite the many decades of research on African trypanosomes, studies on the major pathogens responsible for animal African trypanosomiasis, *Trypanosoma vivax* and *Trypanosoma congolense,* has been lagging behind compared to their sister parasite *Trypanosoma brucei* . Though they share a common ancestry, these parasites have evolved to differ in tropism, motility, morphology and composition of their cell-surface proteome. As the trypanosome bloodstream forms (BSF) are exclusively extracellular, their cell surfaces form a critical interface between the parasite and the host. The parasites use their surface for evasion of the host immune responses and acquisition of nutrients and host-derived macromolecules. Approximately, 10 million copies of an immunogenic glycosylphosphatidylinositol (GPI)-anchored protein, the variant surface glycoprotein (VSG), cover the entire BSF *T. brucei* surface. Although *T. brucei* VSG sequences share minimal similarities, their tertiary structure appears highly conserved. *T. brucei* VSGs are 50-60 kDa proteins, consisting of an elongated N-terminal domain that is important for antigenic variation and a shorter C-terminal domain that confers flexibility to the protein. *T. vivax* and *T. congolense* VSGs also harbour an N-terminal domain, however, they appear to lack the structured regions present in the C-terminal domain of *T. brucei* VSGs. *T. vivax* VSGs are smaller (42-50 kDa) and have been suggested form a less dense coat, probably due to the presence of an extended repertoire of predicted non-VSG surface proteins.
Here we have used the tractable *T. brucei* system as a platform for forward cell surface engineering and comparative analysis of surface coats of African trypanosomes. Whereas a *T. congolense* VSG was readily expressed in *T. brucei ,* a *T. vivax* VSG could only be expressed after modification of its C-terminal signal peptide. A second *T. vivax* VSG could not be expressed in *T. brucei ,* despite similar modifications to its signal

peptide. The *T. brucei* model we have established opens avenues to address fundamental questions; for example, what is the fate of the invariant surface glycoproteins (ISGs) in the presumably shorter trans-species VSG coats? How do different surface components influence VSG diffusion? Can the expression of surface proteins such as *T. congolense* trans-sialidases provide a gain of function to the *T. brucei* VSG coat?

Poster 49 : Overexpression of RNA Binding Protein 5 (RBP5) blocks cell cycle progression in bloodstream form trypanosomes.

Presenter: **Miss Carla Gilabert Carbajo**, *Research Technician, Imperial College London*

C Gilabert Carbajo[1]; L Cornell[1]; J D Bangs[2]; C Tiengwe[1]

[1] Imperial College London, UK; [2] University at Buffalo (SUNY), United States

In eukaryotic cells, RNA binding proteins (RBP) play essential roles in regulating mRNA abundance and fate. This is especially important for Kinetoplastid parasites since gene expression is mostly regulated post-transcriptionally. In African trypanosomes, RBPs (e.g. RBP6, RBP10) have been implicated as key modulators of developmental transition, but very little is known about their role in cell cycle progression. Recently, a quantitative phosphoproteome study identified a number of differentially phosphorylated RBPs suggesting putative functions in regulating the trypanosome cell cycle (Benz et al. PLoS Pathog 15(12): e1008129), but their precise role and mRNA targets remain unexplored. In an attempt to identify trans-acting regulators of transferrin receptor expression, we found by transcriptomics that TbRBP5, an uncharacterised RBP was upregulated in iron-starved cells. TbRBP5 has a single RNA-recognition motif and initial characterisation suggests that it may regulate cell cycle progression in bloodstream form trypanosomes. We epitope-tagged both alleles and show by subcellular fractionation and immunofluorescence that it is cytosolic. Overexpression of Ty-tagged TbRBP5 leads to rapid cell death within 24 hrs and accumulation of cells with precisely 2 nuclei and 2 kinetoplasts without cytokinesis initiation, suggesting that excess TbRBP5 disrupts proper mitotic to cytokinesis transition. Alternatively, overexpression of wild type (WT) TbRBP5 generates an identical but less severe phenotype. The cell cycle defect observed with TbRBP5-Ty or WT overexpression is similar to that reported for VSG knockdown, albeit more severe and more precise. Taken together, these results suggest that levels of TbRBP5 may be tightly regulated, and overexpression may disrupt the mRNA levels of key cell cycle regulators. Whole genomic approaches are underway to identify and characterise TbRBP5 mRNA targets.

Poster 50 : Reduction of susceptibility to Amphotericin B in promastigotes of *Leishmania* braziliensis exposed *in-vitro* to miltefosine.

Presenter: **Dr Maria Echeverry**, *Associate Professor, Universidad Nacional de Colombia*

M Echeverry[3]; M Castaño[3]; S Aponte[1]; C Ovalle-Bracho[2]; C Franco[1]; C H Saavedra[3]

[1] Centro Dermatológico Federio LLeras Acosta, Colombia; [2] Pontificia Universidad Javeriana, Colombia; [3] Universidad Nacional de Colombia, Colombia

Cutaneous leishmaniasis (CL) is still a serious Public Health problem in South America with not many treatment options. First line treatment is provided mainly by antimony derivates, whilst second line treatment varies according to local recommendations, being the most common: pentamindine, miltefosine (Milt), and amphotericin B (AmB). The last two medicaments target molecules associated to the cell membrane structure in the parasite

by mechanisms not totally understood. *Leishmania braziliensis* (*L.b*) is the predominant species producing CL in the region and is intrinsically less susceptible to Milt. The present study was aimed to study the emergence of cross-resistance between Milt and AmB in *L.b* parasites exposed to Milt. Induction of resistance was achieved by stepwise exposure to Milt in clones of *L.b* promastigotes (MHOM/BR/75/M2903). Milt and AmB effective concentration 50 (EC_{50}) against induced-resistant clones and clinical isolates *in-vivo* exposed to Milt, were assessed by colorimetric assays. RTq-PCR was performed to determine mRNA levels of lathoesterol oxidase(LO), sterol methyltransferase (SMT), miltefosine transporter (MiT) and putative Qc-SNARE protein (VAMP) in resistant clones and to compare with un-induced clone. Expression values were determined through the ΔΔCq method using the Gene Study module of the software CFX manager TM version 3.0. Loss of fitness in the "resistant" parasites was evaluated by growth curves and *in-vitro* infectivity. Milt-resistance induction was achieved after 4 months of continuous culture obtaining four clones of *L.b* showing 2.8 to 4.9 fold increase in the Milt EC_{50} (Milt EC_{50} in µM for un-induced clone: 29.17 and for induced clone 1: 84.1, clone 2: 121.2, Clone 3: 143.6 and clone 4: 123.7). These clones also exhibited a change in AmB susceptibility displaying 2 to 3.5 fold increase in the AmB EC_{50} (AmB EC_{50} in ng/ml for un-induced clone: 44, and for induced clone 1: 157.3, clone 2: 159.4, Clone 3:128.2 and clone 4: 90.2). Clones 3 and 4 showed minor loss of fitness as observed by slight slowdown in growth. When comparing with an un-induced clone the four resistant clones presented lower mRNA levels of LO; clones 1, 2 and 3 showed lower mRNA levels of SMT; clones 1 and 2 exhibited down-regulation of VAMP, and clone 1 showed lower mRNA levels of MiT. Milt EC_{50} against *L.b* promastigotes isolated from CL patients (n≤4) that received Milt and did not respond to treatment, showed no differences with the Milt EC_{50} observed in the *L.b* reference strain (MHOM/BR/75/M2903). Interestingly one of those clinical isolates showed a moderate but significant increase in AmB EC_{50} when compared with the reference strain. The present work suggests that cross-resistance for AmB-Milt may arise in *L.b* and that further studies are necessary to understand the two drugs

Poster 51 : Mechanisms of selective translation stimulation and suppression by the multiple eIF4E isoforms of *Trypanosoma brucei*

Presenter: **Prof Christine Clayton**, *Professor, ZMBH*

F Egler[2]; O de Melo Neto[1]; **C Clayton**[2]

[1] Fundação Oswaldo Cruz Recife, Brazil; [2] ZMBH - Heidelberg University, Germany

Trypanosoma brucei has six isoforms of the cap-binding translation initiation factor eIF4E, and five eiF4Gs. EIF4E1 interacts with 4EIP, which suppresses translation and provokes mRNA degradation. 4EIP is required for translation suppression in stumpy forms (PMID: 30124912). EIF4E1, but not 4EIP, is required for survival of procyclic forms. The EIF4E1-4EIP complex recruits a cystosolic terminal uridylyltransferase 3 (TUT3). EIF4E3, 4, 5 and 6 all stimulate expression when tethered, suggesting that they promote translation initiation. EIF4E3 and EIF4E4 may be general translation factors (PMID: 29077018). EIF4E6 interacts with EIF4G5, and is also associated with the stimulatory complex of MKT1, PBP1, XAC1 and LSM12. The MKT complex (PMID:24470144) is recruited to mRNAs via sequence-specific RNA-binding proteins, which include ZC3H11, ZC3H20 and the VSG-mRNA-associated protein CFB2

Poster 52 : Discovery and optimization of cruzain inhibitors using docking and virtual screening

Presenter: **Miss Viviane Correa Santos**, *PhD student, Universidade Federal de Minas Gerais*

V C Santos[2]; P Kolb[1]; R S Ferreira[2]

[1] Philipps-Universität Marburg, Germany; [2] Universidade Federal de Minas Gerais, Brazil

Cruzain is the main cysteine protease of *Trypanosoma cruzi*. It is a validated target for drug development against this parasite. It is associated with evasion of the immune system response, invasion of host cells, and parasite cell differentiation. Herein, we describe the identification of a cruzain inhibitor and the design of its analogues. To do so, we used virtual screening and docking. We screened 372,632 "leads now" molecules from the ZINC12 database. We prepared these molecules with the program Epik at pH 5.5. We prepared the crystal structure of cruzain (PBD ID: 3KKU) using the Protein Preparation Wizard from Schrödinger. Two grids were prepared to vary the protonation states of three residues in the active site: CYS25, HIS162, and GLU208. We did the docking with the program Glide. We used two levels of precision in our docking calculations. First, the molecules were submitted to HTVS docking. We ranked the predicted poses according to the docking score, and we submitted the top 10% to docking SP in Glide. It is possible to compare the docking generated poses with crystallographic structures. We can extract patterns from the crystallographic poses and compare them with docking results. It is interesting because, in the crystals, we can observe the interactions between small-molecule and receptor, which allows us to search them in the predicted poses. By doing so, we can increase the likelihood of this pose to be correct. When we analyzed the crystallographic cruzain inhibitors, we verified the most frequent interactions they were involved in. Then, we selected between SP docking results in which the molecules were making these interactions. After a visual inspection, we selected 35 molecules that were filtered based on some characteristics: water solubility, measured by clogS; hydrophobicity (clogP); chiral center count (maximum one); and we also applied a PAINS filter, that verifies whether the molecule has chemical groups commonly founded to interfere in the enzymatic assays leading to false-positive results. Aided by the docking results, we bought 17 molecules for the *in-vitro assays.* From those, we discovered one competitive cruzain inhibitor. It has an IC50 ≤ 3.09 μM ± 1.6 and a Ki ≤ 4.68 μM. This compound can be synthesized by a condensation reaction between an amine and a carboxylic acid. Based on this information, we designed analogues of this active compound. We found 129 amines and 291 carboxylic acids with a similarity of 0.8 from the active compound in the ZINC15 database. We combined them with the program Reactor; this program simulates chemical reactions. We obtained 30,952 analogues. After filtering them according to the lead-like properties and chiral atom count, 9,296 molecules were docked with DOCK3.7. Our goal now is to inspect the docking results and select compounds for synthesis. The synthesized compounds will be evaluated in enzymatic assays with cruzain.

Poster 53 : An RNA-binding protein complex regulates the purine-dependent expression of the *Trypanosoma brucei* NT8 nucleobase transporter

Presenter: **Dr Antonio M Estevez**, *Staff Scientist, IPBLN-CSIC*

M Rico-Jiménez[1]; G Ceballos-Pérez[1]; C Gómez-Liñán[1]; A M Estévez[1]

[1] IPBLN-CSIC, Spain

We have previously characterized a short RNA stem-loop *cis*-element within the 3'-UTR of the *NT8* nucleobase transporter mRNA (PuRE, Purine Responsive Element) that is necessary and sufficient to confer a strong repression of gene expression in response to purines. In this study, we have identified a protein complex composed of two RNA-binding proteins (PuREBP1 and PuREBP2) that binds to the PuRE *in-vitro* and

to *NT8* mRNA *in-vivo*. Depletion of PuREBP1 by RNAi results in the upregulation of just *NT8* and the mRNAs encoding the amino acid transporter AATP6 paralogues. Moreover, we found that the PuREBP1/2 complex is associated with only a handful of mRNAs, and that it is responsible for the observed purine-dependent regulation of NT8 expression.

