České Budějovice

BSP Trypanosomiasis & Leishmaniasis

Seminar 2016

BIOLOGY
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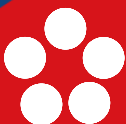
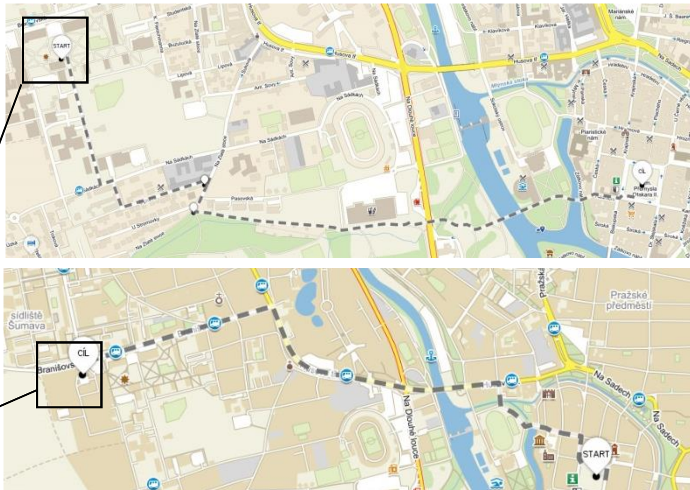


University of South Bohemia

Two routes from the town centre to the campus



The Campus

BIOLOGICKÉ CENTRUM AV ČR, v. v. i.

Sukova
Na Zlaté Stoce
Na Sádkách
Branišovská
J. Opletala
Student Accommodation
Canteen for
BSP meeting
Bus Stop no. 3 from Railways
Bus Stop no. 3 to Railways St.
Zadní vjezd Back entrance
Hlavní vjezd Main entrance
P

BUDOVY BIOLOGICKÉHO CENTRA AV ČR / **BUILDINGS OF BIOLOGY CENTRE ASCR**

No.	Budova	Building
1	Vstupní hala / Recepce	Entrance Hall / Reception
2	Ředitelství	Headquaters
	Středisko služeb	Servis Unit
3	Kongresové centrum	Congress Centre
4	Ústav molekulární biologie rostlin	Institute of Plant Molecular Biology
5	Entomologický ústav	Institute of Entomology
6	Parazitologický ústav	Institute of Parasitology
7	Laboratoř elektronové mikroskopie	Laboratory of Electron Microscopy
8	Hydrobiologický ústav	Institute of Hydrobiology
9	Ústav půdní biologie	Institute of Soil Biology
10	Úsek transferu technologii	Technology Transfer Office
	Garáže	Garages
11	Dílny	Workshops
12	Energocentrum	Energy Centre
13	Ubytovna / Dětská skupina Motýl	Dormitory / Motýl Children Group
14	Skladové haly	Storage Halls

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No.	Budova	Building
15	JU | Rektorát / Filozofická fakulta	USB | Rector's Office / Faculty of Philosophy
16	JU | Akademická knihovna	USB | Academic Library
17	JU | Koleje	USB | Hall of Residence
18	JU | Aula	USB | Lecture Hall
19	JU | Přírodovědecká fakulta, budova A	USB | Faculty of Science, Building A
20	JU | Přírodovědecká fakulta, budova B	USB | Faculty of Science, Building B
21	JU | Přírodovědecká fakulta, budova C	USB | Faculty of Science, Building C
22	JU | Zemědělská fakulta	USB | Faculty of Agriculture
23	Centrum výzkumu globální změny AV ČR	Global Change Research Centre ASCR

BIOLOGY CENTRE ASCR
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Dear friends,

We are happy to welcome you at the Trypanosomiasis and Leishmaniasis Seminar of the British Society for Parasitology held in České Budějovice, the "capital" of South Bohemia. More of you signed up for it than we expected, which is great and shows that the interest in our favorite protists is not waning.
The meeting is organized by the Institute of Parasitology and will be held at the Biology Centre, both part of the Czech Academy of Sciences. The conference site is located on the outskirts of the city (about 90,000 inhabitants), walking distance from the historical centre (~ 25 minutes walk) and is well connected by public transportation. The program will be quite intense, following the traditional single session policy, but there will be enough time for discussions, social events and a party at a chateau just for us.
The organizers will do all they can to ensure this is an enjoyable meeting for all of you, and please feel free to contact us with any questions. We look forward to meeting you all during the conference.
Best wishes,

Julius Lukeš

Sunday (September 4) (3:00-5:00) program **5**
Plenary (7:00 – 7:40 PM) 5
Monday (September 5) program **6**
Session 1 – *Comparative and Population Genomics* (9:00 - 11:00 AM) 6
Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM) 6
Session 3 – *Host-Parasite Interactions* (2:00 till 4:00 PM) 7
Poster Session (4:15 – 6:00 PM) 7
Tuesday (September 6) program **8**
Session 3 – *Cell Biology* (9:00 - 11:00 AM) 8
Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM) 8
Session 4 – *Gene Expression* (2:00 till 4:00 PM) 9
Wednesday (September 7) program **9**
Session 6 - *Interactions in the Field, Epidemiology and Cell Biology* (9:00 - 11:00 AM) 9
Session 2 - *Cell Biology* (11:30 TILL 1:00 PM) 10
Abstracts **11**
Oral Presentations in time order **11**
Sunday (September 4) **11**
Plenary (7:00 – 7:40 PM) 11
Monday (September 5) **12**
Session 1 – *Comparative and Population Genomics* (9:00 - 11:00 AM) 12
Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM) 14
Session 3 – *Host-Parasite Interactions* (2:00 till 4:00 PM) 17
Tuesday (September 6) **20**
Session 3 – *Cell Biology* (9:00 - 11:00 AM) 20
Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM) 22
Session 4 – *Gene Expression* (2:00 till 4:00 PM) 25
Wednesday (September 7) **27**
Session 6 - *Interactions in the Field, Epidemiology and Cell Biology* (9:00 - 11:00 AM) 27
Session 2 - *Cell Biology* (11:30 TILL 1:00 PM) 30
Posters **33**
Index **94**

Program

September 2016 BSP meeting – České Budějovice

Sunday (September 4) (3:00-5:00) program

Kinetoplast Breeders - **Meeting of P.I.'s about joint grant applications, EU calls etc.**
Mark Carrington **Introduction**
(3:00)

Harry de Koning **Academic perspective: the PDE4NPD consortium**
(3:05)

David Bailey **SME perspective: Building international scientific networks to tackle neglected parasitic diseases: the EU NPD Project as a Case Study**
(3:30)

Coffee Break

Gerald Spaeth **LeishDrug, LeishRIIP and LeiSHield**
(4:30)

Keith Gull **Closing remarks**
(5:00)

Registration (4:00 – 8:00 PM)

Plenary (7:00 – 7:40 PM)

František Štěpánek (plenary lecture**) Remote control of reaction-diffusion processes with chemical microrobots**
(7:00 – 7:40 PM)

Welcome Party (7:50 – 12:00 PM)

Monday (September 5) program

Session 1 – *Comparative and Population Genomics* (9:00 - 11:00 AM)

Vyacheslav Yurchenko (invited): **Comparative genomics of trypanosomatids: identification of novel virulence factors and new insights into evolution of *Leishmania*** (A10575); (9:00 – 9:25)

Gerald Spaeth: **Asexual maintenance of genetic diversity in *Leishmania donovani*** (A10958);(9:25 – 9:40)

Michael Grigg (invited): **Extant heterozygosity and genetic hybridization have dramatically impacted "Old World" *Leishmania* natural population genetics** (A10579); (9:40 – 10:05)

Malgorzata Domagalska: **Genome and transcriptome dynamics throughout the life cycle of *Leishmania donovani*** (A10843); (10:05 -10:20)

Louisa Messenger: **Pan-American migration promotes the spread of pathogenic *Trypanosoma cruzi* hybrid strains** (A10793); (10:20 – 10:35)

Caroline Dewar: **What do kinetoplastids need a kinetoplast for? Life cycle progression of *Trypanosoma brucei* in the presence and absence of mitochondrial DNA** (A10625); (10:35 – 10:50)

Coffee Break

Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM)

David Horn (invited): **Decoding antitrypanosomal drug action and resistance** (A10577); (11:25 – 11:50)

Michal Shapira: **A network of cap-binding proteins in the service of stress physiology in trypanosomatids** (A10838); (11:50 – 12:05)

Martin Taylor (invited): **Iron uptake in *Trypanosoma brucei*** (A11141); (12:05 – 12:30)

Susanne Kramer: **Where is the trypanosome decapping enzyme?** (A10673); (12:30 – 12:45)

Helena Reis: **A novel telomere-binding protein, TelBP1, modulates VSG expression site silencing kinetics in *Trypanosoma brucei*** (A11035); (12:45 – 1:00 PM) (12:45 – 1:00 PM)

Lunch (1:00 -2:00 PM)

Session 3 – *Host-Parasite Interactions* (2:00 till 4:00 PM)

Matthew Higgins (invited): **A receptor's tale: structural and functional diversification of the trypanosome haptoglobin-haemoglobin receptor** (A10578)
(2:00 – 2:25 PM)

Jayne Raper: **Germline transmission of targeted baboon apolipoprotein L-1 in mice protects against African trypanosomes** (A11080);
(2:25 – 2:40 PM)

Steven Kelly (invited): **Your genes are what you eat: The metabolic basis of genome composition and codon bias** (A10576);
(2:40 – 3:05 PM)

Elisha Mugo: **The RNA binding protein-RBP10 defines bloodstream form trypanosome identity** (A11051);
(3:05 – 3:20 PM)

Thomas Bartossek: **The complete structures of VSGs MITat1.1 and ILTat1.24: combining density and mobility through flexibility** (A11069);
(3:20 – 3:35 PM)

Pegine Walrad: **Regulating the regulators: LmjPRMT7 in *Leishmania* parasite virulence and trans-regulator methylation"** (A11186);
(3:35 – 3:50 PM)

***Poster Session* (4:15 – 6:00 PM)**

Free Evening

Tuesday (September 6) program

Session 3 – *Cell Biology* (9:00 - 11:00 AM)

Mark C. Field (invited): **Drugs and trafficking in trypanosomes** (A10580);
(9:00 – 9:25)

Lucy Glover: **Replication protein A DNA repair foci persist through the cell-cycle and reflect a 'divide and repair later' strategy in trypanosomes** (A11081);
(9:25 – 9:40)

Brooke Morriswood (invited): **The real flux capacitor? A putative molecular valve in trypanosomes** (A10581);
(9:40 – 10:05)

Olivia J. S. Macleod: **Molecular characterization of the complement factor H receptor in the bloodstream and procyclic forms of *Trypanosoma brucei*** (A11132);
(10:05 – 10:20)

Alena Zíková: **Cultured bloodstream *Trypanosoma brucei* adapt to life without their mitochondrial translation release factor** (A11177);
(10:20 – 10:35)

Tom Beneke: **Discovery of new *Leishmania* motility mutants in a CRISPR-Cas9 knockout screen of the flagellar proteome** (A11091);
(10:35 – 10:50)

Coffee Break

Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM)

Paula MacGregor (invited): **Exploitation of receptor-mediated ligand uptake for the targeted delivery of antibody- toxin conjugates into *Trypanosoma brucei*** (A10582);
(11:25 – 11:50)

Anna Trenaman: **The ZC3H39/40 RNA-binding complex and the control of electron transport chain expression in African trypanosomes** (A11134);
(11:50 – 12:05)

Torsten Ochsenreiter (invited): **Mitochondrial organelle biogenesis during the cell cycle in *Trypanosoma brucei*** (A10584);
(12:05 – 12:30)

Priscila Peña-Diaz: **A leucine aminopeptidase is involved in kinetoplast DNA segregation in *Trypanosoma brucei***
(12:30 – 12:45)

Zdeněk Paris: **Queuosine: The role of an essential tRNA modification in *Trypanosoma brucei*** (A11185);
(12:45 – 1:00 PM)

Lunch (1:00 -2:00 PM)

Session 4 – *Gene Expression* (2:00 till 4:00 PM)

Keith Matthews (invited): **Understanding the signalling pathway controlling African trypanosome quorum sensing and life-cycle differentiation** (A10583);
(2:00 – 2:25 PM)

Vladimír Varga: **The flagella connector of *Trypanosoma brucei* is a kinesin-powered junction distinct from the axonemal capping structure** (A11158);
(2:25 – 2:40 PM)

André Schneider: **The non-canonical mitochondrial inner membrane protein translocase of trypanosomatids contains two essential rhomboid-like proteins** (A10798);
(2:40 – 2:55 PM)

Jack Daniel Sunter: **TrypTag: Genome-wide protein localisation in the trypanosome** (A10807);
(2:55 – 3:10 PM)

Bungo Akiyoshi: **Understanding unconventional kinetoplastid kinetochores** (A10947);
(3:10 – 3:25 PM)

Janaina de Freitas Nascimento: **Codon usage links translation and mRNA decay in *Trypanosoma brucei*** (A11131);
(3:25 – 3:40 PM)

Departure (5:00 PM) Via bus to the evening program at a chateau (6:00 till 11:00)
(Earlier arrivals will be possible)

Wednesday (September 7) program

Session 6 - *Interactions in the Field, Epidemiology and Cell Biology* (9:00 - 11:00 AM)

Michael Miles (invited): **'Mind the gap': the relevance of genetic exchange and comparative genomics to combatting Chagas disease and visceral leishmaniasis** (A10586);
(9:00 – 9:25)

Harry De Koning: **CARP3 is a plasma-membrane and adenylate-cyclase associated regulator of cyclic AMP signalling in *Trypanosoma brucei*** (A10794);
(9:25 – 9:40)

Sarah Schuster: **From solitary swimmers to swarms and back – trypanosomes on thejourney through the tsetse fly** (A11008);
(9:40 – 9:55)

Annette Macleod: **The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes** (A11077);
(9:55 – 10:10)

Frederik Van den Broeck: **Population genomics of New World *Leishmania* along the Andes reveal species diversity and patterns of allopatric speciation** (A11002);
(10:10 – 10:25)

Calvin Tiengwe: **GPI-dependent trafficking of the transferrin receptor (TfR) in African trypanosomes** (A10988);
(10:25– 10:40)

Coffee Break

Session 2 - *Cell Biology* (11:30 TILL 1:00 PM)

Richard Wheeler (invited): **Adaptation of Leishmania parasite shape to different hosts** (A10587)
(11:30 – 11:55)

Carolina Catta-Preta: **Protein kinases that regulate *Leishmania* differentiation** (A10952);
(11:55 – 12:10)

Sue Vaughan (invited): **Basal body connections in *Trypanosoma brucei*** (A10588);
(12:10 – 12:35)

Sandro Kaser: **An outer membrane protein complex mediates mitochondrial genome inheritance** (A11136);
(12:35 – 12:50)

Carolin Wedel: **GT-rich promoters drive RNA pol II transcription and deposition of H2A.Z in African trypanosomes** (A11127);
(12:50 – 1:05 PM)

Lunch & Departure (1:00 -2:00 PM)

Abstracts

Oral Presentations in time order

Sunday (September 4)

Plenary (7:00 – 7:40 PM)

(7:00 – 7:40 PM)

Remote control of reaction-diffusion processes with chemical microrobots - A10585
Presenter: **Prof František Štěpánek**, *University of Chemistry and Technology, Prague*

The ability to control the rates of chemical or biochemical reactions at length-scales comparable to those of single-cell organisms or their sub-cellular compartments would be desirable in a number of situations, ranging from fundamental studies of transfer phenomena to applications such as controlled delivery of actives from functional materials in the pharmaceutical, food, personal care or crop protection products. Strategies for the control of biochemical reactions have been applied to autonomous micro-scale systems called “chemical robots”. A chemical robot is an internally structured microparticle consisting of a semi-permeable membrane that regulates molecular transport between the interior and the surroundings, a system of stimuli-responsive internal compartments that store and release reactants, immobilised enzymes or catalysts for facilitating chemical reactions, and magnetic nanoparticles that act as susceptors and enable receiving radiofrequency signals. The talk will present an implementation of chemical robots based on soft hydrogel bodies produced by drop-on-demand inkjet printing and microfluidic methods, with internal storage reservoirs formed by phospholipid vesicles (liposomes) and immobilised enzyme (laccase) as a bio-catalyst. Super-paramagnetic iron oxide nanoparticles that dissipate heat when exposed to alternating magnetic field in the radiofrequency range are used as susceptors that facilitate the control of local temperature, which in turn controls the diffusion rate of reactants from liposomes and thus the local reaction rate. The talk will cover fabrication methods for the bottom-up assembly of chemical robots, their structural and functional characterisation. Several scenarios will be presented, including simple one-off release of a pre-synthesised chemical payload, repeated on/off release, and finally repeated starting, stopping and restarting of a local chemical reaction that produces a chemically unstable but physiologically active product. The flow and deposition of chemical robots in porous media observed by MRI in the context of spatially specific delivery of actives will be discussed. Examples of the interaction between chemical robots and living cells or their colonies will be shown.

Monday (September 5)

Session 1 – *Comparative and Population Genomics* (9:00 - 11:00 AM)

(9:00 – 9:25)

Comparative genomics of trypanosomatids: identification of novel virulence factors and new insights into evolution of *Leishmania* - A10575
Presenter: **Dr Vyacheslav Yurchenko**, *Associate Professor, University of Ostrava*

Authors: **V Yurchenko**[1;2;] A Butenko[1;2]; A Ishemgulova[1]; J Lukeš[2]
[1] Life Science Research Centre, Faculty of Science, University of Ostrava, Czech Republic; [2] Institute of Parasitology, Biology Centre, ASCR, Czech Republic

While numerous high-quality genomes are available for dixenous (two hosts) trypanosomatid species of the genera Trypanosoma, *Leishmania*, and Phytomonas, only fragmentary information is available for their monoxenous (single-host) cousins. In trypanosomatids, monoxeny is ancestral to dixeny, thus it is anticipated that the genome sequences of the key monoxenous parasites will be instrumental for both understanding the origin of parasitism and the evolution of dixeny. Here, we present a high-quality genome for Leptomonas pyrrhocoris, which is closely related to the dixenous genus *Leishmania*. In addition, several genomes belonging to the different lineages of Trypanosomatidae were compared side-by-side. Using the L. pyrrhocoris genome, we pinpointed genes gained and lost in *Leishmania*. Among those genes, 20 genes with unknown function had expression patterns in the *Leishmania* mexicana life cycle suggesting their involvement in virulence. By combining differential expression data for L. mexicana, L. major and Leptomonas seymouri, we have identified several additional proteins potentially involved in virulence, including SpoU methylase and U3 small nucleolar ribonucleoprotein IMP3.