Poster 54 : Rate of Kill (RoK) experiments to find new drug combinations against *Trypanosoma cruzi*

Presenter: **Mr Juan Cantizani**, *Scientist, GSK*

J Cantizani[1]

[1] GSK, Spain

Drug combination is a widely used for many therapeutic areas including oncology, immunology or antibacterial and antiretroviral therapies. Likewise, it could also be an approach against *Trypanosoma cruzi*. Benznidazole has been shown to be an effective clinical drug but has adverse side effects, poor patience compliance, need a prolonged treatment and is not always efficacious in the chronic stage. Finding active molecules that could work as monotherapy is highly challenging given the limited resources, the poor knowledge of different aspects of the disease and the parasite complexity. All these reasons make the drug combination a good approach to increase the success of the different programs. We envisage a Target Product profile for a new chemical entity which may shorten the length of treatment or the dose of benznidazole in combination, thus improving the safety profile whilst keeping the therapeutic efficacy. In classical parasite *in-vitro* growth assays, low parasite concentrations are used, but do not allow too discern whether a compound is cidal or static. Our goal is to identify cidal compounds with fast speed of action that eradicates the parasite from culture and thus halts relapse. Classical approach to combinations is based on simple IC50 calculations. However, Rate of Kill assays look at the mechanistic mode of action more holistically. This communication presents an enhancer compound whose use is capable of decreasing the dose of benznidazole up to 5 times and increase the speed of action *in-vitro*. Interestingly, similar doses of either compound or benznidazole alone are not able to reduce parasite burden. This result correlates with *in-vivo* experiments in a chronic model validating the enhancer effect. Based on these results, a screening methodology is described to search for benznidazole enhancers. Some of the advantages are that can be applicable to any other molecule of interest, have a high throughput, discriminates new mode of action and give faster results to find new molecules to combat this neglected disease.

Poster 55 : The potential of screening microbial extracts for anti-T. b. brucei drug discovery

Presenter: **Phd Studen Claudia Gomez Liñan** , *Molecular Biology , Instituto de parasitología y biomedicina López Neyra*

F Annang[1]; R Diaz[2]; C Gómez-Liñán[2]; J Martin[1]; O Genilloud[1]; F Reyes[1]; F Vicente[1]; M Navarro[2]

[1] Fundación MEDINA, Spain; [2] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain

Sleeping sickness is a Neglected Tropical Disease caused by *T. b. gambiense* and *T. b. rhodesiense* protozoan parasites. Known as African sleeping sickness, this disease is endemic to Sub-Saharan countries with at risk population of 65 million (36 countries, 67.5% in DRC alone). Recent introduction of oral fexinidazole has led to

95% case reduction (2000-2018), however, melarsoprol (causes death in 5% patients) is still the only drug for Stage II *T. b. rhodesiense* (infection of the Central Nervous System (CNS)), hence need for new safe drugs here. We report here on a previously validated robust High Throughput Screening (HTS) platform with state-of-the-art chemistry capacity established by MEDINA/IPBLN-CSIC for the discovery of novel drugs from microbial extracts against *T. b. brucei*. We have recently screened a subset (about 13,000 extracts) of Fundación MEDINA microbial extracts library (>116,000 strains and 200,000 extracts), and report of the promising results so far obtained from our robust screening strategy and the potential for future novel anti-*T. b. brucei* compounds.

Poster 56 : Transcriptome profiling reveals new essential genes regulated by heme in *Leishmania major*

Presenter: **Miss Graciela Juez-Castillo**, *PhD student, IPBLN-CSIC*

G Juez-Castillo[1]; L M Orrego[1]; P Vargas[1]; M Cabello-Donayre[1]; M Martínez-García[1]; J A García-Sánchez[1]; E Andres-León[1]; J M Pérez-Victoria[1]

[1] IPBLN-CSIC, Spain

Heme is an iron-coordinated porphyrin almost ubiquitous among living beings, in which it serves as a cofactor of hemoproteins involved in a wide range of fundamental physiological processes, and also as a signalling molecule. At the same time, free heme and its precursors are highly cytotoxic due to their ability to generate reactive species of oxygen, so its homeostasis requires very precise regulation. Heme is essential in most aerobic organisms, which are generally able to synthesize it through a broadly conserved metabolic pathway. One of the rare exceptions to this scenario is trypanosomatid parasites such as *Leishmania*, which are auxotrophic for heme since they lost its complete biosynthesis pathway during evolution and must obtain this essential compound from the infected host. Exploiting this heme dependency is a rational way to find new leishmanicidal agents. The aim of this work is to identify and characterize genes/proteins differentially regulated by heme in *Leishmania major*, and evaluate their potential use as new drug target. First, we analysed by RNA-seq the transcriptomic differences between L. major promastigotes cultured in the presence or the absence of heme. Three replicates of each type of sample were sequenced in an Illumina Nextseq 550 using a standard protocol for paired-end 75nt libraries. The generated fastq files were analyzed using miARma-Seq, and quality analysis was performed using fastqc program to identify sequencing errors. Reads were aligned against L. major Friedlin reference genome from TriTrypDB version 31 using three different aligners: Bwa, hisat2 and STAR. Then, aligned reads were summarized into gene expression using FeatureCounts to be subsequently analysed by edgeR to identify differentially expressed genes (DEG) among heme-depleted and control samples. We consider that a gene was differentially expressed if it appears with a value of FDR <0.05 in the three data sets obtained by the three aligners. Our preliminary results show 1,908 DEG (802 up-regulated in the absence of heme and 1,106 down-regulated). 12 DEG were selected for qPCR validation (6 up-regulated and 6 down-regulated) showing a huge correlation between the results obtained by both techniques. In order to perform a functional interpretation of the results, the GO terms of each of the genes were obtained, according to the TriTrypDB database. With these terms, a database was built and a functional enrichment analysis was carried out. Among the most enriched terms (having a p-value <0.05) in the ontology of biological processes we found “regulation of developmental process” and “transmembrane transport” and between the molecular functions ontology: “heme binding” and “metal ion transmembrane transporter activity”. Finally, some of the most up-regulated genes in the heme depletion condition were selected for further analysis, evaluating their intracellular localization and essentiality in the parasite using the CRISPR-Cas9 technology. In situ ta

Poster 57 : Discovery of new phosphonium salts active *in-vivo* against *Leishmania* parasites

Presenter: **Dr Christophe Dardonville**, *Research Scientist, Medicinal Chemistry Institute (IQM-CSIC)*

J I Manzano[4]; E J Cueto-Díaz[5]; A I Olias[2]; A Perea[4]; T Herraiz[3]; J Torrado[1]; J M Alunda[2]; F Gamarro[4]; **C Dardonville**[5]

[1] Facultad de Farmacia, Universidad Complutense, Madrid, Spain; [2] Facultad de Veterinaria, Universidad Complutense, Madrid, Spain; [3] Instituto de Ciencia y Tecnologia de los Alimentos y Nutricion, CSIC, Madrid, Spain; [4] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain; [5] Instituto de Quimica Medica, CSIC, Madrid, Spain

We report the discovery of a new phosphonium lead compound (**1**) showing a strong *in-vivo* activity by oral dosage in a mouse model of visceral leishmaniasis. A focused screening of an in-house series of trypanocidal compounds against *L. donovani* parasites resulted in the discovery of potent leishmanicides. Three 4-hydroxyphenyl-derived phosphonium salts displaying EC_{50} < 0.2 μM against promastigotes and intracellular amastigotes of *L. donovani*, and satisfactory selectivities (SI > 10), were selected for further studies. Since the selected compounds contain a hydrolysable ester bond, which may potentially limit its *in-vivo* efficacy, more metabolically stable analogues **1**–**3** were synthesized. The new compounds showed high efficacy (EC_{50} < 0.28 μM) against promastigote and intracellular amastigote forms of *L. donovani* with SI > 20. Compound **1** (EC_{50} ≤ 90 nM and SI ≤ 31), which was equally effective against antimonials and miltefosine-resistant clinical isolates of *L. infantum*, was selected for *in-vivo* assays in a mouse model of visceral leishmaniasis. Compound **1** reduced the parasite load in spleen (98.9%) and liver (95.3%) of infected mice after an oral dosage of 4 daily doses of 1.5 mg/kg. Mode of action studies showed that compound **1** diffuses across the plasma membrane and targets the mitochondrion of *Leishmania* parasites. Disruption of the energetic metabolism, with a decrease of intracellular ATP levels as well as mitochondrial depolarization together with a significant ROS production, contributes to the leishmanicidal effect of **1**.

Poster 58 : Extracellular vesicles of trypomastigotes of *Trypanosoma cruzi*(DTU I) induce physiological changes in non-parasitized culture cells

Presenter: **Dr Luis Miguel de Pablos Torró**, *Assistant Professor, University of Granada*

L Retana Moreira[1]; A Prescilla Ledezma[1]; L M De Pablos Torró[1]; A Osuna[1]

[1] Departamento de Parasitología, Grupo de Bioquímica y Parasitología Molecular CTS-183, Universidad de Granada, Spain

Extracellular vesicles (EVs) are a diverse group of nanoparticles involved in intercellular communication under physiological and pathological conditions. *Trypanosoma cruzi*, the protozoan that causes Chagas disease, releases EVs that facilitate parasite invasion of the host cell, immunomodulate the host response and help the parasite to evade this response, among other functions. However, little is known about how the host cell is altered. In this work, we isolate EVs of tissue-culture cell-derived trypomastigotes of *T. cruzi* Pan4 strain using differential centrifugation and confirm that these EVs increase cell parasitism. We also demonstrate that EVs affect cell permeability in Vero cells and cardiomyocytes using viability assays and confocal microscopy, as well

as the rise in the intracellular Ca2+levels, causing the disruption of the actin filaments. Finally, an arrest of the cell cycle at the G0/G1 phases produced by these EVs is also confirmed by flow cytometry and Western blot. This work seeks to elucidate the way in which EVs influence certain aspects of the cell physiology that favour the establishment of this parasite inside the host cell.

Poster 59 : Condensin complex contributes to VSG expression, regulation and switching in *Trypanosoma brucei*

Presenter: **Dr Domingo Rojas**, *Post-doctoral researcher, IPB "López-Neyra", CSIC*

D Rojas[1]; A Saura[1]; J M Bart[1]; G Diffendall[1]; M Navarro[1]

[1] Instituto de Parasitología y Biomedicina López-Neyra. Consejo Superior de Investigaciones Científicas, Spain

African trypanosomes evade the host immune response by periodically changing their protein coat, constituted by a single Variant Surface Glycoprotein (VSG), allowing for chronic infections. We have previously published that Cohesin complex regulates in situ transcriptional VSG switching, as partial depletion of Cohesin subunits increases the frequency of antigenic variation. Condensin complex, structurally similar to the cohesin complex, has recently emerged in eukaryotes as a mayor regulator of chromosome architecture, chromatin compaction during interphase, and is greatly enriched near highly expressed genes. Previous results suggest that SUMOylation of chromatin at the active VSG locus may function to nucleate factors to the nuclear body ESB, where VSG transcription occurs. Furthermore, we found by proteomic analysis that TbSMC4, a subunit of the condensin complex, is a consistent and abundant SUMO target. Therefore, it seems probable that Condensin functions in the regulation of VSG monoallelic expression and/or transcriptional switching. Co-IP experiments showed that trypanosome condensin includes well-known subunits CND1 and 2 and SMC4, suggesting a conserved multiprotein complex that localizes in the nucleus, as described in other eukaryotes. In addition, we found distinct SUMOylation sites in the condensin subunits of the infective bloodstream forms. Interestingly, partial depletion of condensin subunits resulted in a significant increase of VSG221 switching off events, reaching up to 10% of the population, a switching frequency higher than previously described for cohesin depletion. Isolated switches corresponded to in situ transcriptional activation events of independent telomeric VSG-ESs. All data suggest that condensin complex has a key role in establishing and/or maintaining the transcriptional state of VSG-ES chromatin.