(9:25 – 9:40)

Asexual maintenance of genetic diversity in the protozoan pathogen *Leishmania donovani*- A10958
Presenter: **Mr Gerald Spaeth**, *Head of the Laboratory, Institut Pasteur*

Authors: P Prieto Barja[1]; P Pescher[2]; G Bussotti[2]; F Dumetz[4]; H Imamura[4]; M Domagalska[4]; D Kedra[1]; F Guerfali[3]; P Bastien[5]; Y Sterkers[5]; J C Dujardin[4]; C Notredame[1]; **G F Späth**[2];
[1] Centre for Genomic Regulation and Universitat Pompeu Fabra, Spain; [2] Institut Pasteur, Paris, France; [3] Institut Pasteur, Tunis, Tunisia; [4] Institute of Tropical Medicine, Belgium; [5] University of Montpellier and Centre Hospitalier Universitaire, France

Natural selection is an important driver of microbial pathogenesis. Infectious agents have evolved various bet hedging strategies to constantly generate genetic diversity that allows for selection of the fittest in changing host environments. As part of the LeiSHield consortium we discovered a novel strategy of evolutionary adaptation in the protozoan parasite *Leishmania* relying on chromosomal amplification and allelic variation. Drawing from the sequenced genomes of 204 *L. donovani* field isolates and conducting evolutionary experiments we uncover highly dynamic and regulated karyotype changes *in vitro* and *in vivo* that allow for the development and selection of beneficial alleles. We demonstrate that haplotype selection regulates transcript abundance and generates considerable phenotypic variability causing fitness gains in culture and infected hamsters in a tissue-specific manner. We further show that allelic diversity is higher for those chromosomes that undergo frequent amplification thus linking aneuploidy to genome evolution and the

generation of new haplotypes. Our data unravel a new microbial bet hedging strategy based on genome instability that drives parasite fitness and its long-term evolution, which may be broadly applicable to other eukaryotic pathogens and questions current approaches towards *Leishmania* epidemiology and drug discovery.

(9:40 – 10:05)

Extant heterozygosity and genetic hybridization have dramatically impacted "Old World" *Leishmania* natural population genetics (A10579)
Presenter: Michael Grigg (invited)

(10:05 -10:20)

Genome and transcriptome dynamics throughout the life cycle of *Leishmania donovani*- A10843
Presenter: **Dr. Malgorzata Domagalska**, *postdoctoral researcher, Institute of Tropical Medicine*

Authors: F Dumetz[3]; H Imamura[3]; M Sanders[4]; V Seblova-Hrobarikova[1]; J Myskova[1]; P Pescher[2]; G Bussotti[2]; G F Späth[2]; J A Cotton[4]; P Volf[1]; J C Dujardin[3]; **M A Domagalska**[3];
[1] Charles University, Czech Republic; [2] Institut Pasteur, France; [3] Institute of Tropical Medicine, Belgium; [4] The Wellcome Trust Sanger Institute

Genome plasticity and its potential role in diversity in natural *Leishmania* populations have been a subject of several studies carried out on isolated and cultivated promastigotes. However, it is unknown whether the results obtained with *in vitro* parasites reflect the *Leishmania* genome dynamics in *in vivo*. In this study we addressed (i) whether genome diversity is generated and selected during the life cycle of *L. donovani*, and (ii) what is the impact of potential genomic changes on the transcriptome. The study was initiated with an aneuploid (8/36) field isolate of reference strain BPK282/0. We observed a minor decrease in aneuploidy (6/36) after the establishment of late stage infection in *Phlebotomus argentipes*, followed by further decrease in somy of one chromosome in amastigotes purified from golden hamster at the end of first passage (2 months). Interestingly, after consecutive 3 and 4 passages in golden hamsters, the aneuploidy pattern changed drastically with all trisomic chromosomes becoming disomic, and the chr8 emerging as trisomic. Thus, we conclude that aneuploidy pattern is flexible and environment-specific. The observed changes in chromosome copy number in each samples correlated with the amount of transcripts per chrosomome, with the exception of chr31. At the same time, when we compared the transcriptomes of amastigotes, and *in vitro* promastigotes characterised by identical low aneuploidy pattern, we found 9.6 % of the genes to be overexpressed in promastigotes, and only 3.2% in amastigotes. Therefore, the transcriptome is shaped by a complex interaction between genome dosage and stage-specific regulation of gene expression.

(10:20 – 10:35)

Pan-American migration promotes the spread of pathogenic *Trypanosoma cruzi* hybrid strains - A10793
Presenter: **Dr. Louisa Messenger**, *Post-doctoral research fellow, London School of Hygiene and Tropical Medicine*

Authors: **L A Messenger**[1]; J D Ramirez[2]; M S Llewellyn[3]; M A Miles[1];
[1] London School of Hygiene and Tropical Medicine; [2] Universidad del Rosario, Colombia; [3] University of Glasgow

Despite the existence of two recent natural hybrid lineages (TcV and TcVI), which are sympatric with severe disease in southern endemic areas, the principal reproductive strategy of *Trypanosoma cruzi* , the aetiological agent of Chagas disease, remains controversial. High resolution nuclear and mitochondrial genotyping of potential hybrid isolates from Colombia was undertaken to resolve their putative status as novel recombinants. All suspected Colombian hybrids were highly heterozygous, minimally diverse and possessed intact parental alleles at each loci. Compared to local strains, Colombian hybrids were distinct from, but more closely related to, southern TcVI isolates. Based on independent inheritance patterns of microsatellite loci, our data support the hypothesis that two recombination events led to the formation of TcV and TcVI. However, more private alleles among Colombian hybrids and the sharing of their mitochondrial haplotypes with southern samples, suggests they are unlikely to be predecessors of southern TcVI strains, but were also not clear descendants, and may instead represent a sibling group, which diverged and anthroponotically dispersed northwards. We discuss the important implications the geographical range expansion of TcVI has for emergent human Chagas disease in Colombia, considering the successful, epidemic establishment of this low-diversity genotype among domestic vectors and human infections in the South.

(10:35 – 10:50)

What do kinetoplastids need a kinetoplast for? Life cycle progression of *Trypanosoma brucei* in the presence and absence of mitochondrial DNA- A10625

Presenter: **Dr Caroline Dewar**, *Postdoctoral research associate, University of Edinburgh*

Authors: **C E Dewar**[4]; P MacGregor[3]; N Savill[4]; A Casas[2]; B Rotureau[1]; A Acosta-Serrano[2]; K Matthews[4]; A Schnaufer[4];

[1] Institut Pasteur, France; [2] London School of Tropical Medicine; [3] University of Cambridge; [4] University of Edinburgh

There is a switch in directionality of the mitochondrial F_1F_o-ATPase between the *T. brucei* procyclic insect form (PCF) and slender bloodstream form (BSF). In PCF, it is thought a need for oxidative phosphorylation requires the enzyme to generate ATP. In slender BSF, the enzyme uses glycolytic ATP to drive proton pumping to maintain the essential mitochondrial membrane potential. F_o-ATPase subunit 6 is critical for proton translocation in either direction and is encoded in the mitochondrial DNA (kDNA). kDNA is therefore essential in BSF, and also in PCF, where it encodes subunits of the respiratory chain. Specific point mutations in the nuclear-encoded F_oF_1-ATPase ? subunit allow BSF survival in the absence of kDNA (Dean et al., 2013). We now show that (i) the L262Pg mutation functionally uncouples the enzyme, with dramatic consequences for F_1F_o structure and kDNA stability; (ii) $kDNA^0$ cells can differentiate to transmissible stumpy forms, but these cells show a decreased lifespan, suggesting a critical role for a kDNA-encoded product; (iii) kDNA is indispensable for progression to PCF, but homozygous L262P? cells (expressing only uncoupled F_1F_o-ATPase) can still differentiate and survive in the tsetse fly midgut. Hence, despite long-held beliefs in the field, oxidative phosphorylation may not be essential in PCF *in vivo*. However, these PCF cells show decreased motility and cell cycle progression *in vitro*, a phenotype that may be caused by ATP starvation.

Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM)

(11:25 – 11:50)

Decoding antitrypanosomal drug action and resistance- A10577

Presenter: **Prof David Horn**, *Prof. of Parasite Molecular Biology, University of Dundee*

RNA interference has been particularly effective for gene knockdown and functional studies in the African trypanosome. Such genetic approaches used in high-throughput mode can be particularly powerful and we have found this to be the case for RNA Interference Target sequencing, or RIT-seq. Genes can be rapidly linked to phenotypes, even when nothing is known about mechanism at the outset. RIT-seq was initially used to generate genome-scale loss-of-fitness profiles, facilitating drug-target prioritization. Used in combination with antitrypanosomal drugs, RIT-seq then revealed the genes associated with drug-resistance. Defective drug uptake emerged as a prominent feature and one particular transporter, an aquaglyceroporin, was found to be responsible for the most widespread form of resistance in trypanosomiasis patients. Other examples of how we are using genetic screens to develop our understanding of druggable biology will be presented.

(11:50 – 12:05)

A network of cap-binding proteins in the service of stress physiology in Trypanosomatids-A10838

Presenter: **Prof. Michal Shapira**, *Group leader, Academic staff, Ben-Gurion University of the Negev*

Authors: R Shrivastava[1]; D C Elimeleh[1]; N Tupperwar[1]; S Meleppattu[2]; A Gilad[1]; I Orr[1]; M Leger-Abraham[2]; G Wagner[2]; **M Shapira**[1];
[1] Ben-Gurion University of the Negev, Israel; [2] Harvard University, United States

Exposure to changing environmental conditions can induce a global translation arrest, to prevent misfolding of de-novo synthesized proteins, and allow the synthesis of selected proteins. Translation arrest is achieved by inhibition of cap-dependent initiation, whereas the synthesis of specific proteins can via cap-independent mechanisms. *Leishmania*s must adapt their proteomic profiles to the changing hosts and environments, this is achieved mostly by control of translation. We found that trypanosomatids encode for an unusual large repertoire of paralogs of translation initiation factors, which vary extensively in sequence, structure and function. To date, six paralogs of the cap-binding protein eIF4E have been identified, and a similar number of paralogs for the eIF4G scaffold proteins. Our studies show that discrete cap-binding complexes have evolved to function under specific stress conditions, which include both abiotic stresses along with a temporary nutritional scarcity, experienced during metacyclogenesis. Furthermore, we have identified unique and novel proteins that interact with specific cap-binding proteins, forming a network that modulates their function. These regulators are unique only to trypanosomatids. They appear to assist in the remarkable adaptation of their protein synthesis machinery to the changing conditions and to the formation of unique RNA storage granules, under conditions of temporary translation arrest.

(12:05 – 12:30)

Iron Uptake in *Trypanosoma brucei*- A11206

Presenter: **Dr Martin Taylor**, *Senior Lecturer in Molecular Biology, London School of Hygiene and Tropical Medicine*

Authors: **M Taylor**[1,2]; J Kelly[1,2];
[1] London School of Hygiene and Tropical Medicine; [2] London School of Hygiene and Tropical Medicine

Iron is an essential nutrient in African trypanosomes. Bloodstream trypanosomes derive iron from host transferrin via the ESAG6/7 transferrin receptor. Iron is subsequently released from transferrin in the lysosome as FeIII. However, the pathway by which FeIII is reduced to FeII, then transported into the cytosol has not been resolved. The minimal requirement for this process would be a ferric reductase and a cation channel/transporter. We have previously shown that the cation transporter TbMLP is involved in iron

transport. We have constructed bloodstream form null mutants of the two transmembrane ferric reductases (FRs) in the *T. brucei* genome. The FRs are orthologues of cytochrome b561 (TbCytb561) and cytochrome b558 (TbFre1), respectively. Both null mutants show no growth defect *in vitro* or in a murine model. The null mutants present growth phenotypes under iron-limiting conditions. TbCytb561 is also dispensable in the procyclic form with a different phenotype to the BSF. We have also constructed cell lines in which both TbCytb561and TbFre1 have been deleted following four rounds of gene deletion in the BSF. Their phenotype is more complex as the response to specific iron binding drugs differs depending on the target protein. Our current model for iron uptake will be presented.

(12:30 – 12:45)

Where is the trypanosome decapping enzyme?- A10673
Presenter: **Dr Susanne Kramer**, *Junior PI, Biozentrum der Universität Würzburg*

Authors: **S K Kramer**[1];
[1] Biozentrum der Universität Würzburg, Germany

5' to 3' decay is the major mRNA degradation pathway in many organisms, including trypanosomes. It starts with cap-removal by the decapping enzyme DCP2 and finishes with 5'-3' decay by the exoribonuclease Xrn1 (XRNA in trypanosomes). Kinetoplastids have decapping activity, but are the only eukaryotes with no obvious orthologue to DCP2. Here we provide evidence for an ApaH-like phosphatase (ALPH1) being the trypanosome decapping enzyme; this protein had been identified as a novel RNA granule component by our recent purification of trypanosome stress granules. ApaH is a subgroup of bacterial phosphatases with diadenosine tetraphosphatase activity: an activity reminiscent of mRNA decapping. I found striking similarities between XRNA and ALPH1: RNAi depletion of both proteins is lethal and causes stabilisation of total mRNAs. Moreover, ALPH1 and XRNA, but no other proteins, co-localise to a special granule at the posterior pole of the cell. I have recently developed a novel fluorescent tool for the detection of mRNA decay intermediates on subcellular resolution. RNAi depletion of XRNA causes an increase in both intact mRNAs and in 5'-3' decay intermediates, consistent with a function in mRNA degradation. In contrast, RNAi depletion of ALPH1 causes an increase in intact mRNAs only, consistent with a function in the initiation step of 5'-3' mRNA decay. Together, these data provide strong evidence for ALPH1 acting upstream of XRNA in the 5'-3' decay pathway, possibly in decapping.

(12:45 – 1:00 PM)

A novel telomere-binding protein, TelBP1, modulates VSG expression site silencing kinetics in *Trypanosoma brucei*- A11035
Presenter: **Helena Reis**, *PhD student, Biozentrum der Universität Würzburg*

Authors: **H Reis**[3]; M Dejung[1]; E Kremmer[2]; F Butter[1]; C J Janzen[3];
[1] Institut für Molekulare Biologie (IMB), Mainz, Germany; [2] Institut für Molekulare Immunologie (IMI), Helmholtz Zentrum München, Germany; [3] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg, Germany

For successful immune evasion the mammalian infectious bloodstream form (BSF) of *Trypanosoma brucei* relies on antigenic variation. This process is based on monoallelic expression of variant surface glycoprotein (VSG) genes and their periodic switching. The active VSG is transcribed from one of 15 subtelomeric expression sites (ES), while the remaining ES are silenced. All ES are inactive in the insect vector stage procyclic form (PCF). Previous reports have shown that the telomere complex components TRF, RAP1 and TIF2 are involved in ES regulation. However, the precise nature of their contribution remains unclear. To determine how telomeres influence the transcriptional control of VSG expression, it is first essential to identify the

complete composition of the telomere-binding protein complexes. We used two approaches to find novel telomeric factors in *T. brucei* - a pull-down assay with telomeric repeat oligonucleotides, and co-immunoprecipitation (CoIP) to find TRF-interacting proteins. Here, we describe a new telomere-binding protein, TelBP1, which was found in both experiments. Indirect immunofluorescence analysis and reciprocal CoIP verified TelBP1 as a telomeric component. Mass spectrometry and immunoblot analyses showed an upregulation of TelBP1 in BSF cells. Interestingly, VSG silencing was faster in TelBP1-depleted BSF cells during their differentiation to PCF cells. Our results suggest that TelBP1 influences ES silencing kinetics

Session 3 – *Host-Parasite Interactions* (2:00 till 4:00 PM)

(2:00 – 2:25 PM)

A receptor's tale: structural and functional diversification of the trypanosome haptoglobin-haemoglobin receptor - A10578
Presenter: **Prof Matthew Higgins**, *Oxford University*

*Authors: H Lane-Serff[1]; H Lane-Serff[2]; P MacGregor[3]; M Carrington[3]; **M Higgins**[1];[2];*
[1] Department of Biochemistry, South Parks Road, University of Oxford; [2] Department of Biochemistry, South Parks Road, University of Oxford; [3] Department of Biochemistry, Tennis Court Road, University of Cambridge

The haptoglobin-haemoglobin receptor (HpHbR) of *Trypanosoma brucei* allows acquisition of haem and provides an uptake route for trypanolytic factor-1, a mediator of innate immunity against trypanosome infection. This talk will present our findings about the structure of the receptor and its mechanism of action in mediating ligand binding. We will describe the adaptations that have allowed TbHpHbR to bind to large ligands in the context of the densely packed VSG coat. We will show the effect of a receptor polymorphism that influences the uptake of trypanolytic factors in human infective T. b. gambiense. Finally, we will reveal the remarkable molecular and cellular adaptations that have occurred during the development of this receptor as it has diverged from an epimastigote expressed haemoglobin receptor in ancestral trypanosomes to a haemoglobin-haptoglobin receptor in *T. brucei.* These stories illustrate how a receptor can operate in the context of the trypanosome surface and how one receptor has evolved different roles in different trypanosome species.

(2:25 – 2:40 PM)

Germline Transmission of Targeted Baboon Apolipoprotein L-I in Mice Protects Against African Trypanosomes- A11080
Presenter: **Jayne Raper**, *Professor, Hunter College, City University of New York*

Authors: **J Raper**[1,5]; J Verdi[1,5]; J Pant[1,5]; M Mugnier[4]; D Kovacsics[1]; M Yu[2]; S Kemp[2]; C Schoenherr[3]; A Economides[3]; D Frendewey[3]; V Lai[3];
[1] Hunter College at the City University of New York, United States; [2] International Livestock Research Institute, Kenya; [3] Regeneron Pharmaceuticals, United States; [4] Rockefeller University, United States; [5] The Graduate Center at the City University of New York, United States;

Many mammals are highly susceptible to infection by African trypanosome species including domestic cattle. 10 million square miles of African land is thereby left uncultivated, as farmers are unable to maintain livestock for haulage, milk, and food. Baboons and other primates are immune to infection by *Trypanosoma brucei* and related species due to the anti-microbial activity of Trypanosome lytic factor (TLF). We propose to combat this debilitating economic and agricultural burden by generating genetically modified cattle expressing the baboon TLF genes in order to create an indigenous breed capable of thriving in sub-Saharan Africa. TLF is a 550-kDa high-density lipoprotein (HDL) incorporating the primate specific haptoglobin-related protein (HPR), which enhances parasite uptake of TLF, which carries the pore-forming toxin apolipoprotein L-I (APOLI). We have generated baboon APOLI expressing transgenic mice via stable germline integration in a C57BL/6N background. Ubiquitin promoter driven expression of APOLI protects mice from infection by T. b. brucei, T. congolense, and T. b. rhodesiense. This protection is augmented by co-expression of baboon HPR via hydrodynamic gene delivery. The mice secrete 10-20 fold less APOLI into their serum compared to baboons. We have not detected any APOLI induced host toxicity; however, this low expression level has selected for baboon APOLI resistant trypanosomes. We are determining the source of the APOLI resistance via RNA sequencing and testing more trypanosome species in our mice. To prevent resistance from developing in cattle, we are generating mice with both APOLI (higher expression) and HPR in order to produce fully protected animals. The information obtained in these studies will be applied to the generation of genetically modified cattle in the near future.