Poster 60 : Hit-to-lead and target identification studies within a novel class of anti-trypanosomal agents.

Presenter: **Dr Rosario Diaz Gonzalaz**, *Postdoctoral researcher, Spanish Research Council*

L Ferrins[2]; R Diaz Gonzalaz[1]; G Ceballos-Pérez[1]; A Saura[1]; G Perez-Moreno[1]; R García-Hernández[1]; C Bosch-Navarrete[1]; F Gamarro[1]; L M Ruiz-Perez[1]; D Gonzalez-Pacanowska[1]; M Navarro[1]; M Pollastri[2]

[1] Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', CSIC. Granada, Spain; [2] Northeastern University, United States

Human African trypanosomiasis (HAT), one of 20 neglected tropical diseases (NTDs) designated by the World Health Organization, affects approximately 8,000 people in the remotest parts of Africa; the disease is devastating to those afflicted. HAT is an insect-borne disease caused by the protozoan parasite *Trypanosoma*

brucei, and once the parasite crosses the blood brain barrier it causes disrupted sleep patterns, brain damage and eventual death. Current treatments for this disease are associated with complex treatment regimens and often result in the development of long-term health issues; in addition, reports of resistance are becoming more frequent, and its immune evasion mechanisms makes challenging the development of vaccines. Combined, these factors contribute to a need for new lead compounds to fill the drug discovery pipeline for HAT.
To this end, we employed a lead repurposing strategy, whereby lead compounds against a human target are screened against the disease-causing parasite. We previously reported the results of a whole organism high-throughput screen of 42,444 inhibitors from the GSK kinase-focused screening collection that led to the identification of 797 sub-micromolar inhibitors of *T. brucei* growth. The structure-activity relationships (SAR), biological profiling and target identification studies within one of these clusters with high potency, excellent selectivity and good absorption, distribution, metabolism, and excretion (ADME) properties will be discussed. The antiparasitic effect of compounds in the series was measured against intracellular *T. cruzi* and *Leishmania donovani* and their host mammalian cell lines, generating a wider view of the potential of these compounds for the treatment of other diseases caused by kinetoplastids such as the leishmaniasis and Chagas disease.

Poster 61 : The epidemiological role of indigenous dogs in transmitting animal and human African trypanosomiasis in Zambia: A case study of dogs from Mambwe district, eastern Zambia.

Presenter: **Mr Malimba Lisulo**, *PhD Student, The University of Edinburgh*

M Lisulo[2]; B Namangala[6]; C Mweempwa[4]; M Banda[1]; K Hayashida[3]; C Sugimoto[3]; M Sutherland[5]; K Picozzi[2]; E MacLeod[2]

[1] Central Veterinary Research Institute, Zambia; [2] Division of Infection and Pathway Medicine, Biomedical Sciences, Edinburgh Medical School, College of Medicine and Veterinary Medicine, The University of Edinburgh, UK; [3] Hokkaido University Center for Zoonosis Control, Japan; [4] Ministry of Livestock and Fisheries - Tsetse and Trypanosomiasis Control, Zambia; [5] School of Biological Sciences and Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, UK; [6] School of Veterinary Medicine - University of Zambia, Zambia

Throughout their long history of domestication, dogs have been sources of parasite exchange between livestock and humans and remain an important source of emerging and re-emerging diseases including trypanosome infections. A cross sectional survey of Canine African Trypanosomiasis (CAT) involving 237 indigenous dogs was conducted in tsetse-infested Mambwe district, eastern Zambia in October 2012. Presence of *Trypanosoma congolense*, T. b. brucei and zoonotic *T. b. rhodesiense* in dog blood was confirmed by microscopy (5.9%; 95% CI: 2.9 – 8.9%) and LAMP (8.4%; 95% CI: 4.9 – 12.0%). Most carriers did not manifest clinical illness, except for 3 dogs with *T. brucei* subspecies infection that developed corneal opacity. These findings suggested that indigenous dogs carrying zoonotic *T. b. rhodesiense* might play a reservoir role in the sporadic human sleeping sickness cases being reported. Current and on-going research: A cohort of 162 dogs from the same area was enrolled in 2018 to understand their interaction with African trypanosomes. The study also measured changes in health and demographic outcomes, as well as risk factors associated with morbidity and mortality. Follow-ups were done at 3 different time-points: June, September and December 2018 representing the cold, hot and rainy season. A total of 41 dogs were lost to follow-up: 31 (died from ill-health, wild-predators and other causes) and 10 (Sold or relocated from study area). Preliminary results show an overall prevalence of 53% trypanosome infection with half involving zoonotic *T. b. rhodesiense*. As earlier observed in 2012, most of the carriers remained asymptomatic throughout the study. Apart from harbouring trypanosomes, several dogs had endo and

ecto-parasites, microfilariae and tick-borne diseases. Molecular characterization of zoonotic *T. b. rhodesiense* and suspected human-infective microfilariae is underway.

Poster 62 : Fully bi-parental inheritance of kDNA minicircles in *Trypanosoma brucei*

Presenter: **Miss Lizzie Wadsworth**, *PhD student, University of Edinburgh*

E Wadsworth[1]; S Cooper[1]; C Clucas[3]; B Bradley[3]; F Van den Broeck[2]; A MacLeod[3]; N Savill[1]; A Schnaufer[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Institute of Tropical Medicine, Antwerp, Belgium; [3] Wellcome Centre for Integrative Parasitology, University of Glasgow, UK

Trypanosoma brucei kinetoplast DNA (kDNA) is an interlocked network of 30-50 homogeneous 'maxicircles' and ~10,000 heterogeneous 'minicircles'. Maxicircles are the equivalent of mtDNA in other eukaryotes, and minicircles code for small RNAs which specify extensive and essential post-transcriptional editing of maxicircle-encoded mRNAs. We previously reported ~400 unique minicircle sequences in a pleomorphic *T. brucei* strain, and loss of complexity over time during bloodstream stage culture. Earlier studies suggest minicircles may be exchanged during *T. brucei* mating in the tsetse fly and this may compensate for minicircle loss in the bloodstream stage.
Using deep sequencing, we now investigate kDNA inheritance in depth. We conclusively demonstrate that hybrids inherit minicircles from each parent in roughly equal proportions, allowing for an increase in minicircle complexity. Thus, kDNA inheritance in *T. brucei* is fully bi-parental.

Poster 63 : Abundance and distribution of tsetse flies in Thuma forest reserve area, Salima district of the central region of Malawi

Presenter: **Prof Zumani Banda**, *Professor, ShareWORLD Open University Malawi*

Z Banda[1];

[1] ShareWORLD Open University Malawi

A survey was carried out in Thuma forest reserve area in Salima district in the central region of Malawi with the main objective of finding out the abundance and distribution of tsetse fly species in the area during the dry season of October, 2012. The tsetse population was observed along four transects consisting of a total number of 35 odour baited epsilon traps deployed at an interval of 200 m apart and four fly round transects cutting across various vegetation types and different altitudes. A total of 821 *Glossina morsitans morsitans* (440 males and 381 females) were caught in traps and fly round transects during the survey. Three hundred and twenty five *G. m. morsitans* (169 males and 156 females) were caught in traps after 72 hrs and 496 *G. m. morsitans* (271 males and 225 females) were caught along the fly round transects. Most of the flies were caught in the middle belt which hosts an abundance of wildlife and consists mainly of the riverine type of vegetation. Less flies were caught in the transect with the bamboo and heterogeneous type of vegetation. The index of apparent abundance (IAA) was higher (7.0) in transect 4 from Nagwathemba stream through the Base Camp to Thobothobo stream and the springs where traps were deployed in the predominantly riverine type of vegetation and lower (1.4) in transect 3 from Nagwathemba stream up the Salt Lick Road consisting mainly of bamboo, miombo (*Brachystegia*) and heterogeneous type of vegetation at an altitude of 735- 767 m. There was an apparent

increase in the abundance of tsetse from the buffer zone towards the middle belt. It is suggested that since tsetse depending on their vectorial capacity can transmit trypanosomiases in both susceptible animal and human hosts, control measures need to be introduced in the area.

Poster 64 : Pathogen box compounds as possible leads for new interventions against leishmaniasis

Presenter: **Dr Wanday Amlabu**, *Lecturer and researcher, West African Centre for Cell Biology of Infectious*

W E Amlabu[1]; C A Antwi[1]; G A Awandare[1]; T M Gwira[1]

[1] West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, P. O. Box LG54, Legon, Accra, Ghana., Ghana

*Leishmania*sis is a disease endemic in the tropics. It is caused by *Leishmania* parasites. The most reliable intervention is chemotherapy. The mainstay drugs are toxic, not readily accessible and costly. Here, we showed the antileishmanial activity and likely mode of action of 68 Medicine for Malaria Venture compounds against *Leishmania donovani*. Growth inhibitory concentrations (IC_{50}) obtained ranged between 10 nM and 95 µM. 24 of the compounds were tested for cytotoxicity against RAW macrophages and a selectivity index of 0.03 to 455 was observed. The growth kinetic and reversibility profiles of 20 of the compounds indicated a cytostatic effect, while 4 were cytocidal, they mediated cell death via apoptosis and altered their cell cycle progression. Fluorescence microscopy study revealed distortion in the mitochondrion (60%) and loss of kinetoplasts DNA (30%). Our findings present useful therapeutic potentials of these compounds in leishmaniasis.

Poster 65 : Antiparasitic Drug Discovery: Microbial extracts as a ready source of potent and novel antiparasitic compounds

Presenter: **Dr Frederick Annang**, *Research Fellow, Fundación MEDINA*

F Annang[1]; G Pérez-Moreno[2]; I Pérez-Victoria[1]; J Martín[1]; C Bosch-Navarrete[2]; L Ruiz-Pérez[2]; O Genilloud[1]; F Reyes[1]; F Vicente[1]; D González-Pacanowska[2]

[1] Fundación MEDINA, Spain; [2] IPBLN-CSIC, Spain

P. falciparum, T. cruzi, T. brucei and *L. donovani* are four key parasitic protozoans implicated in four tropical diseases that affect about half of the world´s population, bringing untold sufferings to millions, leading to the deaths of thousands per year in the poorest tropical regions. The current drugs against these parasites have resistance, toxicity and cumbersome administration problems. There is therefore a pending need for the discovery of next-generation antiprotozoal drugs to replace the current outdated and inadequate ones. Historically, compounds from natural sources have routinely provided novel chemical scaffolds from which new potent antiprotozoal drugs have been developed. Microbial extracts provide a unique underexplored chemical space for the current need of novel compounds in drug discovery. Herein we report the establishment of a valid antiparasitic High Throughput Screening drug discovery platform at Fundación MEDINA/IPBLN-CSIC, and the potential use of this platform to screen over 200,000 microbial extracts of Fundación MEDINA´s natural extracts collection. We also report the exciting new antiparasitic compounds so far discovered from screening of a subset (about 30,000) of this collection.

Poster 66 : Ablation of a pH sensing protein kinase forces haptomonad differentiation in *Leishmania mexicana*

Presenter: **Dr Nicola Baker**, *PDRA, University of York*

N Baker[1]; C Hughes[1]; J Mottram[1]

[1] University of York, UK

Survival of *Leishmania* throughout its life cycle relies on perfectly orchestrated differentiation events, triggered by environmental changes such as nutrients, pH and temperature. Protein kinases are fundamental to sensing these environmental changes and signalling differentiation events. A kinome-wide bar-seq experiment was used to identify >26 protein kinases required for successful differentiation from procyclic promastigotes through to amastigotes. These mutants were grown individually in graces media for 8 days to enrich for metacyclic promastigotes and checked for phenotypic abnormalities. A significant enrichment for haptomonad cells was seen for 2 CAMK mutants named haptomonad inhibitor kinases (HIK). HIK1 null mutant cells differentiate into haptomonads faster in low pH media and differentiate back into leptomonads faster in higher pH suggesting that this kinase is required for pH sensing. The HIK1 null mutant provides a novel tool for investigating the sand fly-specific haptomonad life cycle stage as well as a route towards uncovering other components of the pH signalling pathway.

Poster 67 : Assessment of knowledge on tsetse flies and potential risk factors to trypanosomiasis transmission to humans and livestock, Salima District, Central Malawi

Presenter: **Prof Zumani Banda**, *Professor, ShareWORLD Open University Malawi*

Z Banda[1]

[1] ShareWORLD Open University Malawi

A questionnaire survey was carried out during the dry season in October 2012 in seven (7) villages surrounding Thuma Forest Reserve Area in Salima District, in the Central Region of Malawi, to assess local knowledge about tsetse flies (Glossina spp.) and the potential risk towards trypanosomiasis transmission to humans and livestock. Thirty six (36) farmers participated in the study and the questionnaire focussed on issues related to livestock management, bovine trypanosomiasis, sleeping sickness, and how community livelihoods were linked to Thuma forest and wildlife. The results showed that most of the farmers were knowledgeable about tsetse flies causing trypanosomiases. The majority of the farmers indicated that tsetse flies are common and a problem during the rainy season. The survey also found that the majority of the farmers enter the buffer zone and forest reserve to collect firewood and to cut bamboo poles respectively which predispose them to tsetse fly bites. The study further showed that there are no control measures being undertaken to control the tsetse flies and potential trypanosomiasis transmission. This study was merely a pilot designed to assess local knowledge but a more detailed study on vectoral capacity and disease prevalence in both humans and livestock should be carried out in all the 4 districts surrounding Thuma Forest Reserve to ascertain the need for vector and disease control.

Poster 68 : The *Leishmania infantum* Miltefosine Sensitivity Locus

Presenter: **Dr Juliana Brambilla Carnielli Trindade**, *Postdoctoral Research Associate, University of York*

J Brambilla Carnielli Trindade[1]

[1] University of York, UK

Miltefosine has been used successfully to treat visceral leishmaniasis (VL) in India, but it was unsuccessful in a clinical trial for VL in Brazil (cure rate ≤ 60%).We used a genome-wide association study to identify a molecular marker that predicts VL treatment failure following whole genome sequencing of 26 *Leishmania infantum* isolates, from cured and relapsed patients. The Miltefosine Sensitivity Locus (MSL) in *L. infantum* has a frequency that varies in a cline from 95% in North East Brazil to less than 5% in the South East (n≤188). The MSL was found in the genomes of all *L. infantum* and *L. donovani* sequenced isolates from the Old World (n≤671), where miltefosine can have a cure rate higher than 93% (Carnielli *et al*., 2018 EbioMedicine 36:83-91). We have now generated the first Brazilian *L. infantum* 36 one-contig chromosome reference genome (MHOM/BR/06/MA01A) using nanopore and Illumina sequencing. We show that the MSL contains four genes: 3'-nucleotidase/nucleases LinJ.31.2370 and LinJ.31.2380; helicase-like protein LinJ.31.2380; and 3,2-trans-enoyl-CoA isomerase LinJ.31.2400. To test if any of these genes contributed to natural resistance to miltefosine, the Brazilian *L. infantum* MSL$^+$ MA01A reference cell line was engineered to express Cas9 and T7, enabling CRISPR-Cas9-mediated genome editing. The whole MSL and each individual MSL gene was then deleted to create a series of mutants. Deletion of the whole MSL or both nucleotidases significantly reduced miltefosine susceptibility of the promastigotes *in-vitro*. An increase of LinJ.31.2380 protein level under miltefosine treatment was also observed, pinpointing nucleotide metabolism as a key pathway to be investigated further.

Poster 69 : Trypanosome FLAgellum Member 8 redistribution and function in the mammalian host

Presenter: **Estefania Calvo Alvarez**, *Postdoctoral researcher, Institut Pasteur*

E Calvo Alvarez[1]; S Bonnefoy[1]; T Blisnick[1]; A Salles[1]; A Crouzols[1]; P Bastin[1]; B Rotureau[1]

[1] Institut Pasteur, Paris, France

The African trypanosome flagellum is essential in multiple aspects of the parasite development. In the insect form of this protist, FLAgellar Member 8 (FLAM8) is a large protein located at the distal tip of the flagellum, at the interface with new micro-environments. We demonstrate that FLAM8 targeting to the tip relies on intra-flagellar transport. In contrast, FLAM8 extends along the entire flagellar cytoskeleton in mammalian infective forms. The FLAM8 concentration to the tip occurs during the mammalian to insect form differentiation, illustrating for the first time the specific remodelling of an existing flagellum. Despite this differential distribution, FLAM8 appears as a marker of the flagellum maturation in both stages. During the parasite migration in the tsetse vector digestive tract, FLAM8 further redistributes f

Poster 70 : The Qi site of cytochrome b is a promiscuous drug target in *Trypanosoma cruzi* and *Leishmania* donovani

Presenter: **Dr Sandra Carvalho**, *Biochemist, University of Dundee*

S Carvalho[1]; R Wall[1]; R Milne[1]; S Wyllie[1]

[1] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK

Available treatments for Chagas' disease and visceral leishmaniasis are inadequate and there is a pressing need for new therapeutics. Drug discovery efforts for both diseases principally rely upon phenotypic screening. However, the optimisation of phenotypically-active compounds is hindered by a lack of information regarding their molecular target(s). To combat this issue we initiate target deconvolution studies at an early stage. Here, we describe comprehensive genetic and biochemical studies to determine the targets of three unrelated phenotypically-active compounds. All three structurally-diverse compounds target the Qi active-site of cytochrome b, part of the cytochrome bc1 complex of the electron transport chain. Our studies go on to identify the Qi site as a promiscuous drug target in *Leishmania donovani* and *Trypanosoma cruzi* with a propensity to rapidly mutate. Strategies to rapidly identify compounds acting via this mechanism are discussed to ensure drug discovery portfolios are not overwhelmed with inhibitors of a single target.

Poster 71 : RNase H1, a R-loop resolving enzyme, acts to suppress R-loop mediated DNA replication and limit genome instability in *Leishmania*

Presenter: **Dr. Jeziel Dener Damasceno**, *Research Associte, University of Glasgow - WCIP*

J D Damasceno[3]; E Briggs[2]; J R Cunha[1]; K Crouch[3]; C Lapsley[4]; D Bartholomeu[1]; R McCulloch[3]

[1] UFMG, Brazil; [2] University of Edinburgh, UK; [3] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK; [4] Wellcome Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK

Stable RNA-DNA hybrids (R-loops) act in several genomic processes, but their roles in DNA replication are unclear. By using DRIP-seq, we show that R-loop distribution within each chromosome parallels the spatial, temporal and functional compartments of the unconventional DNA replication programme in *Leishmania*. Strikingly, R-loop levels correlate with chromosome size, which in turn correlate with replication timing. MFA-seq analyses shows that DiCre-mediated *RNase H1* KO results in origin-independent DNA replication initiation, profoundly changing the DNA replication programme. Such alteration leads to genome-wide, chromosome-size dependent instability, including aneuploidy, SNPs and InDels, as revealed by whole genome sequencing. Therefore, our data reveal a crucial role of RNase H1 in controlling R-loop-mediated DNA replication initiation, favouring conventional origin-directed initiation, and places the hybrids as a pivotal player in *Leishmania* global genome instability.

Poster 72 : Flagellar remodeling mediates attachment of kinetoplastid parasites to honeybee hindgut via hemidesmosome-like junction complexes.

Presenter: **Dr Luis Miguel de Pablos Torró**, *Assistant Professor, University of Granada*

L M De Pablos Torró[1]; M Buendía-Abad[3]; P García Palencia[2]; R Martín-Hernández[3]; A Osuna[1]; M Higes[3]

[1] Departamento de Parasitología, Grupo de Bioquímica y Parasitología Molecular CTS-183, Universidad de

Granada, Spain; [2] Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Spain; [3] IRIAF, Centro de Investigación Apícola y Agroambiental (CIAPA), Consejería de Agricultura, JCCM, Spain

Flagellar remodeling into attachment structures is a common mechanism found in the insect stages of kinetoplastid parasites. Within their wide range of hymenopteran hosts, *Lotmaria passim* and *Crithidia mellificae* kinetoplastids are able to infect *Apis mellifera*. These parasites might have potential impact on honeybee health, however their life cycles as well as mechanism of pathogenicity are still unresolved. Here we show that *L. passim* promastigotes and *C. mellificae* choanomastigotes differentiate into cells resembling haptomonad stage in the rectum and ileon of honeybees. In those locations, the parasites attach to the surface epicuticule via zonular hemidesmosome-like structures, as revealed by TEM. Additionally, we have generated *in-vitro* culture conditions to study parasite adherent cells and protocols for isolation from field samples. Finally, we are currently developing fluorescent and bioluminescent parasites for *in-vivo* monitoring of honeybee infections.

Poster 73 : Diversity of trypanosome species found in *Chtonobdella bilienata* leeches from Sydney, Australia

Presenter: **Prof John Ellis**, *Professor of Molecular Biology, University of Technology Sydney*

J T Ellis[5]; J Barratt[5]; A Kaufer[5]; B Armstrong[5]; M S Johnson[5]; R Gough[5]; Y Park[5]; L Downey[2]; L Neill[4]; R Lee[3]; D Stark[1]

[1] Department of Microbiology, St Vincent's Hospital Sydney, Australia; [2] Department of Microbiology, St. Vincent's Hospital Sydney, Australia; [3] Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, Australia; [4] NSW Health Pathology, ICPMR, Westmead Hospital, Sydney, Australia; [5] School of Life Sciences, University of Technology Sydney, Australia

Chtonobdella bilienata leeches were investigated for the presence of trypanosomes. PCR of 28S rDNA showed that > 95% of *C. bilienata* contained trypanosomes. Diversity profiling by deep amplicon sequencing of 18S rDNA indicated the presence of four different clusters related to the Megatrypanum *Trypanosoma theileri*. NNN slopes with liquid overlay were used to isolate trypanosomes into culture. PCR and Sanger sequencing of the 18S rDNA from cultures showed the sequences clustered with those previously obtained from haemadipsid leeches and wallaby, as well as *Trypanosoma cyclops*. In contrast phylogeny of 18S rDNA/GAPDH concatenated sequences indicated the trypanosomes were monophyletic with *T. cyclops* as a sister group. Blood meal analyses of leeches by PCR of 12S rDNA showed that these leeches contained blood from Swamp Wallaby (*Wallabia bicolour*), horse (*Equus*) and human.

Poster 74 : The Mitochondrion of *Trypanosoma brucei*; a new perspective through comprehensive tagging of mitochondrial proteins.

Presenter: **Dr Michael Hammond**, *Post-Doctorate, Institute of Parasitology*

J Pyrih[1]; M Hammond[2]; S Dean[4]; J Sunter[4]; R Wheeler[4]; K Gull[4]; J Lukeš[3]

[1] Charles University in Prague, Czech Republic; [2] Institute of Parasitology, BioCenter, České Budějovice, Czech Republic; [3] Institute of Parasitology, Biology Centre, Czech Republic; [4] University of Oxford, Sir William Dunn School of Pathology, UK

The singular mitochondrion of trypanosomatids has been at the forefront of revolutionary discoveries concerning molecular biology, such as a mitochondrial genome of intercatenated DNA circles and complex post-transcriptional RNA editing. The mitochondrial proteome of *Trypanosoma brucei* is one of the most well investigated in protists, which has established a level of mitochondrial complexity rivalling that of many multicellular organisms. The TrypTag project represents an effort to localise every protein of *T. brucei* via endogenous gene tagging, and is the second unicellular eukaryote to be investigated in such a manner. Using fluorescent protein tagging against both N and C termini, we have localised all proteins in procyclic *T. brucei* that were reported previously as mitochondrial and generated list of approximately 1200 sequences which we class as definitively belonging to the mitochondrion, combined with their sub-organellar distribution. Using this knowledge, we investigate many unexplored mitochondrial pathways. We localise the majority of enzymes involved in beta-oxidation and synthesis of fatty acids to the mitochondrion, and define the amino acid repertoire that can be synthesised and degraded within this organelle. We also establish a new list of Tripartite attachment complex proteins, necessary for proper kinetoplast and cell division. The knowledge gained from this study represents a conclusive step to determining and understanding the mitochondrial proteome of *T. brucei*.