(2:40 – 3:05 PM)

Your genes are what you eat: The metabolic basis of genome composition and codon bias - A10576
Presenter: **Dr Steven Kelly**, *Oxford University*

Genomes are composed of long strings of nucleotide monomers (A, C, G, and T) that are either scavenged from the organism's environment or built from metabolic precursors. In this talk I will discuss how adaptation to different metabolic strategies is a major determinant of the differences in genome composition between parasites, using examples from kinetoplastids and parasitic bacteria. I will also discuss how diet impacts on codon bias and discuss novel mathematical models we have developed that provide new insight into the relationship between diet, environment, gene expression and the nucleotide sequence of a gene.

(3:05 – 3:20 PM)

The RNA binding protein-RBP10 defines bloodstream form trypanosome identity- A11051
Presenter: **Mr Elisha Mugo**, *PhD student, University of Heidelberg*

Authors: **E Mugo**[1]; C Clayton[1];
[1] Zentrum für Molekulare Biologie der Universität Heidelberg, Germany

Transitions towards trypanosomes differentiation are complex and require well co-ordinated gene expression programs. Unlike other eukaryotes, transcription in trypanosome is polycistronic; many basal transcription factors are missing. In absence of RNA polymerase II dependent transcription regulation, what are the regulatory factors that triggers changes in trypanosome gene expression and ensures irreversibility after the initial differentiation signal? We here show that a single *T. brucei* RNA binding protein-RBP10 acts as an on/off switch which defines the trypanosome differentiation state. RBP10 is a cytosolic RNA-binding protein which is expressed only in multiplying bloodstream forms. The bloodstream forms depleted of RBP10 can survive only as procyclic forms. More remarkably, expression of RBP10 in procyclic forms results in their direct conversion to bloodstream forms within 2 days. RBP10 binds to procyclic-specific mRNAs containing the sequence UAUUUUUU, targeting them for translation repression and destruction. The products of RBP10 target mRNAs include not only the major procyclic-specific surface protein and

various enzymes of energy metabolism, but also signaling and RNA-binding proteins required for procyclic-form survival. RBP10 is therefore a paradigm for the definition and maintenance of a eukaryotic cell differentiation state by a post-transcriptional regulatory cascade.

(3:20 – 3:35 PM)

The complete structures of VSGs MITat1.1 and ILTat1.24: combining density and mobility through flexibility- A11069

Presenter: **Mr Thomas Bartossek**, *PhD student, Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg*

Authors: **T Bartossek**[4]; N G Jones[4]; C Schäfer[3]; H Mott[1]; M Brennich[2]; C Kisker[3]; M Carrington[1]; M Engstler[4];

[1] Department of Biochemistry, University of Cambridge; [2] European Synchrotron Radiation Facility, Grenoble, France; [3] Lehrstuhl für Strukturbiologie, Rudolf-Virchow-Zentrum der Universität Würzburg, Germany; [4] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg, Germany

Trypanosoma brucei is protected from immune attack by the densely packed and highly mobile variant surface glycoprotein (VSG) coat. Despite its importance for parasite survival, no complete structure of any VSG has been solved to date and the structure-function relationship remains largely unknown. We employ small angle x-ray scattering (SAXS) in combination with high-resolution domain structures of VSGs MITat1.1 and ILTat1.24 to solve the first complete VSG structures. The models are based on crystal structures of the N-terminal domain and NMR-structures of the C-terminal domain (CTD). Both VSGs show extensive flexibility of the CTD and the domain linker, suggesting that VSGs can adopt relaxed and compact conformations. This allows adaptive responses to obstacles or changes in protein density, while maintaining the function of the VSG coat as an impervious shield. Although the CTD-types of VSGs MITat1.1 and ILTat1.24 influence the overall membrane occupancy of the proteins, relaxed and compact conformations exist for both VSGs. Our findings emphasise the importance of the structural flexibility of the VSG in maintaining a functional trypanosome surface coat.

(3:35 – 3:50 PM)

Regulating the regulators: LmjPRMT7 in *Leishmania* parasite virulence and trans-regulator methylation- A11186

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

Authors: T R Ferreira[2]; E A Ferreira[1]; M J Plevin[2]; A K Cruz[1]; **P B Walrad**[2];

[1] University of Sao Paulo; [2] University of York

Arginine methyltransferases (PRMTs) catalyze the post-translational methylation of a wide spectrum of proteins in different cellular processes. Remarkably, RNA-binding proteins (RBPs) are major substrates of PRMTs and modification may alter regulatory function. The *Leishmania major* genome encodes five PRMT homologs, including PRMT7, which is only found in a restricted group of eukaryotes. Both *Lmj*PRMT7 expression and arginine monomethylation are tightly regulated during promastigote development; displaying procyclic stage-specific expression. In Ferreira et al., 2014 we demonstrated that *Lmj*PRMT7 levels are inversely proportional to parasite virulence; the Δ*lmjprmt7* null mutant led to an increased virulence, while *Lmj*PRMT7-overexpressing parasites displayed attenuated virulence, both *in vitro* and *in vivo*. This work was the first to link PRMT enzymes with virulence and to describe a possible role of *Leishmania* methylation in the regulation of gene expression. We previously identified putative RBP substrates which co-immunoprecipitate with *Lmj*PRMT7. Our biochemical analysis has now validated direct PRMT7 substrates and demonstrates monomethylation requires an intact PRMT7 catalytic domain and target protein RGG amino acid motifs. Results indicate *Lmj*PRMT7 methylates Alba20 protein *in vivo* and reduced enzyme

levels regulate expression of this and other RBP target proteins. We preliminarily present the *Leishmania* methylome for the first time and examine the impact of PRMT7 levels.

Tuesday (September 6)

Session 3 – *Cell Biology* (9:00 - 11:00 AM)

(9:00 – 9:25)

Drugs and trafficking in trypanosomes- A10580
Presenter: **Prof Mark C. Field**, *University of Dundee*

Authors: **M Field**[1;2]; F Leung[1,2]; M Zoltner[1]; M Zoltner[2]; D Horn[1,2];
[1] *Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee;* [2] *Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee*

Intracellular transport is a major aspect of eukaryotic cell physiology, and has its origins with the earliest eukaryotes. The system has been adapted in many lineages and this is presumed to be a feature of the lifestyle and specific ecological niche that each organism occupies. Parasitic trypanosomes have an especial burden, in terms of avoiding the immune response of their hosts. In the African trypanosome there are clear modifications to the basic endocytic machinery. Here I will discuss several of these findings, together with the identification and characterisation of novel proteins involved in endocytosis and which appear trypanosome specific. Further, recent evidence indicates an intimate connection between the parasite endocytic apparatus and sensitivity and uptake to drugs currently in the clinic, suggesting that this system may offer a means to target the parasite therapeutically.

(9:25 – 9:40)

Replication protein A DNA repair foci persist through the cell-cycle and reflect a 'divide and repair later' strategy in trypanosomes- A11081
Presenter: **Dr Lucy Glover**, *Group leader, Institut Pasteur*

Authors: L Glover[1]; O Suska[3]; D Horn[2];
[1] *Intitut Pasteur, France;* [2] *Univeristy of Dundee;* [3] *University of Dundee*

Key to host immune evasion is antigenic variation in the bloodstream, triggered by subtelomeric DNA-breaks adjacent to the single active Variant Surface Glycoprotein (VSG) gene. In mammalian cells, distinct single-stranded DNA (ssDNA)-binding proteins control DNA-recombination and repair at different loci and at different cell-cycle stages by protecting ssDNA and recruiting other repair-associated factors to sites of DNA-damage. Mammalian replication protein A (RPA), for example, is a conserved ssDNA binding-protein, which coats ssDNA in a cell-cycle dependent manner in the S- and G2-phases. We have used *Trypanosoma brucei* RPA DNA-damage associated foci to monitor meganuclease-induced DNA breaks at distinct loci. RPA-foci are restricted to the nucleolus following a break within ribosomal DNA, but are found at extranucleolar sites following breaks at active or silent VSG loci. Telomeres and active and silent VSG damage-foci distribute towards the nuclear interior in bloodstream-form *T. brucei* but tend towards the nuclear periphery in the insect-stage. Thus, DNA damage in *T. brucei* appears to generate ssDNA at all loci tested. Most strikingly though, we found that RPA-foci were readily detected at all cell-cycle stages; foci formed in S-phase and persisted post-mitosis and beyond, even while histone gH2A DNA-repair foci were disassembled. Our findings reveal a 'divide and repair later' strategy, whereby DNA-resection is not linked to

a cell cycle checkpoint in *T. brucei*. This likely suits the parasitic life-style and facilitates the generation of genetic diversity within subtelomeric domains.

(9:40 – 10:05)

The real flux capacitor? A putative molecular valve in trypanosomes - A10581
Presenter: **Dr Brooke Morriswood**, *group leader, University of Wuerzburg*

The flagellar pocket is the site of all endo- and exocytic traffic in trypanosomes. Clustered around the neck of the flagellar pocket on its cytoplasmic face are a number of cytoskeletal complexes whose functions remain relatively unexplored. The best characterised of these is the flagellar pocket collar, which has been shown to be essential for flagellar pocket biogenesis. Situated atop the flagellar pocket collar is the hook complex, a multiprotein fishhook-shaped structure containing the repeat motif protein TbMORN1. The morphology and composition of the hook complex have been previously interrogated using imaging and proximity-dependent biotinylation, respectively. Depletion of TbMORN1 in bloodstream form *Trypanosoma brucei* results in the generation of a BigEye (enlarged flagellar pocket) phenotype and subsequent cell lysis. Functional analysis of these TbMORN1-depleted cells suggests that the hook complex may be acting as a kind of molecular valve to regulate the entry of macromolecules into the flagellar pocket (the "flux capacitor" hypothesis).

(10:05 – 10:20)

Molecular characterization of the complement factor H receptor in the bloodstream and procyclic forms of *Trypanosoma brucei* - A11132
Presenter: **Ms Olivia J S Macleod**, *PhD student, University of Cambridge*

Authors: **O J Macleod**[1]; P MacGregor[1]; L Peacock[3]; S Hester[2]; S Mohammed[2]; W Gibson[3]; M K Higgins[2]; M Carrington[1];
[1] Department of Biochemistry, University of Cambridge, Cambridge; [2] Department of Biochemistry, University of Oxford, Oxford; [3] School of Veterinary Science, University of Bristol, Bristol; School of Biological Sciences, University of Bristol, Bristol

Trypanosoma brucei interacts with its hosts through proteins expressed on the external face of the plasma membrane. For example, the transferrin receptor takes up host transferrin and the haptoglobin haemoglobin (HpHb) receptor takes up host HpHb, as well as primate-specific innate immunity factor TLF1. What other molecular interactions are there between the trypanosome surface and the host? A GPI-anchored surface receptor has been identified that binds host complement factor H (FH). FH is a large glycoprotein of 155 kDa that regulates the innate immune system by inhibiting the alternative complement pathway and preventing cell lysis. We have characterized the trypanosome FH receptor at a molecular level, localizing receptor-ligand interactions to the N-terminal portion of the receptor to two domains of FH. In cultured trypanosomes, we have shown that the receptor is expressed in bloodstream and procyclic forms (BSF and PCF). Interestingly, expression levels in the BSF are rapidly and reversibly titratable depending on the concentration of a serum component. The receptor has been localized to the cell surface, and takes up fluorescently labelled ligands into BSF and PCF cells. These observations are the first identification of a direct interaction between the complement system and *T. brucei*, represent a further mechanism of immune evasion in mammalian blood, and demonstrate novel findings about trypanosome receptor functioning.

(10:20 – 10:35)

Cultured bloodstream *Trypanosoma brucei* adapt to life without their mitochondrial translation release factor- A11177
Presenter: **Alena Zíková**, *Group leader, Biology Centre, Institute of Parasitology*

Authors: M Veselíková[1]; B Panicucci[1]; R Aphasizhev[2]; I Aphasizheva[2]; **A Zíková**[1];
[1] Biology Centre, Czech Republic; [2] Boston University, United States

The mitochondrial (mt) translation release factor (Mrf1) promotes peptidyl-tRNA hydrolysis (PTH) in a codon-specific manner and has been shown to be essential in procyclic *T. brucei*. However, we demonstrate that cultured bloodstream forms (BF) can tolerate the elimination of this protein by adapting their mt bioenergenetics and employing the codon-independent PTH factor, ICT1. Specifically, BF Mrf1 double knock-out (DKO) cells have less assembled F_oF_1-ATP synthase (complex V) monomers and oligomers, suggesting that the impairment to translation termination results in the depletion of functional A6, a mt encoded subunit of this enzymatic complex. This correlates with a decrease in the mt membrane potential ($\Delta\psi_m$), which contributes to the slower growth rate observed in these cells. To compensate for the diminished capacity of complex V to pump protons, the activity and expression levels of the ADP/ATP carrier increase to supplement the $\Delta\psi_m$ by the electrogenic exchange of its substrates. Since the cells also became hypersensitive to oligomycin, an inhibitor of the proton pore created by A6, we analyzed if ICT1 could rescue stalled ribosomes. Indeed, while there is no growth phenotype when ICT1 is depleted in wildtype cells, ICT1 silencing in the Mrf1 DKO cell lines severely inhibits growth. Furthermore, the overexpression of ICT1 in the Mrf1 DKO cell lines partially alleviates the observed phenotypes. These outcomes highlight the ability of the BF mitochondrion to adapt when primarily A6 translation is required for the reduced organellar role during this developmental stage.

(10:35 – 10:50)

Discovery of new *Leishmania* motility mutants in a CRISPR-Cas9 knockout screen of the flagellar proteome- A11091
Presenter: **Mr Tom Beneke**, *PhD Student, University of Oxford*

Authors: **T Beneke**[1]; F Demay[1]; J Valli[1]; R Madden[1]; R Wheeler[1]; J Sunter[1]; E Gluenz[1];
[1] University of Oxford, Sir William Dunn School of Pathology

The *Leishmania* flagellum is a multifunctional organelle used for motility, attachment to the sand fly vector and sensory functions. To discover the flagellar protein composition and enable a dissection of different flagellar functions, we developed a new deflagellation protocol for *L. mexicana* promastigotes. The resulting flagella and deflagellated cell bodies were separated into detergent soluble and insoluble fractions for analysis of all four fractions by mass spectrometry. We detected a total of 2491 proteins and using a label-free quantitation method we identified proteins enriched in each of the four fractions. We validated the proteomics data by fluorescence tagging and knockout using CRISPR Cas9 gene editing. 78 proteins enriched in both flagellar fractions were tagged and 66 localised to the flagellum, identifying novel components of the flagellar axoneme, PFR, matrix and flagellar membrane. To date, 18 of these 66 genes were targeted for knockout, resulting in 13 viable knockout cell lines. Four of the mutants had impaired motility; EM analysis is ongoing and flagellar ultrastructural defects for one mutant have been found. This experimental validation showed that the *L. mexicana* flagellar proteome defined in this study is a rich dataset that will facilitate further dissection of flagellar function in *Leishmania spp*. Moreover, we have shown the potential of targeted CRISPR-Cas9 screens for the discovery of mutant phenotypes.

Session 2 - *Biochemistry and Metabolism* (11:25 till 1:00 PM)

(11:25 – 11:50)

Exploitation of receptor-mediated ligand uptake for the targeted delivery of antibody- toxin conjugates into *Trypanosoma brucei*.- A10582
Presenter: **Dr Paula MacGregor**, *Postdoctoral Research Associate, University of Cambridge*

Authors: **P MacGregor**[2]; **P MacGregor**[3]; A L Gonzalez-Munoz[4]; F Jobe[4]; S Rust[4]; A M Sandcock[4]; O J Macleod[2]; O J Macleod[3]; F D'Hooge[5]; C Barry[5]; P Howard[5]; M K Higgins[1]; T Vaughan[4]; R Minter[4]; M Carrington[2]; M Carrington[3];
[1] Department of Biochemistry, South Parks Road, University of Oxford; [2] Department of Biochemistry, Tennis Court Road, University of Cambridge; [3] Department of Biochemistry, Tennis Court Road, University of Cambridge; [4] Medimmune, Granta Park, Cambridge, CB21 6GH; [5] Spirogen Ltd, The QMB Innovation Centre, New Road, London, E1 2AX

Receptor-mediated nutrient uptake in trypanosomes involves internalisation of surface receptors and their ligands through the endosomal pathway. Toxin-conjugated monoclonal antibodies recognising epitopes on such receptors should therefore be internalised by the trypanosome, providing targeted delivery of toxins into the parasite. To test this hypothesis, the *T. b. brucei* haptoglobin-haemoglobin receptor (HpHbR) was selected as a target receptor. The HpHbR is a low copy number GPI-anchored surface protein that functions in haem acquisition through internalisation of host HpHb and in innate immunity through internalisation of trypanosome lytic factor 1. Single chain variable fragments that recognise recombinant HpHbR N-terminal domain were selected by phage-display then converted into humanised IgGs and fluorescently labelled. Five out of seven selected antibodies were efficiently and specifically internalised into *T. b. brucei* wild-type cells and not into HpHbR null cells. Conjugation of these monoclonal HpHbR IgGs to pyrrolobenzodiazepine (PBD) toxins led to targeted cell killing of trypanosomes, including human infective T. b. rhodesiense, at picomolar concentrations. This work demonstrates the potential for exploitation of receptor-mediated nutrient uptake for targeted delivery of toxins into African trypanosomes using antibody-toxin conjugates.