Poster 75 : Trypanosoma suis, I presume? Recent progress in understanding the biology of a forgotten livestock pathogen

Presenter: **Miss Rachel Hutchinson**, *PhD Researcher, University of Bristol*

R Hutchinson[2]; L Peacock[1]; C Kay[2]; W Gibson[2]

[1] School of Biological Sciences and Bristol Veterinary School, University of Bristol, UK; [2] School of Biological Sciences, University of Bristol, UK

Trypanosoma suis is a pathogenic parasite of pigs and, until recently, was believed to be the sole member of subgenus *Pycnomonas*. With the recent discovery of novel pycnomonads in suids, cervids and bovids, it is more important than ever that we characterise this poorly understood trypanosome. Here we discuss how it is possible for a seemingly prevalent parasite to remain undetected and unacknowledged for decades at a time, and explore our recent progress in understanding the life history of this enigmatic parasite. We present results on the growth and morphology of procyclics and epimastigotes of *T. suis*, its developmental cycle in tsetse, and progress on transfection and genetic analysis. Although *T. brucei* is the closest relative of *T. suis*, these two parasites have markedly different biology.

Poster 76 : Interrogating the mitochondrial acetate pathway in Trypanosoma congolense using 1H-NMR and RNAi

Presenter: **Mr James Iremonger**, *PhD student, University of Edinburgh*

J Iremonger[2]; P C Steketee[2]; E Paxton[2]; A Schnaufer[1]; L Morrison[2]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] Roslin Institute, University of Edinburgh, UK

Drug therapies against the livestock parasite *Trypanosoma congolense* are losing efficacy due to growing resistance. An improved understanding of the metabolism of these parasites will be key to the discovery of novel

compounds. A combination of RNAi and ^{1}H-NMR has enabled us to identify metabolic differences between bloodstream form of *T. congolense* and the more intensively studied species *T. brucei*. Our preliminary data suggest much higher flux of pyruvate through the mitochondrial enzyme pyruvate dehydrogenase (PDH) in *T. congolense* compared to *T. brucei*. In contrast to published data for *T. brucei*, inducible knockdown of this enzyme in *T. congolense* led to a severe reduction in growth, accompanied by a decrease in the secretion of acetate and an increase in the efflux of glucose-derived pyruvate. These data highlight important variations that exist in mitochondrial metabolism between these trypanosome species, contributing to more informed approaches to media formulation and drug design.

Poster 77 : Bioinformatics comparison of thymidylate (TMP) metabolism in camels and Trypanosoma evansi

Presenter: **Dr Mahmoud Kandeel**, *Associate professor, King Faisal University*

M Kandeel[1]; A Al-Taher[1]

[1] King Faisal University, Saudi Arabia

Metabolic and structure differences between the host and parasite protein accounts for a hot spot in drug discovery process. In this study, the deoxythymidine 5'-monophosphate (dTMP) metabolic pathway was compared in camel and *Trypanosoma evansi*. Recycling nucleotides to TMP was predicted with camel but not with *T. evansi* due to the absence of 5'-nucleotidase or 5'-deoxyribonucleotidase. While, thymidine kinase was common in both of camel and *T. evansi* genomes. The trypanosomal enzyme was a large size protein, about 2 folds the size of camel TK. In addition, *T. evansi* TK encoded two thymidine kinase domains. These results suggests targeting the *T. evansi* TK for its unique features.

Poster 78 : Shifting perspectives: a modification to the life cycle of *Trypanosoma brucei*

Presenter: **Miss Jaime Lisack**, *PhD student, Biozentrum der Universität Würzburg, Lehrstuhl für Zell- und Entwicklungsbiologie*

S Schuster[1]; J Lisack[1]; I Subota[1]; M Engstler[1]

[1] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum, Universität Würzburg, Germany

African trypanosomes are the causative agent of Human African Trypanosomiasis (HAT) and the cattle plague, Nagana. As with all vector-borne diseases, transmission is intimately tied to parasite survival and propagation in the vector, the blood-sucking tsetse fly. Two main stages of *T. brucei* live in the mammalian host, the proliferative long slender form and the cell cycle arrested short stumpy stage. The transition from slender trypanosomes into stumpy occurs via a quorum sensing mechanism, mediated by the parasite-excreted stumpy induction factor (SIF). As slender populations grow, the SIF threshold is reached and stumpy trypanosomes form. Aside from morphological and metabolic changes, stumpy trypanosomes also express the *protein associated with differentiation 1* (PAD1) (Matthews, 2009). The switch from slender to stumpy trypanosomes is thought to accomplish two things. First, it auto-regulates parasite density and hence, prolongs survival of the host. Second, stumpy forms are thought to be 'pre-adapted' to survival in the tsetse fly vector. It has long been believed that upon uptake from the mammalian blood, only the 'pre-adapted' stumpy trypanosomes can survive in the fly

midgut, while slender trypanosomes were thought to die. Keeping slender trypanosome populations below the SIF threshold and diluting parasites at different densities for *in-vivo* fly infections, we show that both slender and stumpy trypanosomes can propagate with comparable rates in the tsetse fly. We amassed a large dataset of fly infections and dynamics, further showing that that only one trypanosome, slender or stumpy, is necessary to infect a tsetse fly. Next, we looked at differentiation hallmarks at the early stages of differentiation, both in cell culture and in the fly. Here, we found that upon differentiation, PAD1, thought to indicate stumpy formation in the mammalian host, is expressed during slender trypanosome differentiation in the fly midgut, without cell cycle arrest or morphological transition to the stumpy stage. Thus, both stumpy and slender cells can complete the life and transmission cycle inside the tsetse fly vector. These results not only hold implications regarding the life cycle of *T. brucei* but also on transmission dynamics. This data could help answer the long-held question of how disease incidence can be sustained in chronic mammalian infections, at low blood parasitemia, where stumpy trypanosomes are characteristically absent.

Poster 79 : Divide and conquer: a genome-wide approach to identify novel factors for kinetoplast maintenance in trypanosomes

Presenter: **Dr Migla Miskinyte**, *PostDoc, University of Edinburgh*

M Miskinyte[1]; A Ivens[1]; M Waterfall[1]; T Ochsenreiter[2]; M Klingbeil[3]; A Schnaufer[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK; [2] University of Bern, Switzerland; [3] University of Massachusets, United States

Trypanosomatid parasites cause diverse diseases in humans and their livestock, but share a unique biological feature, the organisation of mitochondrial DNA into a complex structure, named kinetoplast (kDNA), which is crucial for the survival of the parasites. Replication, segregation and expression of kDNA are highly complex processes that involve an estimated ~300 proteins, only a fraction of which have been identified. In order to build a more complete picture of kDNA maintenance and regulation we developed an unbiased genetic screen to identify novel factors involved in these processes using a combination of genome-wide RNA interference, a novel kDNA staining protocol, and fluorescence-activated cell sorting. Our screening approach identified several known kDNA factors as well as novel candidate genes. We will present our screening approach, results from the validation studies, and a detailed characterisation of a confirmed novel kDNA maintenance factor.

Poster 80 : Functionally mapping the diversification of African trypanosomes using spatial proteomics

Presenter: **Dr Nicola Moloney**, *Postdoctoral research associate, University of Cambridge*

N Moloney[1]; K Barylyuk[1]; K S Lilley[1]; R F Waller[1]; P MacGregor[1]

[1] University of Cambridge, Department of Biochemistry, UK

Protein function is often intimately linked with localisation and as a consequence the subcellular distribution of a protein provides information on its role in the cell. We have optimised an effective method for resolving subcellular compartments in African trypanosomes for implementation in the spatial proteomics strategy of hyperLOPIT (hyperplexed localisation of organelle proteins by isotope tagging). This work will provide a comprehensive map of the *Trypanosoma brucei* and *Trypanosoma congolense* spatial proteomes. Individually,

these data sets will guide the determination of uncharacterised protein functions, particularly for *T. congolense*, where high-throughput functional analysis lags behind that of *T. brucei*. Further, comparative analysis of these maps will yield insight into the evolutionary diversification of these species and the effects of specialisation on the molecular biology and subcellular architecture of the parasite cell.

Poster 81 :-Assessment of knowledge, perception, and attitude on lymphatic filariasis among the inhabitants of a rural endemic community in Abia State, South-east, Nigeria

Presenter: **Dr Ofoma Cornelius Amoke,** *Lecturer, Michael Okpara University of Agriculture, Umudike*

O C Amoke[1]; C Anyaike[2]; U F Amoke[3]; N B Ezeji[1]

[1] Dept of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Nigeria; [2] Federal Ministry of Health, Abuja, Nigeria; [3] Dept of Nursing Sciences, Niger Delta University, Bayelsa State, Nigeria

Nigeria was reported as a country with the highest prevalence of Lymphatic Filariasis (LF) in sub-Saharan Africa. Elimination strategies are centred on 3 key areas, viz; delivery of 5 effective rounds of Preventive Chemotherapy (PC) using Ivermectin and Albendazole once annually, vector control and Morbidity Management and Disability Prevention (MMDP). Presently, elimination activities are at varying levels of implementation in most endemic Local Government Areas (LGA) in Nigeria. Ignorance and misbelieve were reported as factors that militates against achieving elimination target in Nigeria. This study evaluated the knowledge, Attitude and Perception (KAP) of the inhabitants of LF endemic communities in Abia State, South-east, Nigeria. A descriptive cross-sectional study was used and 293 respondents (≥ 15) were systematically selected. Information on socio-demographic characteristics and KAP on LF were carefully extracted using structured questionnaires. A majority (n ≤ 216, 73.7%) had knowledge of LF, as many (n ≤ 195, 66.6% and n ≤ 144, 49.1%) maintained that they have seen people with elephantiasis and hydrocoele respectively in their communities. Surprisingly, most of them are ignorant of the vector (n ≤ 153, 52.2%) and the causative agent (n ≤ 149. 50.9%) of the disease. Additionally, a significant proportion (n ≤ 114, 38.9%) insisted on the use of traditional medicine for the treatment LF. The study revealed the presence of high awareness of LF and the symptoms in the study area. However, ignorance of the vector and the belief in the use of traditional medicine for the treatment of the disease raises another fear that the 2024 elimination target set in Nigeria may be jeopardized in some places.

Poster 82 : The application of novel genetic and imaging technology reveals that benznidazole uptake in *Trypanosoma cruzi* is mediated by endocytosis.

Presenter: **Dr Francisco Olmo**, *Assistant Professor, London School of Hygiene and Tropical Medicine*

F Olmo[2]; F Costa[1]; S Alsford[2]; M C Taylor[2]; J M Kelly[2]

[1] London School of Hygiene and Tropical Medicine, UK; [2] London School of Hygiene and Tropical Medicine, UK

For almost 50 years, the nitroheterocyclic agent benznidazole has been the front-line treatment for *Trypanosoma cruzi* infections. Benznidazole is a pro-drug that is activated within the parasite by the bacterial-like mitochondrial-

localised type I nitroreductase TcNTR-1. Reductive metabolism results in the formation of reactive intermediates, ultimately leading to the generation of glyoxal, a mutagen with DNA-glycating and cross-linking activity. Laboratory-induced resistance to benznidazole is readily achievable and has been linked with acquired mutations within the TcNTR-1 gene, or to a reduction in copy-number. Investigations into the mechanisms of benznidazole-resistance have been restricted by the limited flexibility of *T. cruzi* genetic tools and the absence of the genetic machinery for RNA interference (RNAi). Because trypanosomatids share many metabolic processes, *Trypanosoma brucei* RIT-seq genome-wide screening technology can be exploited as a tool to provide insight into drug activity in other parasite species where shared pathways/targets/transporters are involved.
When we used a RIT-seq screen to identify *T. brucei* genes linked with benznidazole-resistance acquired through loss-of-function, we detected several genes encoding subunits of the vacuolar-type proton ATPase (V-type ATPase), a membrane-localised complex that mediates acidification of intracellular vacuoles, including lysosomes and acidocalcisomes. This enrichment of RNAi target fragments corresponding to V-type ATPase subunits suggested a role for the endocytic pathway in drug uptake. To validate this in *T. cruzi*, we used a streamlined CRISPR/Cas9 system to generate a range of V-type ATPase subunit single KO and *null* mutants. These each displayed benznidazole-resistance, implying a common uptake mechanism. To investigate this further, we chemically linked benznidazole to BODIPY (boron-dipyrromethene) and incubated the fluorescently tagged drug with parasites. Uptake via the flagellar pocket was readily detectable in real-time, followed by transit through the endosomal pathway. Benznidazole sensitivity of *T. cruzi* in the presence of the specific V-type ATPase inhibitor bafilomycin was also evaluated. This revealed inhibitor antagonism, demonstrating an association between inhibition of complex activity and reduced sensitivity to benznidazole. Therefore, both genetic and chemical validation experiments confirm a role for the V-type ATPase in benznidazole mode of action in *T. cruzi.* Progress in dissecting the mechanisms of benznidazole action has been facilitated by advances in transfection technology and imaging procedures. These will be described, and their further applications discussed.