(11:50 – 12:05)

The ZC3H39/40 RNA-binding complex and the control of electron transport chain expression in African trypanosomes- A11134

Presenter: **Anna Trenaman**, *Post-Doctoral Research Associate, University of Dundee*

Authors: **A Trenaman**[1]; L Glover[1]; S Hutchinson[1]; D Horn[1];
[1] University of Dundee

We ran an RNAi screen in bloodstream form *T. brucei* that implicated the RNA-binding proteins, ZC3H39 and ZC3H40 in variant surface glycoprotein (VSG) gene silencing. Knockdown of either protein disrupted VSG silencing as determined by western blotting, flow-cytometry and RNA-seq. The presence of a cytoplasmic ZC3H39/40 complex in *T. brucei* was supported by immunofluorescence co-localisation, co-destabilisation and co-immunoprecipitation. A CLIP-seq experiment, surprisingly, identified transcripts encoding multiple components of the electron transport chain, including those for cytochrome oxidase and the F-ATPase. Consistent with this association, these same transcripts were down-regulated in ZC3H39/40 knockdowns as determined by RNA-seq and by stable isotope labelling in cell culture followed by mass-spectrometry and proteomic profiling. Thus, we report an RNA-binding complex that controls the expression of electron transport chain components in *T. brucei*. Our findings also establish an intriguing link between oxidative phosphorylation control and VSG expression control.

(12:05 – 12:30)

Mitochondrial organelle biogenesis during the cell cycle in *T. brucei* - A10584

Presenter: **Dr Torsten Ochsenreiter**, *Group Leader, University of Bern*

Trypanosomes contain a single organelle with a single genome and thus organelle biogenesis is tightly integrated into the cell cycle. Biogenesis of the mitochondrion during the cell cycle requires mass increase,

replication of the organelle´s genome and finally the proper segregation of the genome and the organelle into the two daughter cells. Using a combination of quantitative epifluorescence and super resolution microscopy we suggest a model of organelle mass increase during the cell cycle in the parasite. Furthermore, we have characterized novel components of the mitochondrial genome segregation machinery and propose a model of how this large, two membrane spanning complex is assembled during the cell cycle.

(12:30 – 12:45)

A leucine aminopeptidase is involved in kinetoplast DNA segregation in *Trypanosoma brucei* - A11204
Presenter: **Dr Priscila Peña -Diaz**, *Postdoc, Czech Academy of Sciences*

Authors: **P Pena**[1];
[1] Institute of Parasitology, BioCenter, ČCeské Budějovice, Czech Republic.

The kinetoplast (k), the uniquely packaged mitochondrial DNA of trypanosomatid protists, is formed by a concatenated network of minicircles and maxicircles that divide and segregate once each cell cycle. Although many proteins involved in kDNA replication and segregation are known, several key steps in this complex mechanism remain undescribed at the molecular level. Here we characterize an M17 family leucyl aminopeptidase metalloprotease, termed TbLAP1, which specifically localizes to the kDNA disk. Significantly, TbLAP1 is the first known protein of the nabelschnur or umbilicus, a prominent structure which in the human parasite *Trypanosoma brucei* connects the daughter kDNA networks prior to their segregation; so far the nabelschnur has been characterized solely by morphology. We show that TbLAP1 is required for correct segregation of kDNA and the tripartite attachment complex, and its overexpression leads to loss of kDNA, decreased mitochondrial membrane potential and cell proliferation. We propose that TbLAP1 is required for efficient kDNA division and specifically participates in the separation of daughter kDNA networks.

(12:45 – 1:00 PM)

Queuosine: The role of an essential tRNA modification in parasitic protist *Trypanosoma brucei*- A11185
Presenter: **Dr. Zdenek Paris**, *PI, Biology Centre CAS, Institute of Parasitology*

Authors: S Kulkarni[2]; A Kessler[3]; H Stanzl[2]; J A Alfonzo[3]; **Z Paris**[1];
[1] Biology Centre ASC, Institute of Parasitology, Czech Republic; [2] Biology Centre ASC, Institute of Parasitology and Faculty of Science, University of South Bohemia, Czech Republic; [3] Department of Microbiology and OSU Centre for RNA Biology, The Ohio State University, United States

Transfer RNAs (tRNAs) are extensively post-transcriptionally modified to increase their structural stability or fidelity maintenance. In particular, the modifications in the anti-codon loop, have a crucial role in accurate codon selection and translational frameshifting prevention. Queuosine (Q) is a hyper modified guanosine and may be found at the wobble position 34 of a 5'-GUN-3 anticodon sequence-containing tRNAs (His, Asp, Asn, Tyr). Though Q is present in nearly all forms of life, its exact physiological role remains unclear. In bacteria, queuosine is obtained by modifying GTP through five enzymatic steps; it is then added to the tRNA by a tRNA-guanine transglycosylase (TGT) activity. However, eukaryotes lack the enzymes required for de novo synthesis of queuosine and hence rely on their environment or gut microbiome to obtain queuine (free base of queuosine), which is recognised by eukaryotic TGT. In mice and humans, TGT exists as a mitochondrion-localised heterodimer with queuine tRNA ribotransferase 1 (QTRT1), and its splice variant queuine tRNA ribotransferase domain containing 1 (QTRTD1). The *T. brucei* genome encodes two TGT paralogs, TbTGT1 and TbTGT2. TbTGT1 displays a nuclear localization in the procyclic form (insect gut)

meanwhile in the bloodstream form (in mammalian host) the protein is extra-nuclear. Both TbTGT1 and TbTGT2 are involved in Q formation in tRNAs and TbTGT1 is essential for the growth of the bloodstream form of the parasite. This essentiality has been observed exclusively in trypanosomes so far, as no noticeable phenotype has been observed in the downregulation of the protein in the mammals. Consequently, TGT becomes an ideal target for drug development against diseases caused by trypanosomatid parasites

Session 4 – *Gene Expression* (2:00 till 4:00 PM)

(2:00 – 2:25 PM)

Understanding the signalling pathway controlling African trypanosome quorum sensing and life-cycle differentiation- A10583

Presenter: **Prof Keith Matthews**, *Professor of Parasite Biology, University of Edinburgh*

The African trypanosome undergoes density-dependent differentiation in the mammalian bloodstream to prepare for transmission by tsetse flies. This involves the generation of cell-cycle arrested, quiescent, stumpy forms from proliferative slender forms. The signalling pathway responsible for the quorum sensing response was catalogued using a genome wide selective screen, providing a compendium of signalling kinases phosphatases, RNA binding proteins and hypothetical proteins (Mony et al, Nature, 505, 681&hypen;685; 2014). However, the ordering of these components is unknown, as is the surface receptor that initiates the signalling response and the action of the effectors that drive changes in gene expression once the signal is received. Since our description of the signalling pathway, our lab has been intensively involved in piecing together all of these components to provide comprehensive description of how stumpy formation arises. In particular, using a combination of several distinct genome-wide RNAi screens, combinatorial gene knockout and overexpression analyses, as well as functional analyses of a surface transporter protein and gene regulators we have dissected how stumpy form transcripts are repressed in slender forms, and the dependency-relationships between components in the signalling pathway with respect to one another. The integration of these different approaches to understand stumpy formation is providing the first detailed picture of the structure and interactions in a signalling pathway in trypanosomes.

(2:25 – 2:40 PM)

The flagella connector of *Trypanosoma brucei* is a kinesin-powered junction distinct from the axonemal capping structure. - A11233

Presenter: **Mr Vladimir Varga**, *IMG Fellow, Institute of Molecular Genetics*

The flagella connector (FC) of the protozoan parasite *Trypanosoma brucei* is a mobile transmembrane junction connecting the tip of the growing flagellum to the side of the old flagellum during cell division. This association has been implicated in inheritance of the cell pattern. We developed a novel biochemical and proteomic approach for studying discrete cytoskeletal structures and identified 8 FC constituents. Immunogold and fluorescence labelling showed that these proteins localize to specific FC subdomains. Depletion of the constituents by RNAi led to a precocious loss of flagella connection. Moreover, it revealed that the principal mechanistic components of the structure are two types of kinesins cross-connected via the FC transmembrane junction, which comprises kinetoplastid-specific proteins. Intriguingly, the FC also contains two conserved ULK kinases, the mammalian orthologs of which are implicated in ciliogenesis and the plant orthologs in cell division. Finally, our biochemical approach also led to identification of several constituents of the enigmatic axonemal capping structure, suspected to regulate axonemal growth and present at tips of both new and old flagella. Our results show that the FC and the axonemal capping structure are biochemically distinct, despite both localizing to the new flagellum tip.

(2:40 – 2:55 PM)

The non-canonical mitochondrial inner membrane protein translocase of trypanosomatids contains two essential rhomboid-like proteins- A10798
Presenter: **André Schneider**, *Professor, University of Bern*

Authors: **A Schneider**[2]; A Harsman[2]; C Wenger[2]; S Oeljeklaus[1]; J Huot[2]; B Warscheid[1];
[1] Department of Biochemistry and Functional Proteomics, Universität Freiburg, Germany; [2] Department of Chemistry and Biochemistry, Universität Bern, Switzerland

Mitochondrial protein import is essential for all eukaryotes. Here we show that the early diverging eukaryote *Trypanosoma brucei* has a non-canonical inner membrane protein translocase (TIM) which unlike in other systems appears to mediate import of both presequence-containing and mitochondrial carrier proteins. We have analyzed the composition of the trypanosomal TIM complex. Besides TbTim17, which is the single member of the Tim17/22/23 family in trypanosomes, it contains nine subunits that are co-purified in reciprocal immunoprecipitations and with an import substrate that is trapped in the translocation channel. Two of the newly discovered TIM subunits are rhomboid-like proteins, which are essential for growth and mitochondrial protein import. Rhomboid-like proteins were proposed to form the protein translocation pore of the ER-associated degradation system (ERAD). No further candidates for the translocation channel are found among the subunits of the trypanosomal TIM complex, suggesting that the rhomboid-like proteins may contribute to pore formation.

(2:55 – 3:10 PM)

TrypTag: Genome-wide protein localisation in the trypanosome- A10807
Presenter: **Dr Jack Daniel Sunter**, *Research Fellow, University of Oxford*

Authors: R Madden[1]; K Billington[1]; C Halliday[1]; **S Dean** [1]; **J D Sunter** [1]; **R J Wheeler** [1];
[1] University of Oxford

Trypanosomes are exquisitely ordered and structured cells in which protein localisation can be extremely informative in functional studies of both conserved eukaryotic biology and specific parasite biology. Therefore, we have initiated a project based on high throughput endogenous gene tagging methodologies with the aim of systematically localising all proteins in the trypanosome genome via both N- and C-terminal tagging. Here, we will present data on the first 2000 proteins. Control proteins within our dataset are consistent with previously published localisations and there is also good agreement between N and C-terminal localisation data. We have performed a meta-analysis of this first dataset to inform future strategies. When complete this will be the first flagellated organism to have all its proteins localised. We are making this data publicly available immediately on a dedicated website (www.tryptag.org) and a summary for each tagged gene will be available on TriTrypDB.
Co-Principal Investigators on TrypTag project along with Keith Gull, Mark Carrington, Sue Vaughan and Christiane Hertz-Fowler. The TrypTag project is funded by the Wellcome Trust as a Wellcome Trust biomedical resource grant 108445/Z/15/Z.

(3:10 – 3:25 PM)

Understanding unconventional kinetoplastid kinetochores- A10947
Presenter: **Dr Bungo Akiyoshi**, *Group leader, University of Oxford*

Authors: **B Akiyoshi**[1];
[1] University of Oxford

Faithful transmission of genetic material is essential for the survival of all organisms. Eukaryotic chromosome segregation is driven by the kinetochore, the macromolecular protein complex that assembles

onto centromeric DNA and captures spindle microtubules during mitosis and meiosis. We recently identified 20 kinetochore proteins in *Trypanosoma brucei* and discovered that they constitute kinetochores unique to kinetoplastids. We are currently characterizing these 20 proteins *in vitro* and *in vivo* to understand how they carry out conserved kinetochore functions.

(3:25 – 3:40 PM)

Codon usage links translation and mRNA decay in *Trypanosoma brucei*- A11131
Presenter: **Miss Janaina de Freitas Nascimento** , *PhD Student, University of Cambridge*

Authors: **J F Nascimento**[1]; S Kelly[2]; J Sunter[2]; M Carrington[1];
[1] Biochemistry Department - University of Cambridge; [2] Department of Plant Sciences - University of Oxford

Synonymous codons are not used with the same frequencies within and between different species, generating codon bias. However, the extent of which codon preference impacts on gene expression in trypanosomatids is unclear. In this work we developed a novel codon usage metric called the 'gene expression codon adaptation index' (geCAI) that allows a prediction of mRNA abundance with a coefficient of determination of 0.55. The geCAI metric was validated using a synthetic library of 22 synonymous GFP sequences in procyclic *T. brucei.* Protein expression and mRNA levels were modulated over a ~40-fold range, similar to range measured for endogenous genes. Altering the geCAI value increased the expression level of a VSG mRNA. Investigation of mRNA turnover showed that GFPs with low geCAI values had shorter half-lives, indicating that translational efficiency impacts on mRNA steady-state levels. Impairment of translation led to stabilisation of GFP mRNAs whereas reducing translation initiation frequency had the opposite effect, corroborating the role of translation in regulation of mRNA levels. Analysis of the effect of codon position showed that introduction of rare codons reduced both protein and mRNA levels independently of their position within the mRNA. Taken together, the results show that variation in codon use is a major determinant of mRNA levels in *T. brucei*.

Wednesday (September 7)

Session 6 - *Interactions in the Field, Epidemiology and Cell Biology* (9:00 - 11:00 AM)

(9:00 – 9:25)

'Mind the gap': the relevance of genetic exchange and comparative genomics to combatting Chagas disease and visceral *Leishmania*sis. - A10586
Presenter: **Prof Michael Miles**, *London School of Hygiene and Tropical Medicine*

Authors: **M Miles**[4]; C Talavera-López[3]; M Lewis[4]; J Sadlova[2]; J Cotton[5]; L Messenger[4]; J Myskova[2]; V Seblova[2]; A Hailu[1]; T Gelanew[1]; C Durrant[5]; M J Saunders[5]; M Berriman[5]; P Volf[2]; B Andersson[2]; M Yeo[4]
[1] Addis Ababa University, Addis Ababa, Ethiopia; [2] Charles University, Prague, Czech Republic; [3] Karolinska Institute, Stockholm,, Sweden; [4] London School of Hygiene and Tropical Medicine; [5] Wellcome Trust Sanger Institute

Chagas disease (American trypanosomiasis) and visceral *Leishmania*sis (VL) are major public health scourges that warrant intense research and strenuous international efforts at disease control. Astonishing progress with molecular genetics and comparative genomics is changing perceptions of the biology and molecular epidemiology of the disease agents, *Trypanosoma cruzi* and *Leishmania*. The clonal theory of parasitic protozoa considered genetic exchange to be absent or rare and of no epidemiological

consequence in *T. cruzi* and *Leishmania*. On the contrary, in both instances population genetics has revealed evidence of parents and hybrids among sympatric populations. With putative parents selected from such natural populations, we have achieved experimental genetic crosses for *T. cruzi* and for *Leishmania donovani*. Experimental progeny were initially proven and characterised by a combination of targeted multilocus DNA sequencing and microsatellite analysis. Subtetraploid hybrid progeny derived from an intralineage genetic cross of *T. cruzi* I (TcI; DTU I) in mammalian cell culture, show progressive genome erosion over hundreds of generations of axenic growth *in vitro*. Crosses of *L. donovani* strains in two different sand fly species have yielded scores of diverse viable diploid progeny clones, indicating multiple exchange events. Whole genome sequencing allows detailed comparisons of such parents and progeny. For the first time we show that advanced genome sequencing and population genomics have resolved complex repetitive gene families of *T. cruzi* , giving insight into their structure, function, reassortment and post genetic exchange evolution. It is clear that genetic exchange can have a profound epidemiological impact, on the emergence and spread of virulent and drug resistant strains, on adaptation to new vectors, hosts and ecological niches. Furthermore understanding the impact of genetic exchange on genetic diversity will inform both diagnostics discovery and vaccine research. A major challenge remains to bridge the gap between such pioneering research, and the delivery of simple, proven mechanisms of controlling these neglected diseases. Funded by the EC Marie Curie EUROLEISH-NET GA No. 6426; the Swedish Research Council; the Wellcome Trust, the Ministry of Education of the Czech Republic, and institutional support.

(9:25 – 9:40)

CARP3 is a plasma-membrane and adenylate-cyclase associated regulator of cyclic AMP signalling in *Trypanosoma brucei*- A10794

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

Authors: D N Tagoe[3]; M K Gould[1]; T Kalejaiye[3]; S Bachmaier[1]; R J Burchmore[3]; D Horn[2]; M Boshart[1]; **H P De Koning**[3];

[1] Ludwig-Maximillians-University of Munich, Germany; [2] University of Dundee; [3] University of Glasgow

cAMP plays an important role in the cell biology and the life cycle progression of *Trypanosoma brucei*. It is generated by a large number of adenylate cyclases, at least some of which appear to be constitutively active, and broken down by phosphodiesterases. Both classes of proteins have highly conserved active domains. However, almost nothing is known about *how* cAMP regulates key cellular functions including cytokinesis in cell division. Recently we reported on a set of proteins that appear to be involved in cAMP signalling in trypanosomes and named them cAMP Response Proteins (CARPs). Here, we focus on the role of CARP3 in cAMP signalling and regulation in *T. brucei*. Deleting *CARP3* reduces sensitivity to the PDE inhibitor (CpdA) and increases both intracellular and extracellular cAMP levels. Conversely, overexpressing *CARP3* sensitises the cells to CpdA. Co-immunoprecipitation revealed that CARP3 is associated with several adenylate cyclases, and CARP3 influenced adenylate cyclase expression in the presence of CpdA. These results validate a role for *CARP3* as a regulator of adenylate cyclases in trypanosomes and as a key component of the cAMP signalling cascade.

(9:40 – 9:55)

From solitary swimmers to swarms and back – trypanosomes on their journey through the tsetse fly- A11008

Presenter: **Ms Sarah Schuster**, *PhD student, Biozentrum der Universität Würzburg*

Authors: **S Schuster**[2]; T Krüger[2]; I Subota[2]; S Thusek[1]; A Beilhack[1]; B Rotureau[3]; M Engstler[2];

[1] IZKF Forschergruppe für Experimentelle Stammzelltransplantation, Medizinische Klinik und Poliklinik II & Universitäts-Kinderklinik Würzburg, Germany; [2] Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum

der Universität Würzburg, Germany; [3] Trypanosome Cell Biology Unit, INSERM U1201 & Department of Parasites and Insect Vectors, Institut Pasteur, France

Trypanosoma brucei undergoes a complex life cycle in the tsetse fly vector. The parasite´s development occurs during a journey through the different microenvironments of the fly´s interior organs. For the trypanosome it involves crossing various barriers and confined surroundings, concurrent with major morphological changes. The unicellular flagellate´s motility, which is directly dependent on morphology, is essential for its survival and successful development. This work details cell motility, morphology and collective behaviour of different developmental stages using high spatio-temporal resolution microscopic analysis. Swimming patterns of solitary swimmers were analysed *in vivo* and *in vitro,* as well as collective motion at the single cell level *in vivo* by using fluorescently labelled parasites. Light sheet fluorescence microscopy was established as a powerful tool for the 3D analysis of the infection process in the tsetse fly´s digestive tract. This provides information about tissue topology with unprecedented resolution. We are able to visualise fluorescent trypanosomes inside the surprisingly complex folding of the peritrophic matrix and can begin to correlate the microenvironments and trypanosome behaviour to high-speed motility data. We propose that the infection process is a succession of solitary and collective motion and represents an adaption to the varying and convoluted microenvironments trypanosomes have to face inside the tsetse fly.

(9:55 – 10:10)

The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes - A11077

Presenter: **Annette Macleod**, *Wellcome Trust Senior Fellow, university of Glasgow*

Authors: B Rotureau[1]; **A MacLeod**[2];
[1] Institut Pasteur, France; [2] University of Glasgow

The role of mammalian skin in harbouring and transmitting arthropod-borne protozoan parasites has been overlooked for decades as these pathogens have been regarded primarily as blood-dwelling organisms. Intriguingly, infections with low or undetected blood parasites are common, particularly in the case of Human African Trypanosomiasis caused by *Trypanosoma brucei* gambiense. We hypothesise; therefore, the skin represents an anatomic reservoir of infection. Here we definitively show that substantial quantities of trypanosomes exist within the skin following experimental infection, which can be transmitted to the tsetse vector, even in the absence of detectable parasitaemia. Importantly, we demonstrate the presence of extravascular parasites in human skin biopsies from undiagnosed individuals. The identification of this novel reservoir requires a re-evaluation of current diagnostic methods and control policies. More broadly, our results indicate that transmission is a key evolutionary force driving parasite extravasation that could further result in tissue invasion-dependent pathology.

(10:10 – 10:25)

Population genomics of New World *Leishmania* parasites along the Andes reveal species diversity and patterns of allopatric speciation- A11002

Presenter: **Dr. Frederik Van den Broeck**, *Postdoctoral researcher, Institute of Tropical Medicine*

Authors: **F Van den Broeck**[2]; H Imamura[2]; J Arevalo[3]; V Adaui[3]; M Jara[3]; L Garcia[1]; M J Sanders[4]; M Berriman[4]; J A Cotton[4]; J C Dujardin[2];
[1] CUMETROP, Bolivia; [2] Institute of Tropical Medicine, Belgium; [3] Instituto de Medicina Tropical A. von Humboldt, Peru; [4] Wellcome Trust Sanger Institute

New World *Leishmania*sis is caused by parasites that show an extensive phenotypic diversity in terms of evolution, ecology and clinical outcome. The two main *Leishmania* species encountered in Peru illustrate this diversity and present an ideal study system for a better understanding of speciation in the Neotropics, the most-species rich region in the world. Here we sequenced 116 *L. braziliensis* and *L. peruviana* clinical isolates that were collected in Peru and Bolivia. SNP calling revealed 237,721 variable sites in *L. braziliensis*, while only 32,110 variable sites were detected in *L. peruviana*. The genomes of *L. peruviana* were almost devoid of heterozygous sites, resulting in long tracts of homozygosity. Phylogenomic and population genomic analyses revealed strong population substructure in *L. peruviana* that matched biogeographical regions present along the Western slopes of the Andes. We found that the most northern *L. peruviana* population diverged firstly from *L. braziliensis*, followed by a southbound colonization of *L. peruviana* into each biogeographical region. Altogether, these results suggest an allopatric speciation of *L. peruviana* following colonization of the Western slopes of the Andes through the Porculla pass in the North of Peru, resulting in serious founder effects. Further work is ongoing to study the evolutionary history of these parasites based on genome structural variations.

(10:25– 10:40)

GPI-dependent trafficking of the Transferrin Receptor (TfR) in African trypanosomes - A10988

Presenter: **Dr Calvin Tiengwe**, *Postdoctoral Fellow, University of Buffalo*

Authors: **C Tiengwe**[1]; J D Bangs[1];

[1] *University at Buffalo (SUNY), United States*

Bloodstream form trypanosomes encode two structurally related GPI-anchored proteins, variant surface glycoprotein (VSG) and transferrin receptor (TfR), that are both critical for survival in the mammalian host. VSG is central to antigenic variation; TfR internalizes transferrin (Tf) for iron acquisition. Both are expressed by the active telomeric expression site, but ~20% of all TfR transcripts derive from 'silent' sites. VSG is a homodimer; TfR is a heterodimer of ESAG6 (E6, GPI-anchored) and ESAG7 (E7). We have shown that progression of GPI-anchored proteins correlates with GPI-valence: homodimeric VSG (GPI^2) is a surface protein; heterodimeric TfR (GPI^1) is in the flagellar pocket; and truncated VSG (GPI^0) is degraded in the lysosome. Overexpressed TfR accesses the plasma membrane suggesting a saturable mechanism for retention in the flagellar pocket. However, surface TfR is non-functional for Tf binding and we proposed that such TfR represents GPI^2 E6 homodimers that are unable to bind transferrin - mimicking native VSG [*J. Cell Sci.* (2005) 118:5499]. We now create RNAi cells for suppression of native TfR subunits. Silencing is lethal and is complemented by placing resistant ($RNAi^R$) E6/E7 genes into the active TfR locus. When expressed alone each subunit conforms to the valence model: GPI^2 E6 homodimers appear on the surface like native VSG; GPI^0 E7 homodimers are delivered to the lysosome like GPI-minus VSG. Finally, we created an $RNAi^R$ GPI^2 TfR by fusing the C-terminal domain of E6 to E7 ($E7^{GPI}$). When expressed together ($E6{:}E7^{GPI}$) these proteins form functional GPI^2 heterodimers that rescue growth, mediate Tf binding and uptake, and localize to the cell surface. Collectively our results with GPI-modified TfR validate the GPI valence hypothesis.

Session 2 - *Cell Biology* (11:30 TILL 1:00 PM)

(11:30 – 11:55)

Adaptation of *Leishmania* parasite shape to different hosts - A10587

Presenter: **Dr Richard Wheeler**, *Sir Henry Wellcome Postdoctoral Fellow, Oxford University*

Leishmania parasites undergo a dramatic shape change from the insect life cycle stages to adapt to their mammalian host, changing from a long thin promastigote to a short, near-spherical, amastigote. The flagellum also changes from having a long motile 9+2 axoneme to a short collapsed 9+0 (9v) axoneme, lacking the central pair, presumably linked with changes to the flagellar pocket structure. These changes are likely vital for pathogenesis. We used electron microscopy, electron tomography and fluorescence microscopy of tagged proteins to determine the changes to the ultrastructure and molecular composition of the flagellum and flagellar pocket. This revealed a 9+2 axoneme can restructure to 9+0, and that the concurrent restructuring of the flagellar pocket involves the reorganisation of the *Leishmania* flagellum attachment zone (FAZ), a structure in the flagellar pocket neck homologous to the trypanosome FAZ. We are now expanding this analysis to the sub-pellicular microtubules which define cell shape, using serial block face scanning electron microscopy and electron tomography of whole cells. This is revealing how modular self-organising cytoskeletal elements are used by trypanosomes to achieve the restructuring of the flagellum, the flagellar pocket and FAZ, and the sub-pellicular microtubules to allow *Leishmania* parasites adapt their shape to different hosts.

(11:55 – 12:10)

Protein kinases that regulate *Leishmania* differentiation- A10952
Presenter: **Dr Carolina Catta-Preta**, *Research Associate, University of York*

Authors: **C Catta-Preta**[1]; J Mottram[1];
[1] University of York

Leishmania encounters a number of challenges during its life cycle following environmental changes that trigger the transition from an intracellular amastigote that replicates inside a parasitophorous vacuole in the mammalian host to the extracellular insect stages, a replicative procyclic promastigote and an infective metacyclic. The differentiation process is thought to be regulated in part by phosphorylation resulting in dramatic changes in protein expression, which allows adaptation. Here we aim to identify and characterize protein kinases involved in this process, as well as understand which mechanisms *Leishmania* uses to overcome stress and regulate its life cycle. We have applied the CRISPR-Cas9 genome editing system to *L. mexicana* in order to investigate 2 protein kinases previously characterized in *Trypanosoma brucei* as regulators of bloodstream to procyclic form differentiation, RDK1 and RDK2 (Repressor of Differentiation Kinase) [1]. Our data show that while RDK1 is non-essential for *L. mexicana* promastigotes, its absence impacts on amastigote proliferation and differentiation to promastigotes *in vitro*, but doesn't affect infectivity in mice. RDK2 is located on supernumerary Chromosome 30, making it a challenging gene to study. Progress on the application of CRISPR-Cas9 technology to generate protein kinase null mutants and investigate their involvement in *Leishmania* life cycle progression will be discussed. [1] Jones et al., 2014. PMID: http://www.ncbi.nlm.nih.gov/pubmed/24453978

(12:10 – 12:35)

Basal body connections in *Trypanosoma brucei* (A10588);
Presenter: **Sue Vaughan**, *Oxford Brooks University*

The flagellum of Trypanosomes is important for many aspect of pathogenicity. They assemble from basal bodies, which are located at the proximal end of the flagellum inside the flagellar pocket. Basal bodies are crucial for flagellum function and exist as a pair that are physically connected to each other. During the cell cycle, basal bodies duplicate and segregate to the two daughter cells and connections between them must re-organise and sever in order for the basal body pairs and two flagella to segregate. The structural organisation of the connections and the molecular characterisation are poorly understood. We have used

Electron tomography to characterise the connections through the cell cycle. In addition we have discovered a hypothetical protein that localises between the basal bodies and is important for basal body segregation.

(12:35 – 12:50)

An outer membrane protein complex mediates mitochondrial genome inheritance- A11136
Presenter: **Mr Sandro Käser**, *PostDoc, University of Bern*

Authors: **S Käser**[1]; M Willemin[1]; S Oeljeklaus[3]; F Schnarwiler[1]; B Warscheid[2]; A Schneider[1];
[1] Department of Chemistry and Biochemistry, University of Bern, Switzerland; [2] Department of Chemistry and Functional Proteomics and Centre for Biological Signaling Studies, University of Freiburg, Germany; [3] Department of Chemistry and Functional Proteomics, University of Freiburg, Germany

The mitochondrial genome encodes essential proteins involved in oxidative phosphorylation and thus, the genes for these proteins must be faithfully inherited during cell division. However, only little is known about the molecular mechanisms of mitochondrial genome inheritance. Here, we have discovered an outer mitochondrial membrane (OM) protein complex, which is crucial for mitochondrial genome inheritance in the parasitic protozoan *Trypanosoma brucei*. The tripartite attachment complex (TAC) functions as the mitochondrial genome segregation machinery in trypanosomes, which spans both mitochondrial membranes and physically links the basal body of the flagellum to the single-unit mitochondrial genome. TAC40 is a β-barrel membrane protein and reciprocal pulldown experiments demonstrate that it occurs in a distinct and stable complex with two novel TAC components, namely TAC60 and TAC42. By immunofluorescence analysis (IFA) we show that TAC60 depends on its N-terminus for proper TAC localization. Moreover, we demonstrate that the kinetoplastid-specific TAC60 and TAC42 proteins are both OM proteins and intriguingly, that TAC42 represents an additional β-barrel membrane protein in the TAC. Thus, the mitochondrial genome segregation machinery in trypanosomes includes a specialized OM protein complex, which is composed of three proteins, including two distinct β-barrel proteins.

(12:50 – 1:05 PM)

GT-rich promoters drive RNA pol II transcription and deposition of H2A.Z in African trypanosomes- A11127
Presenter: **Ms Carolin Wedel**, *PhD candidate, University of Wuerzburg*

Authors: **C Wedel**[2]; K U Förstner[1]; R Derr[2]; T N Siegel[2];
[1] Core Unit Systems Medicine, University of Wuerzburg, Germany; [2] Research Center for Infectious Diseases, University of Wuerzburg, Germany

Genome-wide transcription studies are revealing ever more 'dispersed promoters' that unlike 'focused promoters', lack well-conserved sequence motifs and tight regulation. Dispersed promoters are nevertheless marked by well-defined chromatin structures, suggesting that specific sequence elements must exist in these unregulated promoters. Here, we have analyzed regions of transcription initiation in the eukaryotic parasite *Trypanosoma brucei,* in which RNA polymerase II transcription initiation lacks regulation and occurs over broad (~10kb) regions without distinct promoter motifs. Using a combination of site-specific and genome-wide assays, we identified GT-rich promoters that can drive unidirectional transcription and promote the targeted deposition of the histone variant H2A.Z. In addition, mapping nucleosome occupancy at high resolution, we find that nucleosome positioning may affect RNA maturation, pointing to a previously unrecognized layer of gene regulation in trypanosomes. Our findings show that even highly dispersed, unregulated promoters contain specific DNA elements able to induce transcription and changes in chromatin structure.

Posters

The significance of respiratory complex I and mitochondrial retrograde signalling in the transition of *Trypanosoma brucei* from bloodstream to insect form- Poster 1 : A10592
Presenter: **Miss Gloria Amegatcher**, *PhD student, University of Edinburgh*

Authors: **G Amegatcher**[1]; K Matthews[1]; A Schnaufer[1];
[1] *Institute of Immunology and Infection Research, University of Edinburgh*

Mitochondrial retrograde signalling in many organisms have been shown to use specific signalling pathways to convey information on their developmental and physiological status to the nucleus and modulate expression of nuclear genes accordingly. It is not known if similar signalling pathways exist in trypanosomes. We are using pleomorphic *Trypanosoma brucei* strain devoid of mitochondrial DNA (akinetoplastic, or AK cells) to investigate presence of retrograde signalling in these organisms. As *T. brucei* differentiates from the slender bloodstream forms to the procyclic form it undergoes dramatic remodelling of its morphology and metabolism, including mitochondrial activity. Respiratory complexes III (cIII, cytochrome bc1 complex) and IV (cIV, cytochrome C oxidase), composed of both mitochondrially and nuclearly-encoded subunits, are repressed in slender forms but fully active in procyclic forms. mRNAs for nuclearly encoded cIII and cIV subunits become detectable in transitional stumpy forms and the corresponding proteins and complexes only become detectable after differentiation into PCF forms. Using northern blotting we now show that AK *T. brucei*, lacking the mitochondrial encoded cIII and cIV subunits, still upregulate cIV subunit COX VI mRNA upon differentiation into stumpy forms, potentially indicating a lack of communication between mitochondrion and nucleus. We are currently comparing the global transcriptome of wild type and AK *T. brucei* during differentiation from slender to stumpy forms to obtain a comprehensive view of the potential effects of mitochondrial dysfunction on nuclear gene expression in these parasites.

Involvement of an RNA binding protein containing Alba domain in the stage-specific regulation of beta-amastin expression in *Trypanosoma cruzi* - Poster 2 : A10752
Presenter: **Dr. Leticia Perez-Diaz**, *Alba domain protein in T. cruzi , School of Sciences, UdelaR*

Authors**: L Perez-Diaz**[1]; T Silva[2]; S M Teixeira[2];
[1] *Facultad de Ciencias, UdelaR, Uruguay;* [2] *Universidad Federal de Minas Gerais, Brazil*

Amastins constitute a group of small surface glycoproteins, first identified in amastigotes of *T. cruzi* but later found to be expressed in several *Leishmania* species, as well as in *T. cruzi* epimastigotes. Amastin differential expression results from regulatory mechanisms involving changes in mRNA stability and/or translational control. Although distinct regulatory elements were identified in the 3' UTR of *T. cruzi* and *Leishmania* amastin mRNAs, RNA binding proteins involved with amastin gene regulation have only being characterized in L. infantum where, through RNA affinity chromatography, an Alba-domain protein (LiAlba20) was demonstrated to bind to the 3' UTR of a δ-amastin mRNA contributing with stage-regulated stability of amastin transcripts. Here we investigated the role of TcAlba30, the LiAlba20 *T. cruzi* ortholog, in the post transcriptional regulation of amastin genes. TcAlba30 protein is expressed in all stages of the *T.*

cruzi life cycle. A transfected cell line expressing a cMyc tagged TcAlba30 was generated. RNA immunoprecipitation using anti-Myc antibody followed by RT-PCR revealed TcAlba30 β-amastin RNA interaction. Besides, amastin steady state mRNA levels were altered in these transfectants through TcAlba30 interaction with its 3'UTR. Analysis of changes in the parasite transcriptome resulting from ectopic TcAlba30 expression reveals that this protein modulates steady state mRNA levels from other genes that co-localize in the same chromosomal region. GO analysis for downregulated transcripts reveals a significant ($p<0.01$) enrichment in mRNAs encoding proteins involved in translation processes with many transcripts encoding ribosomal proteins and translation factors.

The mitochondrial genome and gRNA-ome of pleomorphic *Trypanosoma brucei*- Poster 3 : A10627 Presenter: **Mr Sinclair Cooper**, *PhD Student, University of Edinburgh*

Authors: **S Cooper**[2]; N Savill[2]; T Ochsenreiter[1]; A Schnaufer[2];
[1] Institute of Cell Biology, University of Bern, Switzerland; [2] Institute of Immunology and Infection Research, University of Edinburgh

We present the first near-complete mitochondrial genome (kDNA) and corresponding small RNA transcriptome of *T. brucei.* The kDNA (or kinetoplast) represents the most complex mtDNA in nature. It is composed of ~23-kb maxicircles (~50 homogenous copies), analogous to mitochondrial genomes of other eukaryotes, and thousands of highly heterogeneous ~1-kb minicircles, unique to kinetoplastida. Maxicircles encode subunits of respiratory chain complexes as well as a mitoribosome subunit and rRNAs. mRNAs for 12 maxicircle genes require post-transcriptional RNA editing to become functional, a process mediated by minicircle-encoded guide RNAs (gRNAs). We have now generated the first nearly complete (~99%) kDNA genome assembly of *T. brucei*, including predictions of gRNA genes and associated minicircle motifs, and determined the small RNA transcriptome before and after differentiation from bloodstream to procyclic form. We show that many minicircle genes encode transcripts that do not match sequences in the known editing space. These gRNA-like molecules bear many of the characteristics of known gRNAs, but also have distinct features, suggestive of a functionally distinct role. Although the life cycle stages of *T. brucei* in the mammal and in the tsetse fly depend on different subsets of mitochondrial mRNAs, we find that both life cycle stages express sufficient gRNAs to cover the entire known editing space.