Poster 83 : A new member of Curvicollide family kills African trypanosomes by inhibiting transcription

Presenter: **Ms Matilde Ortiz González**, *PhD student, Ibs Granada y Universidad de Almeria*

M Ortiz-González[2]; I Pérez-Victoria[1]; M Soriano[3]; J Martín[1]; C Rodrigues-Poveda[2]; N de Pedro[1]; J Maceira[2]; F Vicente[1]; F Reyes[1]; J A García-Salcedo[2]

[1] Fundación Medina, Spain; [2] Instituto de Investigación Biosanitaria de Granada, Ibs. Granada, Spain; [3] Universidad de Almería, Spain

Sleeping sickness or African trypanosomiasis is a serious health problem with an added socio-economic impact in sub-Saharan Africa, due to direct infection in both humans and their domestic livestock. There is no vaccine available against these protozoan parasites African trypanosomes and the main reason is the ability of the parasite to change the major surface glycoprotein (VSG) avoiding the antibody-mediated response. The current drugs used to treat the disease are effective, but most of them present resistance, toxicity and specificity problems. Therefore, there is a clear need of novel, safe, and affordable treatments. In a search for new molecules with trypanocidal activity, we have performed a high throughput screening of 2000 microbial extracts from a fungi and actinomycetes natural products library. Several known active molecules were identified, including Cordycepin, Curvicollide A-C, Chaetocin, 11-Deoxyverticillin A and Verticillin and also a new member of

the curvicollide family with an unreported molecular structure that we have named Curvicollide D. The new compound showed an effective concentration 50 (EC50) of 1 mM. This concentration range had no effect on the growth of the human hepatoma G2 cells, being the EC50 for this cell line 16-fold higher. Treatment of African trypanosomes with Curvicollide D induced cell cycle arrest, causing an accumulation of cells in the G2/M phase. The new compound also had an effect on the morphology of the trypanosomes by inducing the appearance of aberrant cells that contain several nuclei and kinetoplasts. At intracellular level, Curvicollide D caused the disruption of the nucleolar structure after 3hour of treatment. An analysis of nucleolus function revealed that RNA polymerase I (Pol I) transcription was inhibited at the ribosomal locus, but also at the locus where the variant surface glycoprotein is expressed, which is also transcribed by Pol I. We next study the effect on RNA Pol II transcription, finding that it was inhibited too. Finally, FID assay demonstrated that curvicollide D is an intercalator of duplex DNA. Taken together, this data demonstrate that Curvicollide D binds DNA and inhibits transcription, causing cell death. In addition, these results provide for the first time an insight on the mechanism of action of the members of the Curvicollide family.

Poster 84 : Drug target deconvolution studies in *Leishmania* donovani

Presenter: **Miss Luciana Paradela**, *PhD student, University of Dundee*

L Paradela[2]; S Carvalho[2]; R Wall[2]; J Martin[1]; I Gilbert[2]; S Wyllie[2]

[1] Global Health R&D, GlaxoSmithKline, Spain; [2] School of Life Sciences, University of Dundee, UK

Visceral leishmaniasis (VL) is caused by protozoan parasites from the *Leishmania* genus and is potentially fatal if left untreated. Despite the significant impact of VL on morbidity and mortality in endemic countries, there are current few efficacious antil*eishmania*l drugs available. New drugs are urgently needed, however, the development of effective treatments for VL is impeded by the lack of robustly validated drug targets in *Leishmania* parasites. Phenotypic high-throughput screening of 1.8 million compounds against *L. donovani* was performed by GlaxoSmithKline leading to the identification of approximately 200 compounds demonstrating antil*eishmania*l activity. These compounds were subsequently assembled and made available to the scientific community for further study (Leish-box). Since there is no associated information regarding the molecular targets of these promising phenotypically-active compounds, studies to understand their mechanism(s) of action (MoA) are required. With this in mind, we selected seven Leish-box compounds for comprehensive drug target deconvolution studies. Here, we will focus on the outcome of our studies with compound GSK2920487A (C5). Using a variety of genome-wide genetics, chemical proteomics and biochemistry-based approaches, we identified the likely molecular target of C5 as lanosterol synthase, a key enzyme in sterol biosynthesis. Details of these studies and our subsequent work to validate lanosterol synthase as the target of C5 will be presented. Further, we will discuss the feasibility of exploiting lanosterol synthase as drug target in *Leishmania*.

Poster 85 : Intricate subcellular trafficking of queuosine modified tRNAs in *Trypanosoma brucei*

Presenter: **Dr Zdenek Paris**, *Principal investigator, Biology Centre CAS, Institute of Parasitology*

Z Paris[1]; E Hegedusova[1]; S Kulkarni[1]; J Alfonzo[2]

[1] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; [2] The Ohio State University, United States

Proteins and RNAs are routed across the nuclear envelope via the nuclear pore complex and require transport receptors belonging to the karyopherin family (exportins). Only a limited set of export factors, conserved in other organisms, is identifiable in trypanosomes by bioinformatics. Thus our knowledge of tRNA nuclear export in these organisms remains limited. We show here that, like in other eukaryotes, canonical tRNA exporter Xpo-t is not essential in trypanosomes and resulted neither in disruption of mature tRNA export to the cytoplasm nor intron-containing tRNA accumulation in the nucleus; phenotypes commonly observed with analogous yeast mutants. Also similar to yeast, Mex67-Mtr2, the main mRNA transporter in other systems, has a role in tRNA export in *T. brucei* with one major distinction, in *T. brucei* there is a clear separation of functions between Mex67 and Mtr2. The latter still serves a general role as a tRNA and/or mRNA export factor, but down-regulation of its partner, Mex67, leads to the specific accumulation of queuosine (Q)-containing tRNAs in the nucleus. In addition, compared to cytosol, tRNAs fully modified with Q, are preferentially imported into the mitochondria. The absence of Q in mitochondria-imported tRNAs also leads to a decrease in *de novo* synthesized mitochondrial proteins implying the role of Q in mitochondrial translation and physiology. Taken together, our data highlights the significance of intracellular trafficking in determining the fate and function of tRNAs via differential modification.

Poster 86 : Highly efficient conditional gene deletion combining di-CRE and CRISPR-Cas9 systems in Trypanosomatids

Presenter: **Mr Yvon Sterkers**, *PI, MiVEGEC Univ. Montpellier*

Y Sterkers[2]; A Yagoubat[2]; M F Lévêque[2]; R M Corrales[2]; L Berry[1]; P Bastien[2]

[1] LPHI & MEA, CNRS University of Montpellier, France; [2] MIVEGEC, IRD, CNRS University of Montpellier, France

CRISPR-Cas9 genome edition has been successfully implemented to Trypanosomatids. To knockout essential genes, we have developed an inducible system combining the Di-CRE recombinase and CRISPR-Cas9. We used a *Leishmania mexicana* cell line expressing Di-CRE, Cas9 and T7 polymerase and provided donor DNAs and sgRNAs as PCR products. With our system, LoxP sequences can classically flank the GOI. However, since UTR modifications can interfere with the function and localization of certain proteins of interest, we propose to preserve the UTRs. To do so we introduced the LoxPs in the least transcribed areas of the intergenic regions surrounding the GOI. Our methodology is versatile due to the development of 'universal' template plasmids for donor DNA cassettes, PCR based and highly efficient. We are developing the same strategy in *T. brucei* as an alternative for RNAi.

Poster 87 : Analysis of released peptidases and their role in the transmission biology of African trypanosomes

Presenter: **Miss Mabel Deladem Tettey**, *PhD Student, The University of Edinburgh*

M D Tettey[1]; F Rojas[1]; K R Matthews[1]

[1] Institute of Immunology and Infection Research, The University of Edinburgh, UK

Molecules released by African trypanosomes have an identified role in parasite virulence and quorum sensing and may also be important at various stages of the parasite lifecycle. Some of these secreted proteins include

peptidases, which contribute to creating the density-sensing signal in the bloodstream. Here, we analysed proteins released by the various lifecycle stages of the parasite. Mass spectrometric analysis of the *T. brucei* secretome identified a total of 12 peptidases significantly enriched in stumpy forms and at 3 hours into synchronous differentiation to procyclic forms. We validated the release of these 12 peptidases using CRISPR mediated *in situ* gene tagging in pleomorphic *T. brucei* . Exploring the roles these peptidases may play in parasite differentiation highlighted 3 peptidases that triggered enhanced parasite differentiation in the mammalian bloodstream with 2 potentially being major contributors to the quorum sensing signal.

Poster 88 : Iron-dependent control of transferrin receptor expression in *Trypanosoma brucei*

Presenter: **Dr Calvin Tiengwe**, *Research fellow, Imperial College London*

C Tiengwe[1]; C Gilabert Carbajo[2]; Y Madbouly[2]; M Tinti[3]

[1] Imperial College London, UK; [2] Imperial College London, UK; [3] School of Life Sciences, University of Dundee, UK

Iron is an essential co-factor for many enzymatic reactions but is potentially cytotoxic in excess. Consequently, intracellular iron levels must be tightly regulated. This typically occurs via interactions of trans-acting iron regulatory proteins (IRPs) and cis-acting iron responsive elements (IRE) modulating mRNA stability of major components of iron uptake, storage, and recycling pathways. In *T. brucei*, an evolutionarily divergent transferrin receptor (TbTfR) mediates uptake of host transferrin as a nutritional source of iron, and its expression is modulated by iron availability. However, previous work indicates TbTfR mRNA regulation does not follow the IRP/IRE paradigm, although it is mediated via its 3'-UTR. Here, we address the underlying mechanism for TbTfR upregulation in response to iron deficiency. We show that blocking transcription (actinomycin D) and splicing (Sinefungin) leads to a significant increase in TbTfR mRNA half-life consistent with a requirement for a trans-acting stabilising factor. Conversely, blocking translation (cycloheximide) does not affect TbTfR protein decay suggesting that active protein synthesis is required for maintaining high TbTfR expression. To understand how *T. brucei* controls TbTfR mRNA expression, we performed genome-wide transcriptome and proteomic studies and identified parasite-specific iron responsive factors: two RNA binding proteins (RBP), an RNA helicase, a phosphatase and four hypothetical proteins. We independently validated these putative IRPs using quantitative-PCR and immunoblotting following iron starvation. Finally, we show that one candidate TbRBP5 and TbTfR mRNA and protein levels are co-ordinately regulated suggesting that TbRBP5 may regulate a subset of genes with an iron-related function. Studies are underway to characterise its mechanism.

Poster 89 : Can drug resistance in *Leishmania* have an impact on the transmission potential by the sand fly vector?

Presenter: **Miss Lieselotte Van Bockstal**, *PhD student, University Antwerp*

L Van Bockstal[3]; S Hendrickx[3]; J Sadlova[1]; H Aslan[3]; P Volf[1]; S Kamhawi[2]; L Maes[3]; G Caljon[3]

[1] Department of Parasitology, Charles University, Prague, Czech Republic; [2] Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States; [3] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium

Introduction: The acquisition of drug resistance may be accompanied by changes in the fitness of *Leishmania* parasites, hence impacting on the propagation of the trait in nature. Former studies already indicated that paromomycin resistant (PMM-R) visceral *Leishmania* strains display enhanced *in-vitro* and *in-vivo* intramacrophage growth potential, while miltefosine-resistant (MIL-R) strains exhibit a decreased fitness. To understand the epidemiological implications of resistance, the development of MIL-R and PMM-R *L. infantum* and *L. donovani* strains was studied in the sand fly vectors *Lutzomyia longipalpis and Phlebotomus pernisiosus* or *Phlebotomus argentipes*. The aim was to estimate the potential of these strains to circulate, similar to reports about transmission potential of antimony-resistant *L. infantum*.
Methods: Development of wildtype and MIL-R/PMM-R isogenic strains was investigated in the natural sand fly vectors by recording percentage of infected flies, gut/stomodeal valve colonization and proportion of infective metacyclics. Stability of the R-phenotype after sand fly passage and transmission studies to rodents were conducted as well.
Results: The experimentally selected MIL-R *L. infantum* strain (LEM3323), harboring a frameshift mutation in the MIL transporter (*MT*) gene, failed to efficiently infect sand flies and BALB/c mice. In contrast, a clinically isolated MIL-R strain (LEM5159) with a frameshift mutation in the *ROS3* gene (encoding a subunit of the MT-complex), efficiently developed mature metacyclic infections and could be successfully transmitted to mice although showing a low infectivity. Similarly, experimentally selected PMM-R *L. infantum* and *L. donovani* strains (with unknown mechanisms of resistance) were able to complete development in the sand fly vectors. The MIL-R and PMM-R phenotypes were both maintained after sand fly passage.
Conclusion: These results highlight the transmission risk of MIL-R and PMM-R *Leishmania* strains by sand flies. The obtained data indicate that this risk largely depends on the resistance-conferring (genetic) alterations.