FoF1-ATP synthase subunit α – A tale of two fragments- Poster 5 : A10675
Presenter: **Dr Karolina Subrtova**, *Postdoc, University of Edinburgh*

Authors: **K Subrtova**[1]; O Gahura[2]; M Saldivia[3]; B Panicucci[2]; J Mottram[3]; A Zikova[2]; A Schnaufer[1];
[1] Institute of Immunology and Infection Research, University of Edinburgh; [2] Institute of Parasitology, Biology Centre, ASCR, Czech Republic; [3] University of York, Centre for Immunology and Infection

The FoF1-ATP synthase is a reversible nanomotor synthesizing ATP in bacteria and eukaryotic mitochondria. The core catalytic F1 moiety of this multisubunit complex is formed by a globular hexamer of alternating subunits α and β sitting on a central stalk consisting of subunit γ and small subunits δ and ε [1]. The composition and structure of the core F1-ATPase is believed to be strictly conserved throughout evolution [1], however this notion is based on the established structures of FoF1-ATP synthase complexes of bacteria and model eukaryotes and may not reflect full eukaryotic diversity. Several reports have indicated that the Euglenozoa F1-ATPase subunit α is split into two fragments, presumably by proteolytic cleavage [2–5]. Both fragments stay associated with the complex. This feature appears to have no parallel in any other group of organisms. In this project, we are investigating whether α cleavage results in novel features of this

key enzyme, that are important for the structure/function of FoF1-ATP synthase in trypanosomes. We also aim to identify the protease responsible.

[1]J.E. Walker, Biochem. Soc. Trans. 41 (2013) [2]A. Zikova, et al , PLoS Pathog. 5 (2009) [3]D. Speijer, et al , Mol. Biochem. Parasitol. 85 (1997)[4]R.E. Nelson, et al , Mol. Biochem. Parasitol. 135 (2004) [5]E. Perez, et al , Mitochondrion 19 (2014)

Identifying highly divergent glycosyltransferases in the African trypanosome- Poster 6 **:** A10681
Presenter: **Samuel Duncan**, *Postdoctoral Researcher, The University of Dundee*

Authors: **S M Duncan**[1]; M A Ferguson[1];
[1] The University of Dundee

Trypansoma brucei is a protozoan parasite that infects humans and cattle via a tsetse fly vector. Key to parasite survival during progression through this complex life cycle is the expression of cell surface and endocytic pathway glycoproteins, modified with glycosylphosphatidylinisitol (GPI) membrane anchors and/or N-linked oligosaccharides. We estimate that protein glycosylation in this parasite requires at least 38 distinct glycosyltransferases (GTs), only a few of which can be predicted by bioinformatics. Interestingly, a family of 21 putative trypanosome GTs has been identified that share a single beta 1-3 transferase ancestor but catalyse a diverse array of glycosidic linkages. Inhibition of such highly divergent GTs is therefore a promising therapeutic avenue, yet 17 of these putative TbGTs require characterisation. This project aims to identify their function by utilising reverse genetics, mammalian complementation and RIT-Seq approaches.

Kinetic of detection of *Leishmania donovani* DNA in the brain during experimental infection in mice. - Poster 7 : A10693
Presenter: **Miss Fernanda Grecco Grano**, *PhD student, University of York*

Authors: **F G Grano**[1]; J Doehl[1]; P M Kaye[1];
[1] Centre for Immunology and Infection, Department of Biology and Hull York Medical School, University of York

Visceral leishmaniasis (VL) is a systemic disease caused by the Leishmania donovani complex, with spleen, liver and bone marrow being major sites of infection. Little is known, however, about whether this parasite reaches the central nervous system, despite some evidence in the literature that suggests this possibility. The aim of this study was to investigate the presence of *L. donovani* DNA in the brain at various time after infection (14, 28, 35 and 56) in C57BL/6 mice. Using qPCR for kinetoplastid DNA, the peak of infection was observed at d28 post infection ($3x10^5 \pm 4x10^5$, $2x10^5 \pm 3x10^5$, $8x10^4 \pm 1x10^5$ and $2x10^4 \pm 1x10^4$ parasites / 25mg tissue in cerebellum, frontal lobe, diencephaplon and occipital lobe, respectively). In addition to a reduction in parasite load at later times of infection, parasite clearance from the brain could also be accelerated by administration of low dose lipopolysaccharides (LPS; 4ng/mouse from d14 to d28), collectively suggesting that brain parasite load may be controlled by immune mechanisms. These data provide strong evidence of the presence of the Leishmania parasite in the brain, contributing to a new perspective of the pathogenesis of VL

Characterizing genes involved in function and biogenesis of mitochondrial DNA in *Trypanosoma brucei*- Poster 8 : A10701
Presenter: **Dr Migla Miskinyte**, *Postdoc, University of Edinburgh*

Authors: **M Miskinyte**[2]; **C Schaffner-Barbero**[2]; M K Gould[3]; L Glover[1]; J C Mottram[4]; D Horn[1]; A Schnaufer[2];
[1] University of Dundee; [2] University of Edinburgh; [3] University of Munich, Germany; [4] University of York

Survival of tsetse-transmitted trypanozoon species crucially depends on maintenance and expression of their mitochondrial genome, termed kinetoplast (kDNA). Replication, segregation and expression of kDNA are extraordinary complex processes that involve an estimated ~300 proteins, only a minority of which have been identified [1,2]. In addition, very little is known about how these processes are regulated and integrated into cell and life cycle of the parasite. We are using genetic RNA interference target sequencing approaches (RIT-seq [3]) with kinome-wide and genome-wide RNAi libraries to identify novel factors involved in kDNA maintenance and expression and have identified several candidates. We will report on our progress in validating these candidates and in elucidating their specific biological roles using assays ranging from subcellular localisation to measuring the effects of gene knockdown on kDNA composition, maintenance, and expression.

1. Jensen & Englund (2012) Annu Rev Microbiol 66:473-91.
2. Lukes et al. (2011) IUBMB Life 63(7):528-37.
3. Glover et al. (2015) Nat Protoc 10(1):106-33.

Chemical V-ATPase inhibition fully compensates for ablation of mitochondrial RNA editing ligase 1 in *Trypanosoma brucei*- Poster 9 : A10702
Presenter: **Dr Claudia Schaffner**, *Postdoctoral associate, University of Edinburgh*

Authors: **C Schaffner-Barbero**[2]; **M Miskinyte**[2]; D Horn[1]; A Schnaufer[2];
[1] University of Dundee; [2] University of Edinburgh

The survival of *Trypanosoma brucei* depends on maintenance and function of the mitochondrial genome (kinetoplast, or kDNA). However, a single point mutation in subunit γ of the mitochondrial F1FO ATPase can abolish the requirement of kDNA for survival of the bloodstream form of the parasite (Dean et al., 2013), and it was found that genetic or chemical inhibition of the vacuolar ATPase (V-ATPase) could have a similar effect (Baker et al., 2015). Here we present detailed studies of the effects of V-ATPase inhibitors on parasite growth after genetic or chemical interference with kDNA function. We found that treating bloodstream form *T. brucei* cells with bafilomycin A1 fully rescued loss of viability induced by depletion of the essential RNA editing ligase REL1. This confirmed a connection between V-ATPase function and kinetoplast dependency and suggests that V-ATPase inhibitors could be used as chemical tools in the study of kinetoplast biology. However, only limited rescue of the growth phenotype was found upon addition of bafilomycin A1 to cells treated with a low concentration of ethidium bromide that caused specific kinetoplast loss. Furthermore, this rescue was not robust beyond 3 days. Work to explain these seemingly contradictory data is in progress and will also be presented at the meeting.

Phenotypic and functional genetic analysis of virulence attenuation in *Leishmania donovani*- Poster 10 : A10719
Presenter: **Ms Laura Piel**, *PhD student, Institut Pasteur*

Authors: **L Piel**[1]; P Pescher[1]; G Bussotti[1]; L Ma[1]; C Bouchier[1]; G F Späth[1];
[1] Institut Pasteur, Paris, France

Leishmania donovani cycles between insect and mammalian hosts and has evolved strategies to survive inside phagocytic cells by subverting host antimicrobial activities through expression of virulence factors.

Mechanisms that govern intracellular Leishmania survival remain widely unknown and only few parasite virulence factors have been identified to date. Our previous results revealed a progressive loss of L. donovani infectivity during culture adaptation. A phenotypic characterization of virulent promastigotes derived from hamster splenic amastigotes (culture passage P2) and attenuated long-term cultured parasites (culture passage P20) showed differences in vitro in terms of growth and stress resistance. In order to assess mechanisms underlying loss of infectivity and identify novel virulence factors, we use our experimental Leishmania system for a functional genetic screen (CosSeq). Genomic DNA from promastigotes freshly derived from hamster splenic amastigotes was used to generate a cosmid library. Using HTseq analysis, we validated nearly complete coverage of the Leishmania genome in our library, with only few genes missing. P20 parasites were transfected with the homologous cosmid library and injected into hamsters for in vivo selection of parasites carrying a cosmid with genes that enhance or restore infectivity. The hamster weight is monitored weekly and parasites will be extracted 10 weeks after inoculation. After mapping of virulence genes encoded in the cosmid inserts by transposon mutagenesis, genes will be individually validated in vivo and in vitro for their capacity to increase parasite infectivity and resistance to various cytotoxic host activities.

Functional characterisation of six essential protein kinases in *Trypanosoma brucei* reveals 3 that are required for cytokinesis- Poster 11 : A10740
Presenter: **Miss Helena De la Torre Olvera**, *PhD student, Institute of Infection, Immunity and Inflammation*

Authors: **H M De la Torre**[1]; J C Mottram[2]; **T C Hammarton**[1];
[1] Institute of Infection, Immunity and Inflammation, University of Glasgow; [2] University of York

Trypanosoma brucei is a protozoan parasite that causes sleeping sickness (in humans) and nagana (in cattle), which result in major health and economic problems in Sub-Saharan Africa. The life cycle of the parasite is complex involving a mammalian host and an insect vector (tsetse fly, Glossina spp.). In an RNAi screen (Jones et al., 2014) of the protein kinome of the bloodstream form (BSF) of *T. brucei*, 6 protein kinases were identified as playing a key role in the cell cycle of the parasite. Here, more detailed analysis reveals that 3 of these kinases are essential for cytokinesis.

Importance of trypanosome motility in the early infection- Poster 12 : A10797
Presenter: **Dr Christelle Cren**, *Post-doc, Institut Pasteur*

Infective forms of *Trypanosoma brucei* parasites are transmitted by the tsetse fly bite and develop in the lymph and blood as extracellular forms, inducing the typical symptoms of the disease. To characterize these early steps of development, mice were infected with fluorescent and bioluminescent parasites and the infection was daily monitored by intravital imaging. After natural transmission, parasites seem to be rapidly drained from the site of injection and firstly observed in the extravascular compartment before being detected in blood. Trypanosome flagellum being proposed as a virulence factor, we have investigated its importance in parasite migration/proliferation and avoidance of host immune system. Conditional knockout parasites for the dynein light chain 1 (LC1) of the dynein motor complex were generated. These mutants express a WT version of the LC1 gene in all insect stages except in metacyclic forms. In these forms as well as in mammalian bloodstream forms, only a non-functional version of LC1 will be expressed, preventing parasites from swimming forward. After natural transmission of these mutants, the early steps of infection will be scrutinized and compared with WT parasites. This study will provide invaluable information on the initiation of the disease that could be helpful for diagnosis improvements.

Evolutionary diversification of the trypanosome haptoglobin-haemoglobin receptor from an ancestral haemoglobin receptor- Poster 13 : A10814

Presenter: **Miss Harriet Lane-Serff** , *PhD student, University of Oxford*

Authors: **H Lane-Serff**[3]; P MacGregor[2]; L Peacock[1]; O Macleod[2]; C Kay[1]; W Gibson[1]; M Carrington[2]; M K Higgins[3];
[1] University of Bristol; [2] University of Cambridge; [3] University of Oxford

The haptoglobin-haemoglobin receptor of *Trypanosoma brucei* is expressed by the bloodstage form of the parasite, allowing acquisition of haem. We show that in T. congolense, the major species responsible for causing nagana in cattle, this receptor is instead expressed in the epimastigote developmental stage that occurs in the tsetse fly, where it acts as a haemoglobin receptor. Additionally, we present the structure of the T. congolense receptor in complex with haemoglobin. We propose an evolutionary history for this receptor, identifying the changes that took place as it adapted from a role in the insect to a new role in the mammalian host. The haptoglobin-haemoglobin receptor of *Trypanosoma brucei* is expressed by the bloodstage form of the parasite, allowing acquisition of haem. We show that in *T. congolense, the major species responsible for causing nagana in cattle,* this receptor is instead expressed in the epimastigote developmental stage that occurs in the tsetse fly, where it acts as a haemoglobin receptor. Additionally, we present the structure of the *T. congolense* receptor in complex with haemoglobin. We propose an evolutionary history for this receptor, identifying the changes that took place as it adapted from a role in the insect to a new role in the mammalian host. The haptoglobin-haemoglobin receptor of *Trypanosoma brucei* is expressed by the bloodstage form of the parasite, allowing acquisition of haem. We show that in *T. congolense, the major species responsible for causing nagana in cattle,* this receptor is instead expressed in the epimastigote developmental stage that occurs in the tsetse fly, where it acts as a haemoglobin receptor. Additionally, we present the structure of the *T. congolense* receptor in complex with haemoglobin. We propose an evolutionary history for this receptor, identifying the changes that took place as it adapted from a role in the insect to a new role in the mammalian host.

An improved *Leishmania donovani* reference genome after PacBio SMRT sequencing: the road to Whole Genome Sequence of the parasite- Poster 14 : A10815

Presenter: **Prof. Jean-Claude Dujardin**, *Dept of Biomedical Sciences, Head, Institute of Tropical Medicine*

Authors: **H Imamura**[1]; M Domagalska[1]; F Dumetz[1]; M Vanaerschot[1]; J Cotton[3]; M Berriman[3]; J Vermeesch[2]; J C Dujardin[1];
[1] Institute of Tropical Medicine, Antwerp, Belgium; [2] KU-Leuven, Belgium; [3] Wellcome Trust Sanger Institute

In 2011, the first *L. donovani* reference genome, based on a clinical isolate from the Indian Subcontinent (BPK282/0cl4), was assembled using a combination of 454 and Illumina sequencing. Using the Pacbio RSII sequencer and P5-C3 chemistry, we have re-sequenced that genome, yielding around 616,900 post-filtered reads with an average length of 8.4 kb (131x coverage). SMRTanalysis tools were used to assemble the reads and final base/indel correction was carried out by ICORN using Illumina reads, while annotations were added using Companion. The PacBio assembly resulted in 36 chromosomes and, for the first time, a full maxicircle. At the same time, quality increased by reducing gaps from 2142 in the previous reference to 20. Complex regions were refined like intra-chromosomal amplicons on chromosome 23 (H-locus) and 36 (MPK1) or other repetitive regions such as the HSP70 and miniexon tandem-arrays. Further improvements were also made in telomeric regions. In addition, inversions and miss-assemblies in the old reference

sequence were corrected, reducing a substantial number of false positive genetic variations. These improvements led to a better gene annotation and provided new insights into previously poorly characterized regions. Those particular features are required for a thorough investigation of the genome stability and plasticity of the *Leishmania* genome at population and single cell levels.

Parasite Secretomics: Investigating the secretome of *Leishmania* mexicana to elucidate mechanisms of intracellular survival- Poster 15 : A10823
Presenter: **Miss Hazel Hamilton**, *PhD Student, University of Glasgow*

Authors: **H J Hamilton**[1]; R J Burchmore[1];
[1] *University of Glasgow*

Leishmania communicate with their host environments by the uptake and secretion of proteins and metabolites. Secreted molecules from the parasite are hypothesised to play a critical role in parasite establishment and survival through amelioration of host challenges or augmentation of nutrient availability. Our aim is to identify effector molecules and survival mechanisms used by the parasite to maintain infection. We have optimised methods of controlled serum-free secretome extraction to minimise the contribution of serum and cell lysis proteins to the proteome of the cell conditioned media. Results show the identification of proteins enriched in the secretome of both promastigotes and amastigotes of *L. mexicana.* In addition, differences between the secreted proteins of the two forms are highlighted, indicating a defined role for these secreted proteins in each of the distinct host environments. Furthermore, attenuated parasites which can infect but do not survive in macrophages demonstrate significant differences in their secreted proteins to that of the wild type. This indicates a key role for secreted proteins in parasite survival in the host cell. Investigating the host parasite interaction furthers our understanding of the factors that determine the growth and persistence of these parasites in the body to aid in the development of drug interventions.

Mutational analysis of the phosphorylation sites of the *Leishmania* mexicana kinesin homologue LmxKin29- Poster 16 : A10827
Presenter: **Mrs Suad Al Kufi**, *PhD student, Strathclyde University*

Authors: **S Al-Kufi**[1]; J Emmerson[1]; H Rosenqvist[1]; M Wiese[1];
[1] *Strathclyde Institute of Pharmacy and Biomedical Sciences*

Kinesins are ATP-dependent microtubule binding molecular motor proteins essential for many key cellular processes in eukaryotic cells. They are involved in the movement of cell organelles, chromosomes, protein complexes, and mRNA to specific destinations within the cell. Kinesins can be modulated by mitogen-activated protein (MAP) kinase signal transduction cascades. Here, we focus on the kinesin homologue, LmxKin29 from *Leishmania mexicana*. It has been found to be phosphorylated at serine 548, serine 551 and serine 554 using whole cell lysates in phosphoproteomics analyses. A peptide carrying these phosphorylation sites is phosphorylated by activated recombinant LmxMPK3, a *Leishmania* MAP kinase involved in flagellar length control. A null mutant for LmxMPK3 showed flagella reduced in length to approximately 1/5 of the length of a wild type flagellum. We cloned and expressed LmxKin29 fused to glutathione-S-transferase and a number of putative phosphorylation site mutants. This allowed us to prove that LmxMPK3 can phosphorylate full length LmxKin29 exclusively on serine 554. To understand the function of LmxKin29 in *L.mexicana* we generated homozygous null mutants. Moreover, we generated cell lines expressing N- or C-terminally GFP-tagged LmxKin29 in the wild type and knock out background. Microscopic analyses indicate that LmxKin29 predominantly localises close to the flagellar pocket. Further phenotypic analyses are under way.