Poster 90 : Within-host interactions between *Trypanosoma brucei* and *Trypanosoma congolense* in chronic coinfections *in-vivo*.

Presenter: **Mr Frank Venter**, *PhD Student, University of Edinburgh*

F Venter[1]; E Silvester[1]; K R Matthews[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, UK

Surveys of different geographical regions show that African livestock are often infected with multiple trypanosome strains and, indeed, species. This generates the potential for interaction and competition between distinct trypanosome isolates and species. To explore this, we employed marked trypanosome lines to follow co-infection dynamics between *T. brucei* and *T. congolense in-vivo*. This indicated, firstly, that *T. brucei* exhibits enhanced quorum sensing (QS) in response to *T. congolense* in the environment. Conversely, in multiply-replicated infections sustained over 40 days, *T. brucei* initially suppresses *T. congolense*, but subsequently *T. congolense* dominates until *T. brucei* eventually re-emerges potentially as a result of selection. These oscillations indicate a complex interaction between coinfecting species likely contributed by shared QS signals, distinct within-host compartmentation and immune interaction. In the field, dynamic parasite competition in response to coinfection or trypanocides could generate unpredictable consequences for disease virulence, transmissibility and zoonotic potential.

Poster 91 : *Leishmania* major ATR kinase modulates the parasite response to replication stress.

Presenter: **Mr Gabriel Lamak Almeida da Silva**, *Phd Student, University of São Paulo*

G Almeida da Silva[1]; M S Bastos[1]; S Virgilio[1]; J A Black[2]; K Crouch[2]; J D Damasceno[2]; R McCulloch[2]; L R Tosi[1]

[1] University of São Paulo, Brazil; [2] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK

The ATR (Ataxia-Telangiectasia and Rad3-related) protein kinase is a master regulator of the eukaryotic response to DNA injuries that orchestrates checkpoint activation, cell cycle arrest, replication fork stabilization and DNA repair recruitment, thereby mediating genome maintenance and stability. The function of ATR not been well explored in the protozoan parasite *Leishmania*, whose plastic genome presents hallmarks of instability, such as gene and chromosome copy number variation, mosaic aneuploidy and chromosome rearrangements. In our attempt to characterize the activities provided by the ATR pathway in *Leishmania major*, we have endogenously epitope tagged the gene encoding of ATR, revealing that the protein is mostly located in the nuclear compartment. Attempts to select an ATR-null mutant in *L. major* were unsuccessful, which suggests the protein provides essential functions in the cell. Genome editing was used to generate heterozygous cells in which the ATR kinase domain was truncated in one of its two alleles, generating an ATR-deficient cell line (ATR+/-). ATR deficiency significantly affected the pattern of accumulation and resolution of single-stranded DNA, as well as other genotoxic markers, such as gH2A (phosphorylated histone H2A). We also mapped the genomic localisation of the single stranded DNA-binding protein RPA in response to replication stress using chromatin immunoprecipitation followed by sequencing (ChIP-seq). ATR-deficient cell lines showed decreased RPA chromatin enrichment upon replication stress especially around the single map origin in each chromosome and at chromosome ends. All together this indicating the kinase activity is required for proper recruitment and/or stabilisation of RPA at sites of genome instability.

Poster 92 : Miltefosine restores the infectivity of miltefosine resistant *Leishmania* parasites by attenuating the innate immune response

Presenter: **Mr Dimitri Bulté**, *PhD Student, University of Antwerp*

D Bulté[1]; L Van Bockstal[1]; L Maes[1]; G Caljon[1]

[1] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium

Introduction: Miltefosine (MIL) is an oral drug that is used to treat visceral leishmaniasis (VL) but is failing to permanently clear parasites in an increasing number of patients. Especially immunocompromised patients are prone to relapse. Parasites isolated from these relapse cases do not seem to display an increased resistance, but are hypothesized to interact differently with the immune system in favor of survival inside macrophages even under drug pressure. Only a limited number of MIL resistant (MIL-R) parasites have been isolated so far, although mutations in a single gene can render parasites MIL resistant. In this study, the infection characteristics of parasites with acquired resistance were explored under conditions with and without MIL pressure.
Methodology: To study the impact of drug treatment on survival of MIL sensitive (MIL-S) and MIL-R parasites, double-reporter lines were generated expressing both the PpyRE9 (*in-vivo* bioluminescent imaging) and the DsRed gene (flow cytometry, fluorescence microscopy). *In*-vivo bioluminescent infection studies were complemented with the analysis of cellular immunological responses in the liver and spleen. Major inflammatory cytokine responses were monitored in plasma and correlated with the host infection dynamics. The role of selected immune cell types [natural killer (NK) cells, NKT cells] during the early stage of infection was assessed

by specific depletion protocols and by flow cytometry. The impact of MIL was evaluated by *in-vitro* pretreatment of parasites prior to inoculation of C57Bl/6 and BALB/c mice.
Results: The MIL-R parasite line showed a reduced infectivity when compared to the isogenic MIL-S line. This reduced infectivity was accompanied by an increased innate immune response during the early stage of the infection, characterized by elevated IFN-γ levels leading to the rapid clearance of MIL-R parasites from the liver and abrogating further dissemination to the spleen and bone marrow. NK and/or NKT cells depletion experiments identified these cells as the main source of IFN-γ during infection onset and, in the absence of these cells, infectivity of the MIL-R line was partially restored. Finally, infections with MIL-R parasites under drug pressure revealed that both *in-vivo* MIL-treatment and *in-vitro* MIL pre-exposure significantly rescues the *in-vivo* infectivity. Upon infection under MIL-treatment, the early induction of IFN-γ was less prominent, indicating a reduced activation of NK and NKT cells and a reduced clearance of the MIL-R parasite. These observations emphasize the risk of MIL treatment in sustaining infections with MIL-R parasites.

Poster 93 : Influence of the draining lymph nodes and organized lymphoid tissue microarchitecture on susceptibility to intradermal *Trypanosoma brucei* infection

Presenter: **Dr Omar Alfituri**, *Postdoctoral Research Fellow, The Roslin Institute*

O Alfituri[1]; E Paxton[1]; B M Bradford[1]; L J Morrison[1]; N A Mabbott[1];

[1] The Roslin Institute, University of Edinburgh, UK

African trypanosomes are single-celled extracellular protozoan parasites that are transmitted via the tsetse fly vector. *Trypanosoma brucei* subspecies cause trypanosomiasis in humans and animals across sub-Saharan Africa, inflicting substantial disease and economic strains. Mammalian infection begins when the tsetse fly injects trypanosomes into the skin dermis. The parasites invade the circulatory and lymphatic systems, reaching the draining lymph nodes before disseminating systemically. How this occurs is not known. Lymphotoxin-β-receptor signaling (LTBR) is essential for lymphoid organogenesis and the maintenance of secondary lymphoid tissue microarchitecture. For example, LTB-/- mice lack most lymph nodes and have grossly disturbed splenic microarchitecture. As a consequence of these disturbances LTB-/- have impaired antibody isotype class-switching. Here, LTB-/- mice were used to determine the influence of the draining lymph node and antibody isotype class-switching in susceptibility to intraperitoneal and intradermal infection with African trypanosomes. Data show that in wild-type mice the route of inoculation and parasite dosage greatly impacts on the infection dynamics. Furthermore, disease susceptibility and pathogenesis were exacerbated in LTB-/- mice. This effect coincided with their ability to produce significant serum levels of Ig isotype class-switched parasite-specific antibodies. Thus, our data suggest that organized splenic microarchitecture and the production of parasite-specific Ig isotype class-switched antibodies are essential for the control of intradermal African trypanosome infections.

Poster 94 : *Leishmania* kinesin FAZ7 is required for survival in promastigote and amastigote stages, motility, proliferation in the sand fly vector and virulence in the mammalian host

Presenter: **Mrs Rosa Corrales**, *Leishmania kinesin FAZ7, University of Montpellier*

R Corrales[1]; S Vaselek[2]; R Neish[3]; L Berry[4]; L Crobu[1]; N Kuk[1]; P Volf[2]; JC Mottram[3]; Y Sterkers[1,5]; P Bastien[1,5]

[1]MiVEGEC, Univ. Montpellier, IRD, CNRS, Montpellier, France, [2]Charles University, CZ-12844 Prague, Czech Republic; [3]Centre for Immunology and Infection, University of York, York YO10 5DD, United Kingdom, [4] LPHI - Laboratory of Pathogen Host Interactions & MEA - Microscopie Electronique Analytique, CNRS, Univ. Montpellier, Montpellier, France, [5]Department of Parasitology-Mycology, Montpellier, France

The flagellum attachment zone (FAZ) is a key morphogenetic structure regulating both cell length and organelle positioning in *Trypanosoma brucei*. Intriguingly, *Leishmania* genomes encode homologs of most FAZ proteins albeit lacking the laterally attached flagellum characteristic of *T. brucei.* In *Leishmania*, the flagellum emerges from the flagellar pocket at the cell anterior end and possesses a discrete FAZ filament that undergoes structural reorganization in the transition from the (insect) promastigote to (mammalian) amastigote form. However, the role of FAZ proteins in *Leishmania* is largely unknown. Among the 34 genes encoding FAZ proteins, FAZ7 contains a kinesin motor domain and localizes to the distal end of the *T. brucei* FAZ filament; its function also remains unknown. Strikingly, kinesin FAZ7 orthologs are duplicated in *Leishmania* genomes. Here, we show that *Leishmania* kinesin FAZ7 orthologs display different localizations in *Leishmania mexicana*. Kinesin FAZ7A localizes at the basal body and kinesin FAZ7B localizes at the distal part of the flagellar pocket, suggesting functional differences for these two paralogs. By using a PCR-based CRISPR-Cas9 approach, we generated both FAZ7 single and double knock-out parasites. Null mutants of basal body-resident kinesin FAZ7A displayed normal growth rate and unchanged flagellar length. By contrast, deletion of kinesin FAZ7B and double knock-out impaired cell growth in both promastigote and amastigote forms to a similar extent. *In situ* complementation of the FAZ7B knock-out with a FAZ7B mutated in the ATP binding site of the motor domain was unable to restore growth rate in promastigote and amastigote stages, suggesting that FAZ7B is a functional kinesin. In the promastigote form, FAZ7B null mutants grew significantly longer flagella and displayed reduced motility. In addition, they exhibited a grossly perturbed flagellar pocket and reduced endocytosis rate. Finally, loss of FAZ7B altered the localization of the flagellar pocket collar proteins Bilbo and FPC4. This pleomorphic phenotype has important consequences in the insect-vector and the host: FAZB null mutants displayed reduced proliferation and development in the sand fly and lower virulence in mice. These data highlight the role of FAZ proteins as important factors for a successful life cycle within the sand fly and the mammalian host.

Poster 95 : UBC2 and UEV1 are a ubiquitin E2 enzyme and E2 variant respectively that are essential for L. mexicana amastigote differentiation.