Structural and functional studies of *Trypanosoma brucei* TbMORN1 protein- Poster 17 : A10828
Presenter: **Ms Sara Sajko**, *PhD student, University of Vienna*

Authors: **S Sajko**[2]; B Morriswood[1]; K Djinovic-Carugo[2];
[1] Department of Cell and Developmental Biology (Zoology I), Biozentrum, University of Würzburg, Austria; [2] Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Austria

TbMORN1 is an essential protein in BSF *T. brucei*. It is present in the hook complex at the flagellar pocket neck of the parasite, lying just below its plasma membrane. TbMORN1 is composed of 15 tandem Membrane Occupation and Recognition Nexus (MORN) repeats. MORN repeat proteins are found throughout the tree of life, examples including a family of lipid kinases in plants, the junctophilin proteins found at triad junctions in mammalian muscle, and TbMORN1´s closest homologue, TgMORN1 in *T. gondii*. Although the function of MORN repeats remains elusive, they have been proposed to interact with lipids, in particular phosphatidic acid, PtdIns(4)P and PtdIns(4,5)P_2. We are investigating the TbMORN1 capacity to bind phosphoinositides. Lipid blots indicated that TbMORN1 interacts with different phosphoinositide species in a phosphorylation-dependent manner. Based on the TbMORN1 3D model and bioinformatics analysis, 4 PtdIns(4,5)P_2 binding sites were predicted in MORN repeats 5, 6, 13 and 14. Sequence alignment showed that these binding sites are conserved among TbMORN1 and TgMORN1. Affinity of PtdIns(4,5)P_2 to TbMORN1 and TgMORN1 is being investigated by the fluorescence anisotropy. Preliminary determinations of K_D values indicate that TbMORN1 binds to PtdIns(4,5)P_2 with micromolar affinity. Crystallization experiments yielded diffraction quality crystals and diffraction data of TbMORN1 construct encompassing MORN repeats 7-15, and first crystallization hits of TgMORN1 constructs encompassing repeats 2-15 and 7-15. Optimization of crystallization conditions and of diffraction data is in progress.

Screening for antiparasitic leads from a library of natural products from temperate zone plants- Poster 18 **:** A10830
Presenter: **Dr Helen Price**, *Lecturer in Bioscience, Keele University*

Authors: H Hameed[1]; **H P Price**[3]; R Nash[2]; P D Horrocks[1];
[1] Institute for Science and Technology in Medicine, Keele University; [2] Phytoquest Limited; [3] School of Life Sciences, Keele University

There is an urgent need to identify and evaluate novel chemical scaffolds to seed the drug discovery pipeline for parasitic diseases. The UK-based SME PhytoQuest has produced a library of about 1000 molecules representing a unique resource for lead discovery of high value chemicals from temperate zone plants. The library comprises pure compounds selected from a wide range of chemical classes, with a high degree of functionality and physiochemical properties that match Lipinski's Rule of Five. A subset of 650 compounds have been screened against axenic amastigotes of *Leishmania mexicana*, bloodstream form *Trypanosoma brucei* and the intraerythrocytic stages of *Plasmodium falciparum*. Here we report a characterization of our hits against *L. mexicana* and *T. brucei*, with additional testing of selected compounds on *L. donovani* and human cell lines (THP-1 and HepG2).

Trypanosomatid parasites rescue heme from endocytosed hemoglobin through lysosomal HRG transporters- Poster 19 : A10844
Presenter: **Dr José M. Pérez-Victoria**, *Research Scientist, IPBLN-CSIC*

Authors: M Cabello-Donayre[1]; S Malagarie-Cazenave[1]; J Campos-Salinas[1]; F J Gálvez[1]; L M Orrego[1]; M Martínez-García[1]; A M Estévez[1]; **J M Pérez-Victoria**[1];
[1] IPBLN-CSIC, Spain

An Achilles' heel of pathogenic trypanosomatid parasites is their absolute dependence on scavenging heme from their human hosts. Here, we unravel the mechanism used by *Trypanosoma brucei* and *Leishmania* to rescue heme from endocytosed hemoglobin. We show that parasite HRG transporters localize to the endolysosomal compartment, where hemoglobin is trafficked, and promote heme bioavailability after hemoglobin endocytosis. Using heme auxotrophic yeast mutants we demostrate that parasite HRG proteins enable yeast cells to exploit hemoglobin as a source of heme, increasing hemeprotein activity and rescuing the yeast growth defect. As these transporters are essential in *T. brucei* and *Leishmania*, their specific targeting could represent a novel method for controlling the neglected diseases caused by these parasites.

Data mining using TriTrypDB resources- Poster 20 : A10925
Presenter: **Dr Kathryn Crouch**, *EuPathDB, University of Glasgow*

Authors: **K Crouch**[1] ;**,F Silva**[2]; C Hertz-Fowler[2]; D S Roos[3];
[1] University of Glasgow ; [2] University of Liverpool; [3] University of Pennsylvania, United States

TriTrypDB (http://tritrypdb.org) is a free, online data mining resource that facilitates the discovery of biological relationships from large volumes of data by integrating pre-analyzed omics data with advanced search capabilities, data visualization and analysis tools. TriTrypDB supports over 25 organisms from the Kinetoplastida. These include many species of *Leishmania* and *Trypanosoma* (including multiple strains of *T. brucei*, *T. cruzi* , and others) from a variety of hosts, as well as the related genera *Leptomonas*, *Crithidia* and *Endotrypanum*. TriTrypDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing clinical and field isolates, and data that inform host-pathogen interactions. Data are analyzed using standard workflows and an in-house analysis pipeline generates data including domain predictions, orthology profiles and GO term associations. Our unique strategies system offers over 100 structured searches that query the pre-computed data. Individual search results can be combined into strategies that merge evidence from diverse data types and across organisms. Accessible tools enhance the search strategy system and include dynamic data visualization, comparative genome analysis, population genetics tools, and functional or pathway enrichment. Forthcoming features include a private user work-space for primary data analysis, functional analysis tools for result summarization, genome browser and query improvements. This comprehensive resource places the power of bioinformatics with the scientific community supporting hypothesis driven research. Updates include: completely revamped gene pages, alternative transcripts represented in gene pages and strategies, new search categories with a "Find a Search" tool and additional data. To learn more visit our booth during any poster session.

TbSmee1 regulates hook complex biogenesis in procyclic *Trypanosoma brucei*- Poster 21 **:** A10930
Presenter: **Jenna Perry**, *Graduate Student, Brown University*

Authors: **J A Perry**[1]; A N Sinclair-Davis[1]; C L de Graffenried[1];
[1] Brown University, United States

TbSmee1 (Tb927.10.8820) is a putative substrate of Polo-like kinase and a component of the *Trypanosoma brucei* hook complex, which wraps around the apex of the flagellar pocket. We show that depletion of TbSmee1 in the insect-resident procyclic form causes slow growth and multinucleated cells, suggesting a defect in cytokinesis. Cells lacking TbSmee1 undergo altered hook complex biogenesis and have accelerated endocytic uptake, as measured by the membrane marker FM4-64FX. These results suggest that hook complex biogenesis is essential for regulating endocytic flux.

Forward Genetics of Suramin Resistance in African Trypanosomes- Poster 22 : A10933
Presenter: **Ms Natalie Wiedemar**, *PhD student, Swiss TPH*

Authors: **N Wiedemar**[1]; T Wenzler[1]; R S Schmidt[1]; C Kunz Renggli[1]; M Cal[1]; E Ndomba[1]; F E Graf[1]; P Mäser[1];
[1] *Swiss Tropical and Public Health Institute, Switzerland*

African trypanosomes are able to recover from high suramin pressure *in vitro* and grow new cultures with a massively lower drug susceptibility. To unravel the mechanisms leading to this phenomenon, forward genetic approaches were applied using mRNA sequencing of one *T. b. rhodesiense* clone and four independently selected lines. Gene-expression analysis revealed very few differentially expressed transcripts. Interestingly, the selected lines showed upregulation of the identical VSG-like gene.

Functional analysis of TOEFAZ1 uncovers protein domains essential for cytokinesis in *Trypanosoma brucei*- Poster 23 : A10934
Presenter: **Amy Sinclair-Davis**, *Graduate Student, Brown University*

Authors: **A N Sinclair-Davis**[1]; C L de Graffenried[1];
[1] *Brown University, United States*

Trypanosoma brucei polo-like kinase (TbPLK) is a master regulator of cytoskeletal morphogenesis. We identified the potential TbPLK substrate TOEFAZ1, which localizes to the new extending FAZ, as essential for cleavage furrow ingression in cytokinesis. Analysis of TOEFAZ1 has identified the function of discrete domains, including cell cycle regulation and localization. An RNAi complementation strategy shows that α-helical and zinc finger domains are essential for TOEFAZ1 function. As TOEFAZ1 is conserved in kinetoplastids, elucidating its function will shed light on their unique cytokinetic process.

A cAMP response protein necessary for *Trypanosoma brucei* social motility- Poster 24 : A10941
Presenter: **Mr Aris Aristodemou**, *MSc student, Ludwig Maximilians University - Munich*

Authors: A Aristodemou[1]; S Bachmaier[1]; M Gould[1]; M Boshart[1];
[1] *Biocenter, Section Genetics, Ludwig Maximilians University Munich, Martinsried, Germany*

African trypanosomes exhibit non-canonical cAMP signaling pathways. A recent genome wide RNAi screen for genes implicated in resistance to elevated cAMP has identified several putative cAMP response proteins (CARPs). Given that adenylate cyclase activity was reported to influence trypanosome social motility (SoMo), we functionally screened the identified proteins for SoMo defects using inducible RNAi mediated knockdown. At least one CARP is involved in SoMo as demonstrated by complete loss of group motility in knockdown and knockout cell lines.

Multiple Mechanisms of kDNA Maintenance by Polymerase IC in *Trypanosoma brucei*.- Poster 25 : A10951
Presenter: **Jonathan Miller**, *Graduate student, University of Massachusetts Amherst*

Authors: **J C Miller**[1]; L Vuong[1]; M M Klingbeil[1];
[1] *University of Massachusetts Amherst, United States*

Among the three essential kDNA polymerases, POLIB and POLID have roles in replication, while the role of POLIC in kDNA maintenance remains unclear. POLIC RNAi resulted in loss of fitness (LOF) and a decrease in mini- and maxicircle copy number. RNAi also resulted in an ancillary kDNA phenotype, suggesting that POLIC may have roles besides its DNA polymerase activity. Additionally, POLIC forms foci at the antipodal sites (AP) during kDNA S-phase via unknown mechanisms. Here, we addressed the requirements of DNA polymerase activity and an uncharacterized N-terminal region (UCR) by complementing POLIC RNAi with overexpression of POLIC variants. Importantly, RNAi complementation with WT-POLIC rescued the LOF, restored kDNA copy number and AP foci formation. In contrast, complementation with an inactive-POLIC mutant caused a rapid LOF compared to the parental RNAi cell line, a loss of kDNA, and localization to the kDNA disk throughout the cell cycle, verifying that the polymerase domain is essential to maintain kDNA. Overexpression of inactive-POLIC in a WT background indicated a dominant-negative phenotype, while that with the UCR deletion did not impact fitness. Lastly, complementation with the UCR deletion could not rescue LOF and abolished AP foci formation, TdT incorporation, and caused a stark increase in ancillary kDNA compared to parental RNAi. Together these results show the UCR and DNA polymerase activity are both indispensable for maintaining kDNA integrity.

Ferroptosis in African trypanosomes- Poster 26 : A10964
Presenter: **Ms Marta Bogacz**, *PhD student, Heidelberg University*

Authors: **M Bogacz**[1]; R L Krauth-Siegel[1];
[1] *Biochemie-Zentrum der Universität Heidelberg, Germany*

Ferroptosis is a form of non-apoptotic cell death described in mammalian cells, characterized by iron-dependent lipid peroxidation and glutathione peroxidase 4 (GPx 4) is its main regulator. *Trypanosoma brucei* possess three tryparedoxin peroxidases (Px I-III), related to GPx 4. Flow cytometry analysis of procyclic Px I-III KO cells using MitoSOX revealed reactive oxygen species production in the mitochondrion that was prevented by the iron chelator deferoxamine. Following 10-nonyl acridine orange fluorescence indicated cardiolipin peroxidation. As superoxide anions induce the formation of labile iron, the effect of overexpressing mitochondrial superoxide dismutase on Px I-III KO cells is currently investigated, as are different ferroptosis inhibitors. This work will shed light on the mechanism of iron-mediated cell death in *T. brucei*.

Gene expression regulatory networks in *Trypanosoma brucei*: the RBPome awakens- Poster 27 : A10969
Presenter: **Dr Esteban Erben**, *Postdoc, University of Heidelberg*

Authors: S Lueong[2]; C Merce[3]; B Fischer[1]; J Hoheisel[1]; **E D Erben**[4];
[1] *DKFZ, Germany;* [2] *IARC, France;* [3] *NCT, Germany;* [4] *ZMBH, Germany*

RNA-binding proteins (RBPs) are key modulators of gene expression. Indeed, whether a particular mRNA is translated, repressed, or degraded, depends largely on its RBP interactions. This is particularly relevant for

Kinetoplastids, which relies mainly on post-transcriptional mechanisms for regulation of gene expression. Here, we provide an overview of the proteins that bind to mRNAs and their putative functions in the pathogenic bloodstream form of *Trypanosoma brucei*. We used tethering assays to screen for proteins that play a role in post-transcriptional control. We found 90 proteins displaying a clear effect on the mRNA reporter expression, defining a catalogue of the most relevant trypanosome regulators. This list included both canonical RBPs and also proteins without RNA-related ontology. Several of the discovered repressors interacted with components of the CAF1/NOT1 deadenylation complex. To identify the repertoire of RBPs, we next obtained the mRNA-bound proteome. We identified 155 high-confidence candidates, revealing dozens of novel RBPs. Twenty-seven of these proteins were also found to modulate reporter expression in the tethering screen. Finally, we showed that 37 RBPs are evolutionary conserved from ancient trypanosome to human. Our study provides novel insights into the trypanosome mRNPs composition, architecture and function.

Evidence for a cullin RING ligase ubiquitylation switch modulating the surface proteome in African trypanosomes- Poster 28 : A10973
Presenter: **Martin Zoltner**, *senior research assistant, University of Dundee*

Authors: **M Zoltner**[2]; K F Leung[1]; R C del Pino[2]; D Horn[2]; M C Field[2];
[1] Department of Pathology, University of Cambridge, Cambridge; [2] Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee

We have demonstrated that ubiquitylation pathways play a crucial role in modulating the surface architecture of bloodstream-form *Trypanosoma brucei*. The deubiquitylating enzyme TbUsp7 controls the abundance of a precise cohort of membrane proteins, including invariant surface glycoprotein ISG75 and the acid phosphatase MBAP1, that is required for endocytosis and exocytosis. Recently we discovered a functional link between TbUsp7 and the conserved cullin adaptor TbSkp1, the mammalian orthologue of which is part of the Skp1-Fbox-Cullin1-Rbx1 complex. Both TbSkp1 and TbUsp7 silencing blocked endocytosis and the impacts on the global proteome were highly similar. Additionally, TbSkp1 knockdown dramatically decreased TbUsp7 abundance. These findings add a cullin RING ligase to this ubiquitylation pathway critical for controlling surface protein composition and affecting suramin sensitivity.

Characterisation of the hook complex protein TbSmee1 (Tb927.10.8820)- Poster 29 : A10995
Presenter: **Ms Daja Schichler**, *Master student, University of Würzburg*

Authors: **D Schichler**[1]; B Morriswood[1];
[1] Department of Cell & Developmental Biology (Zoology I), Biocentre, University of Würzburg, Germany

The flagellar pocket of *T. brucei* is the site of all endo- and exocytic traffic. Clustered around the neck of the flagellar pocket on its cytoplasmic face are a number of cytoskeletal complexes. One of these, the hook complex (containing the repeat motif protein TbMORN1), was recently implicated in regulating macromolecule access to the flagellar pocket. Previous work using proximity-dependent biotin identification (BioID) provided a list of new hook complex components. One of these, Tb927.10.8820, was also recently determined to be a candidate binding partner and substrate of the mitotic kinase TbPLK. Tb927.10.8820 was named TbSmee1 due to its presence in the hook complex. A preliminary characterisation of TbSmee1 in bloodstream form cells is presented here.

A chemical tool to characterise essential catabolic functions within the trypanosomatid lysosome.- Poster 30 : A10999
Presenter: **Dr Simon Young**, *Research Fellow, Centre for Biomolecular Sciences*

Authors: **S A Young**[1]; T K Smith[1];
[1] *University of St. Andrews*

Current treatments for kinetoplastid related diseases are inadequate and there is an urgent need for lead compounds that can be translated into safe, cheap, and easy to administer drugs. The causative agent of Human African Trypanosomiasis, *Trypanosoma brucei*, relies upon endocytosis and degradation of host macromolecules from the mammalian bloodstream to acquire metabolites vital for its proliferation and survival. The terminal compartment of the endocytic pathway, the lysosome is critical to this macromolecular digestion. However, very little is known about this organelle with only a handful of proteins characterised. To successfully study essential processes and identify new drug targets within it, the lysosome should be isolated. Standard cellular fractionation is ineffective unless a non-digestible macromolecule is employed which accumulates in *T. brucei* lysosomes and alters their density so they can be more easily purified. Sucrose gradient centrifugation produced a distinct fraction that contained electron dense particles comparable in morphology to lysosomes. Proteomic analysis of the lysosome-like particles identified known lysosomal markers and proteins with digestive, structural and transport related functions. We are utilising this approach to investigate lysosomal catabolic processes in detail and test compounds that will disrupt the function of this essential *T. brucei* organelle.

Stage-specific reporter gene expression in *Trypanosoma cruzi* - Poster 31 : A11004
Presenter: **Ms Anna Fesser**, *PhD student, Swiss Tropical and Public Health Institute; University of Basel*

Authors: **A F Fesser**[1]; R S Schmidt[1]; M Cal[1]; C Kunz[1]; P Mäser[1]; M Kaiser[1];
[1] *Swiss Tropical and Public Health Institute, University of Basel, Switzerland*

There is a persisting need for new drugs against *Trypanosoma cruzi*. Our plan is to render *in vitro* assays against *T. cruzi* more predictive by the inclusion of stage-specific fluorescent reporters. To obtain a suitable transgenic parasite line, amastigote- and trypomastigote-specific genes were identified and chosen as loci of integration for reporter constructs. Having optimized the transfection protocol, we proceed to establish a *T. cruzi* line expressing stage-specific reporters.

mRNAs encoding ribosomal proteins might be regulated by a novel RNA-binding protein-
Poster 32 : A11026
Presenter: **Mrs Kathrin Bajak**, *PhD Student, University of Heidelberg*

Authors: K Bajak[1]; E Erben[1]; C Clayton[1];
[1] *Zentrum für Molekulare Biologie, Germany*

Gene expression in trypanosomes is mainly regulated by post-transcriptional mechanisms, which makes them an excellent system for studying eukaryotic mRNA translation and degradation. RNA-binding proteins (RBPs) are known to be important in this regulation. Previously, a tethering assay was performed to identify putative trypanosome post-transcriptional regulators (1). The tethering screen yielded numerous candidate effectors, including the protein encoded by Tb927.10.14150. This protein has some similarities to yeast Bfr1p, an ER- and polysome-associated protein. Tb927.10.14150 protein is an up-regulator in the tethering

screen, and showed *in vivo* mRNA binding (2) although it lacks canonical RNA-binding domains. We could show that the protein is essential in the bloodstream form trypanosomes, since RNAi mediated knock-down led to a growth defect. Immunofluorescence microscopy to detect a tagged version suggested that it is located in the cytoplasm, but perhaps also in the mitochondria. After pull-down and RNA sequencing, we found that most of the enriched mRNAs encode for ribosomal proteins. The regulation of mRNAs encoding for ribosomal proteins after stress is very different from that of most proteins, since they are never found in RNA-protein stress granules. We speculate that the Tb927.10.14150 protein might keep the enriched mRNAs attached to the ribosomes and thus prevent sequestration in granules.