Presenter: **Miss Rebecca Burge**, *PhD student, The University of York*

R Burge[1]; V Geoghegan[1]; A Damianou[1]; J Hilton[2]; T Wilkinson[1]; B Rodenko[3]; J Mottram[1];

[1] The University of York, UK; [2] Ubiquigent Limited, UK; [3] UbiQ Bio BV, UK

Ubiquitination is a regulatory post-translational modification found in all eukaryotic cells. For ubiquitination to occur, the sequential action of E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin ligase enzymes are typically required. However, little is known about the role of these enzymes in Leishmania. Here we introduce Leishmania mexicana UBC2 and UEV1, catalytic and non-catalytic E2 family members respectively, as conserved orthologues of human UBE2N and UBE2V1/UBE2V2. A bar-seq screen of 46 CRISPR/Cas9-generated ubiquitin system null mutants revealed that UBC2 and UEV1 are essential for amastigote differentiation, suggesting a Leishmania-specific function. However, our structure of the UBC2/UEV1 heterodimer reveals highly conserved interactions in the UBC2/UEV1 interface, highlighting the importance of stable UBC2/UEV1 interaction across evolution. In vitro, UBC2 is able to receive ubiquitin from the L. mexicana E1 UBA1 but not UBA2, demonstrating a level of specificity for E1-E2 interactions in this species, and UBC2/UEV1 can form K63-linked diubiquitin in vitro. Interestingly, UBC2 catalyses substrate ubiquitination in cooperation with human E3s including RNF8 and BIRC2, with UEV1 inhibiting this ability. In summary, we present a biochemical and structural analysis of two ubiquitination proteins essential for Leishmania differentiation. Hints towards potential pathway components and function provided by the UBC2 and UEV1 interactomes will also be discussed.

Poster 96 : Anatomy of a channel: how TbAQP2 facilitates entry of pentamidine and melaminophenyl arsenicals into *Trypanosoma brucei* spp.

Presenter: **Prof Harry De Koning**, *Professor of Parasite Biochemistry, University of Glasgow*

H P De Koning[3]; A H Alghamdi[3]; J C Munday[3]; D Gurvič[1]; G D Campagnaro[3]; M Carrington[2]; U Zachariae[1]

[1] Computational Biology Centre for Translational and Interdisciplinary Research, University of Dundee, UK; [2] Department of Biochemistry, University of Cambridge, UK; [3] University of Glasgow, Institute of Infection, Immunity and Inflammation, UK

Aquaglyceroporins are normally incapable of transporting molecules larger than glycerol through their pores. Cations are also precluded, by the presence of an Arg residue in the pore. However, TbAQP2 has been implicated in the sensitivity to pentamidine melaminophenyl arsenical drugs. Conversely, resistance to these drugs is associated with mutations in TbAQP2. Conflicting models of channel-like uptake and receptor-mediated endocytosis have been proposed. Here, we present evidence that pentamidine does, uniquely, traverse the AQP2 pore, aided by an unusually facilitating selectivity filter and the absence of the Aromatic/Arginine motif. We performed a mutational analysis of selectivity filter residues and molecular dynamics modelling. In addition, we conducted a thorough SAR with diamidine analogues to delineate the determinants for transport by TbAQP2. Phylogenetic analysis suggests that the selectivity filter changes were non-random.

Poster 97 : A case report of Trypansoma vivax in a sheep in Trinidad, West Indies

Presenter: **Dr Candice Sant**, *graduate student , The University of the West Indies*

C Sant[2]; V Kissoon[2]; M A Driscoll[2]; F Mohammed[2]; A Bally[2]; A K Persad[2]; R Suepaul[2]; I Pargass[2]; K Georges[2]; R Charles[2]; L Gyan[1]; V Lashley[1]; D Kangaloo[1]; M J Morris[2];

[1] Ministry of Agriculture, Land and Fisheries, Trinidad and Tobago; [2] The University of the West Indies, Trinidad and Tobago

Trinidad is just seven km off the coast of Venezuela. With the current humanitarian crisis, there has been an upsurge in the movement of people and livestock between Venezuela and Trinidad. The two main species of *Trypanosoma* in the ruminant population of South America are *T. evansi* and *T. vivax*, although to date, neither species have been identified in ruminants in Trinidad. These agents are mainly transmitted by biting dipteran flies. A 2 year old West African ewe presented to the UWI Veterinary Teaching Hospital with inappetence and lethargy. Physical examination revealed cachexia (body condition score of 1.5 / 5), pale mucous membranes and an undulating fever was noted on hospitalisation. Microscopic examination of a Wright-Giemsa stained blood smear revealed 1-3 trypanosomes/field at 100X. The parasite showed the rapid forward progressive movement characteristic for *T. vivax* which was confirmed by sequencing.

Poster 98 : Heme metabolism at the parasite-host interface: exploiting porphyrin auxotrophy in *Leishmania*

Presenter: **Dr José M. Pérez-Victoria**, *Staff Scientist , IPBLN-CSIC*

J M Pérez-Victoria[1]; M Cabello-Donayre[1]; L M Orrego[1]; I Cabello-Donayre[1]; G Juez-Castillo[1]; B Cautain[2]; F Vicente[2]

[1] IPBLN-CSIC, Spain; [2] MEDINA Foundation, Spain

Leishmania is transmitted to a mammalian host by a sand fly vector, and it develops as an intracellular parasite within macrophages. This process requires the acquisition of nutritional heme from the host as *Leishmania* lacks the capacity for *de novo* heme synthesis. Heme is an iron-coordinated porphyrin that serves as a prosthetic group of hemoproteins involved in many fundamental physiological processes, including cellular respiration, oxygen transport and oxidative stress response. In addition, heme can also be a cellular messenger involved in the regulation of important processes such as gene expression, differentiation, proliferation and signal transduction. Due to this essential role of the heme group, a detailed understanding of how these parasites harness host pathways for survival may lay the foundation for promising new therapeutic intervention against leishmaniasis. Accordingly, we try to identify the proteins involved in the use of host porphyrins by *Leishmania*, and validate these as potential drug targets. Thus, we modulate the expression levels of candidate proteins in the parasite (through Crispr-Cas9, etc.) and analyse their role in heme traffic and metabolism. We confirm their function using heterologous systems such as bacteria, yeast, *Trypanosoma* and/or *Xenopus* oocytes. We determine the viability of modified *Leishmania* parasites and their ability to infect and multiply in macrophages, to develop in the insect vector and to produce disease in animal models. We also use knock-out mice for genes important for heme metabolism in the host to analyse their role in *Leishmania* pathogenesis. Finally, we use some of these validated therapeutic targets, functionally expressed in yeast, for target-directed high throughput screening (HTS) in a cellular context to find inhibitors that kill the parasite. Our results show that many proteins involved in *Leishmania* heme transport and metabolism are crucial for the parasite's growth and replication within the host, and that it is possible to identify inhibitors of these proteins with potent leishmanicidal activity through an inexpensive yeast-based HTS platform that can help to bridge the gap between phenotypic and biochemical HTS assays.

Poster 99 : Withdrawn

Poster 100 : Withdrawn

Poster 101 : Trypanosomatid selenophosphate synthetase structure, function and interaction with selenocysteine lyase

Presenter: **Prof Otavio Thiemann**, *Associate Professor, São Carlos Physics Institute, University of São Paulo*

O Thiemann[3]; M T Alves da Silva[6]; I R Silva[6]; N K Bellini[6]; M L Pereira[6]; A L Lima[6]; T C Leandro de Jesus[6]; F C Costa[6]; T F Watanabe[4]; S R Valentini[4]; C F Zanelli[4]; N W Andrews[1]; J C Borges[2]; J P Chagas da Cunha[5]

[1] Department of Cell Biology and Molecular Genetics, University of Maryland, Brazil; [2] São Carlos Institute of Chemistry, University of São Paulo, Brazil; [3] São Carlos Physics Institute, University of São Paulo, Brazil; [4] School of Pharmaceutical Sciences, São Paulo State University, Brazil; [5] Special Laboratory for Applied Toxinology, Butantan Institute, Brazil; [6] Universidade de Sao Paulo, Brazil

Early branching eukaryotes have been used as models to study the evolution of cellular molecular processes. Strikingly, human parasite of the Trypanosomatidae family (*Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*) conserve the complex machinery responsible for selenocysteine biosynthesis and incorporation in selenoproteins (SELENOK/SelK, SELENOT/SelT and SELENOTryp/SelTryp), although these proteins do not seem to be essential for parasite viability under laboratory controlled conditions. Selenophosphate synthetase (SEPHS/SPS) plays an indispensable role in selenium metabolism, being responsible for catalyzing the formation of selenophosphate, the biological selenium donor for selenocysteine synthesis. We solved the crystal structure of the *L. major* selenophosphate synthetase and confirmed that its dimeric organization is functionally important throughout the domains of life. We also demonstrated its interaction with selenocysteine lyase (SCLY) and showed that it is not present in other stable complexes involved in the selenocysteine pathway, namely the phosphoseryl-tRNA^{Sec} kinase (PSTK)-Sec-tRNA^{Sec} synthase (SEPSECS) and the tRNA^{Sec}-specific elongation factor (eEFSec)-ribosome. Endoplasmic reticulum stress with ditiothreitol (DTT) or tunicamycin upon selenophosphate synthetase ablation in procyclic *T. brucei* cells led to a growth defect. On the other hand, only DTT presented a negative effect in bloodstream *T. brucei* expressing selenophosphate synthetase-RNAi. Although selenoprotein T (SELENOT) was dispensable for both forms of the parasite, SELENOT-RNAi procyclic *T. brucei* cells were sensitive to DTT. Together, our data suggest a role for the *T. brucei* selenophosphate synthetasein regulation of the parasite's ER stress response.

Poster 102 : Genome-wide profiling reveals post-transcriptional regulation by hundreds of 3'-untranslated sequences

Presenter: **Dr Anna Trenaman**, *Postdoctoral Research Assistant, University of Dundee*

A Trenaman[1]; R Wall[1]; D Horn[1]

[1] School of Life Sciences, University of Dundee, UK

Trypanosomatids constitutively transcribe almost all genes and utilise RNA-binding proteins (RBPs) to modulate gene expression. Relatively few regulatory UTRs have been identified and the RBPs that bind them remain largely uncharacterised. We developed UTR-seq, a genome-scale screening platform to profile regulatory 3'-UTRs in *T. brucei*. All known regulatory UTRs reported the expected positive or negative regulatory behaviour. Our screens also identified hundreds of new regulatory UTRs. The dataset reveals the specific UTR segments where regulatory capacity resides. As an example, cohorts of positive regulatory UTRs in the bloodstream-form are associated with genes encoding RBPs, VSGs, glycolytic enzymes, translation initiation factors, chaperones and cell cycle regulators. UTR-seq has now been applied to two major life cycle stages of *T. brucei*. The approach reveals the regulatory segments that underpin prominent regulons and stage-specific gene expression profiles.

Poster 103 : The Chagas Antigen and Epitope Atlas: deep serological surveys of human Chagas Disease populations

Presenter: **Dr Fernán Agüero**, *Assistant Professor, Universidad de San Martin -- CONICET*

F Agüero[5]; A D Ricci[5]; L E Bracco[5]; E Salas-Sarduy[5]; J Ramsey[3]; M Nolan[9]; F Torrico[1]; J Altcheh[2]; N Kesper Jr[8]; J C Villar[4]; J D Marco[6]; I Marcipar[7]

[1] Fundacion CEADES, Bolivia; [2] Hospital de Niños Ricardo Gutierrez, Argentina; [3] Instituto Nacional de Salud Pública, Mexico; [4] Universidad de Bucamaranga, Colombia; [5] Universidad de San Martin -- CONICET, Argentina; [6] Universidad Nacional de Salta, Argentina; [7] Universidad Nacional del Litoral, Argentina; [8] Universidade de São Paulo, Brazil; [9] University of South Carolina, United States

During an infection, the immune system produces antibodies against pathogens. With time, the immune repertoires of infected individuals become specific to the history of infections and thus represent a rich source of diagnostic markers. Until now, a complete description of antibody specificities in different individuals has been hindered by the lack of powerful tools. Here, using high-density peptide arrays we examined the global human antibody repertoire developed by Chagas Disease patients. Arrays displaying 2.8 million unique peptides from *Trypanosoma cruzi* strains CL-Brener (TcVI) and Sylvio X10 (TcI) were assayed with serum samples from infected subjects from Argentina, Bolivia, Brazil, Colombia, Mexico and the US, and negative and l*eishmania*sis samples. In the presentation we will describe this collection of antigens and epitopes. This dataset will enable serological studies of human antibody repertoires in Chagas Disease at an unprecedented depth and granularity.

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City Metro Map

Hotel Metro Stop

We would like to express ours gratitude to Alberto Sanchez López who donated unselfishly these pictures of Granada.