(1) Erben et al, PLoS Pathogens 10: e1004178
(2) Lueong et al, Mol MIcrobiol 100: 457-471
(3) Fritz et al, Nucleic Acids Res 43: 8013-8032; and Minia et al (submitted).

Natural compounds: A potential source of novel chemical entities to kill *Leishmania*?-
Poster 33 : A11042
Presenter: **Nilma Fernandes**, *PhD student, University of York*

Authors: **N Fernandes**[2]; V C Desoti[3]; Y C Silva[1]; C V Nakamura[3]; J C Mottram[4]; V F Veiga-Junior[1];
[1] Federal University of Amazonas, Brazil; [2] Federal University of Amazonas/ University of York; [3] State University of Maringa, Brazil; [4] University of York

Therapeutic options for treatment of *Leishmania*sis have severe side effects and low efficacy, so the search for new effective chemotherapeutic agents is a high priority. The interest in natural products has intensified to find new medicines. We have been evaluating different species of plants in order to find bioactive compounds against *Leishmania* . Lauraceae plants should be highlighted since they produce compounds, including alkaloids, with a huge variety of biological activities. We performed *in vitro* screening against *Leishmania* amazonensis using ethanolic extracts of Lauraceae from the Amazon. Extracts of Aniba panurenis showed highest activity against L. amazonensis promastigotes, which lead the isolation of a new alkaloid, C1. We also evaluate changes induced by C1 on L. amazonensis promastigotes. Parasites were treated with the 95.7 µM of C1, after 24h of exposure, reactive oxygen species production and accumulation of lipids bodies were higher in the treated samples compared to the control. After 72h, promastigotes exhibited morphological changes with multiple flagella and nuclei and blocked cytokinesis. Increase in autophagic vacuoles was also observed. Our results demonstrated that C1 can induce changes in the parasites that lead to cell death and could be used as a template to synthesize new compounds with higher activity. Further studies to identify possible targets of anti-*Leishmania*l compounds will be performed.

Dynamic regulation of the *Trypanosoma brucei* transferrin receptor is mediated via the 3'UTR- Poster 34 : A11044
Presenter: **Dr Michael Urbaniak**, *Lecturer, Lancaster University*

Authors: W Lo[1]; H Benns[1]; **M D Urbaniak**[1];
[1] Lancaster University, Biomedical and Life Sciences

Trypanosoma brucei obtains the essential element iron by uptake of host transferrin through its own transferrin receptor. Under iron starvation conditions, expression of the transferrin receptor rapidly increases equally at the mRNA and protein level, suggesting that regulation occurs through an unknown post-transcriptional mechanism (Fast et al., 1999). The *T. brucei* transferrin receptor is a heterodimer of the glycoproteins ESAG6 and ESAG7, with the former containing a GPI anchor to facilitate attachment to the plasma membrane. We identified the *ESAG6* 3'UTR by reverse transcription of mRNA, and to study its

function we fused the 3'UTR onto reporter genes encoding GFP or firefly Luciferase (fLUC) and inserted them into the 2T1 bloodstream form cell line at the tagged RRNA locus. Iron starvation conditions, induced by addition of iron chelators or by switching from bovine to canine serum, resulted in rapid upregulation of the reporter proteins consistent with the magnitude and timing of the previously reported upregulation of the transferrin receptor. We conclude that i. the dynamic regulation of the *T. brucei* transferrin receptor is mediated via its 3'UTR, and ii. the effect is independent of the ES body, Pol I transcription and proximity to the telomere.
References: Fast et al, 1999, Biochem.

Molecular Characterization of Lifetime Infections with Trypanosomes in Individual Cattle in Ghana - Poster 35 : A11046
Presenter: **Ms Jennifer Afua Ofori**, *Student, University of Ghana*

Authors: **J A Ofori**[3]; S M Bakari[3]; G Aning[2]; G Awandare[3]; M Carrington[1]; T M Gwira[3];
[1] Department of Biochemistry, University of Cambridge; [2] School of Veterinary Medicine, University of Ghana, Ghana; [3] West African Centre for Cell Biology of Infectious Pathogens and Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Ghana

Livestock rearing is an integral component of farming systems and contributes significantly to food and economic security in developing countries. Trypanosomiasis is the most economically important constraint to livestock productivity in sub-Saharan Africa. In Ghana, the most common trypanosome species which have been detected in cattle include;*Trypanosoma brucei, T. congolense, T. vivax and T. simiae.* The prevalence of the disease in cattle is estimated between 5-50%. Despite the impact of the disease, there is no study on lifetime infections with trypanosomes in cattle in Ghana. This study aims to characterise trypanosome species throughout the natural infection cycle in cattle in Ghana over a 2 year period. Two herds of cattle (20 each) at Accra and Adidome have been selected based on their geographical location, tsetse fly density, prevalence of trypanosomiasis and the breed of cattle available. Blood is collected at approximately five (5) weeks intervals and the infecting trypanosomes are identified and characterised using internal transcribed spacer (ITS)-based nested polymerase chain reaction (PCR) and multiplex nested PCR targeting part of the trypanosome tubulin gene cluster. The identification is further confirmed by nucleotide sequencing. Preliminary data shows *Trypanosoma theileri* and *Trypanosoma vivax* as the major infecting species at the Accra and Adidome sites, respectively. However, *Trypanosoma brucei brucei*, which was previously reported to be prevalent in Ghana, was not detected. The data generated from this study will provide invaluable information on the biology of trypanosome infection and help inform control measures in the infected area in Ghana.

***Trypanosoma cruzi* RNA polymerase I: The trypanosomatid specific subunit TcRPA31 bears a functional nuclear localization signal**.- Poster 36 : A11047
Presenter: **Dr Roberto Hernandez**, *Proffesor, Instituto de Investigaciones Biomedicas UNAM*

Authors: I Canela[1]; A M Cevallos[1]; I López-Villaseñor[1]; **R Hernández**[1];
[1] Instituto de Investigaciones Biomédicas, UNAM, Mexico

RNA polymerase I from trypanosomes has raised the interest of several colleagues in the field of molelcular parasitology, especially in the case of African species of trypanosomes where this enzymatic complex transcribes the well-studied variant surface glycoprotein encoding genes, in addition to rRNA transcription units. Our research work group has been interested in the analysis of rRNA genes transcription in the American species *Trypanosoma cruzi .* In a recent line of research we are conducting the general

characterization of the orthologous RNA pol I subunit TcRPA31. The deduced primary structure of this protein shows the occurrence of a potential nuclear localization signal (NLS). With the aid of a tagged fluorescent version of this protein, we have been able to demonstrate its nucleolar localization in transfected *T. cruzi* cells. A deletion of the referred NLS in the transfected chimeric genes, produced a fluorescent protein present throughout the cellular body of the parasites. Therefore, we conclude that this NLS is functional in *T. cruzi ,* thus providing an experimental marker to further analyze the import of proteins in this pathogen.

***Leishmania* Strain Panel Development for Phenotypic Screening**- Poster 37 : A11048
Presenter: **Dr Lorna Maclean**, *Post-doctoral scientist, University of Dundee*

Authors: **L M MacLean**[1]; M De Rycker[1];
[1] *Drug Discovery Unit, University of Dundee*

Visceral *Leishmania*sis (VL) is caused by protozoan parasites *Leishmania donovani* and *L. infantum*. These are transmitted by the bite of infected female sandlflies. VL is fatal if untreated with an estimated 310 million people at risk of infection and >50,000 deaths annually. 90% of VL cases are recorded in Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan. The current drugs have issues with toxicity, painful injections, cost, and long treatment regimen, therefore, new treatments are urgently required. We have developed an *in vitro* screening cascade to identify new phenotypic hits against *L. donovani* which could progress to new clinical candidates for VL. Our cascade starts with an axenic amastigote luminescence assay, followed by a high-content image-based intracellular assay which uses *L. donovani* LdBob strain and PMA differentiated THP1 host cells. However, to identify compounds that are effective against clinically relevant strains from different geographical locations development of a *L. donovani/ L. infantum* strain panel was undertaken. This assay employs human peripheral blood monocytes (M-CSF differentiated) infected with low passage metacyclic parasites which are dispensed into 96 well plates for 96h. A single dye staining and analysis protocol was developed to generate potency and host cell toxicity data.

ARF regulating proteins as novel drug targets against kinetoplastids- Poster 38 : A11059
Presenter: **Mr Jaksha Chandrathas**, *PhD student, Life Sciences - Keele University*

Authors: **J P Chandrathas**[1]; H P Price[1];
[1] *Keele University*

Several members of the ADP-ribosylation factor (ARFs) family of the small GTPases are known to be essential for viability in *T. brucei* bloodstream form cells. However, the molecular interactions of these proteins have not been fully characterised in *T. brucei* and there is a high level of identity shared between *T. brucei and human* ARF protein sequences, thus impacting on their potential as drug targets. An alternative method of indirectly targeting of ARFs may be through their regulators, the guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). These regulating molecules are responsible for maintaining the active/inactive state ARFs and are highly divergent in *T. brucei*. The aim of this study is to identify ARF regulators in *T. brucei* and to investigate whether they are essential in the parasite using RNAi. Further studies will determine which ARFs are regulated by these proteins, towards development of an assay for inhibitor screening

Serum Biochemical Parameters and Cytokine Profiles Associated with Animal African Trypanosomiasis in Naturally Infected Cattle in Ghana- Poster 39 : A11063
Presenter: **Dr Theresa Manful Gwira**, *Lecturer, University of Ghana*

Authors: S M Bakari[3]; J A Ofori[3]; K A Kusi[4]; G Aning[2]; G Awandare[3]; M Carrington[1]; **T M Gwira**[3];
[1] Department of Biochemistry, University of Cambridge; [2] School of Veterinary Medicine, University of Ghana, Ghana; [3] West African Centre for Cell Biology of Infectious Pathogens-Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Ghana; [4] West African Centre for Cell Biology of Infectious Pathogens-Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Animal African Trypanosomiasis (AAT) affects livestock production in sub-Saharan Africa with prevalence estimated between 5 – 50% in Ghana. This study established the levels of serum biochemical parameters and cytokine profiles in naturally infected cattle over a period of six months. Nested Internal Transcribed Spacer (ITS)-based PCR and sequencing were used to characterize trypanosome infection in cattle (n = 40) at two areas, Adidome and Accra, of different endemicities. Levels Creatinine, Cholesterol, Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Total bilirubin and Total protein, and cytokines (IL-10, IL-4, IFN-γ, TNF-α and IL-12) were measured in serum samples collected every 4-5 weeks over 6 months. The predominant trypanosomes detected in Accra (non-endemic) and Adidome (endemic) were *Trypanosoma theileri* and *Trypanosoma vivax* respectively. Serum biochemical parameters were similar between infected and uninfected cattle in Accra. Infected cattle at Adidome had significantly higher levels of ALP ($p = 0.02$) and Total Bilirubin ($p = 0.04$) and significantly lower levels of cholesterol ($p = 0.02$) at specific time points. At basal levels and during infection, significantly higher pro-inflammatory/anti-inflammatory (Th1/Th2) cytokine ratios were observed in cattle at Adidome compared to Accra. Levels of IL-10 and TNF-α were significantly elevated in infected cattle in Accra. These findings suggest that, cattle in an endemic area repeatedly infected with parasites of different species and different antigenic types demonstrate high pro-inflammatory (Th1) immune response whereas cattle in a non-endemic area with chronic infections demonstrate high anti-inflammatory (Th2) immune response.

Cep164 genes in the transition zone of the mature basal body- Poster 40 : A11066
Presenter: **Dr Jiri Tyc**, *Postdoctoral Research Scientist, Oxford Brookes University*

Authors: **J Tyc**[1]; M Atkins[1]; S Vaughan[1];
[1] Department of Biological and Medical Sciences, Faculty of Health and Life Science, Oxford Brookes University, Oxford, OX3 0BP, UK

Cep164 proteins are essential components of the transitional fibres (or distal appendages) of basal bodies and centrioles. The transition zone docks the mature basal body to the plasma membrane and without it the basal body cannot nucleate a functional flagellum. Cep164 is one of the most conserved genes found in the basal bodies across the evolutionary spectrum and Kinetoplastid flagellates possess three rather diverse orthologues. Using immunofluorescence microscopy and endogenous tagging approaches all three proteins, both C and N terminally tagged versions, localize to the distal end of the mature basal body in a ring around the barrel of the basal body. Two of the Cep164 proteins are found in all mature basal bodies, but the third one is absent from the newly maturing basal body during the cell cycle and the signal appears only after abscission.

Magnetic Hyperthermia as a Novel Approach for Treatment of Cutaneous *Leishmania*sis-
Poster 42 : A11083
Presenter: **Miss Sarah Oates** , *Research Assistant, School of Life Sciences*

Authors: **S L Oates**[1]; N D Telling[1]; C Hoskins[1]; H Price[1];
[1] Keele University

The use of magnetic nanoparticles to produce heat (magnetic hyperthermia) has gained interest as a novel therapeutic. The increased sensitivity of cancerous cells to heat shock has meant magnetic hyperthermia has gained considerable interest as a potential alternative therapy. In the current study, we are analysing the potential of magnetic hyperthermia to be applied to the treatment of cutaneous *Leishmania*sis as an inducible, controlled and localised form of thermotherapy. We have confirmed there are differences in temperature sensitivity between *L. mexicana* axenic amastigotes and the human monocytic cell line THP-1. A stable ferrofluid has been produced by coating maghemite nanoparticles with citric acid. These nanoparticles are readily taken up by differentiated THP-1 cells, and an increase in temperature of up to 10°C can be achieved upon application of an alternating magnetic field. We are now investigating the effects of magnetic hyperthermia on *L. mexicana* amastigotes, both axenically and inside macrophages.

Elutriation of PCF and BSF *Trypanosoma brucei* allows separation of cell cycle stages- Poster 43 : A11084
Presenter: **Dr Corinna Benz**, *Senior Research Associate, Lancaster University*

Authors: **C Benz**[1]; M D Urbaniak[1];
[1] *Lancaster University*

Centrifugal counter-flow elutriation is a superior, non-invasive method to synchronise cell populations. The centrifugal force within the centrifuge is counterbalanced by a simultaneous flow of buffer that is being pumped through a special elutriation chamber in the opposite direction. Particles are thus separated by size, with smaller ones eluting first at low buffer flow rates if the centrifuge speed is kept constant. Since *Trypanosoma brucei* (procyclic form) PCF cells increase by several microns in length during the transition from G1 to S phase of the cell cycle, the isolation of extremely pure (>95%) G1 fractions by elutriation is possible. These cells can then be put back into culture and will synchronously proceed through the cell cycle. Bloodstream form (BSF) cells are not only smaller than PCF but also highly motile, and have thus far not been reported to separate efficiently during elutriation. Here we present data showing that isolation of cell cycle phase enriched populations of BSF cells by elutriation is possible and more reproducible than hydroxyurea-mediated synchronisation. However, greater care has to be taken that the starting population is in the logarithmic phase of growth to ensure subsequent synchronous progression through the cell cycle. Our overall goal is to use these optimised elutriation procedures to produce cell cycle stage enriched samples from both BSF and PCF cultures for comparative proteomic and phosphoproteomic analysis to identify novel cell cycle regulators in the parasite.

Can folate/pteridine transporters transport pyrimidine in protozoa?- Poster 44 : A11086
Presenter: **Mr Khalid Jamaan H Alzahrani**, *PhD student, University of Glasgow*

Authors: **K J Alzahrani**[1,2]; H P de Koning[2];
[1] *Department of Clinical Laboratory, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia;* [2] *Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom*

Most protozoa are capable of both salvaging performed pyrimidines and *de novo* pyrimidine biosynthesis. This study seeks to identify the gene (family) encoding the protozoan pyrimidine transporters using next-generation RNA-sequencing (RNA-seq). Resistance to 5-fluorouracil (5-FU) was generated in both *T. b. brucei* BSF s427- wild type and *Leishmania mexicana* promastigotes, yielding clonal lines Tbb-5FURes and Lmex-5FURes, respectively. RNA-seq was performed to identify and characterize the differentially expressed genes between the sensitive and resistant strains. Five folate/pteridine transporters genes

(TbbPters) out of nine were down-regulated in Tbb-5FURes, and two folate/pteridine genes (LmexPter) out of eleven genes were down-regulated in Lmex-5FURes. Interestingly, overexpression of TbbPter1 (Tb927.1.2820) in Tbb-5FURes caused a 3-fold increase in 5-FU sensitivity ($P < 0.0001$). Overexpression of TbbPter1, TbbPter3, TbbPter4 and TbbPter5 in Tbb-5FURes revealed a statistically significant decrease ($P < 0.05$) in sensitive to 6-azauracil, indicating a restored uracil salvage pathway and decreasing reliance on de novo synthesis. Characterization of LmexPters and generation of double gene deletions of TbbPters (dKO) is ongoing, and transport and sensitivity assays will be performed. Identification of pyrimidine transporter genes in protozoa will aid in the therapeutic targeting of pyrimidine metabolism of protozoa.

Are vesicles from the *Leishmania* flagellum a source of virulence factors?- Poster 45 : A11090
Presenter: **Miss Laura Makin**, *PhD student, University of Oxford*

Authors: **L Makin**[1]; E Gluenz[1]; S A Cowley[1];
[1] *University of Oxford, Sir William Dunn School of Pathology*

Leishmania are transmitted between mammalian hosts by the sand fly. Upon injection into the mammalian host promastigote-form parasites are phagocytosed by macrophages, where they differentiate into amastigotes. Although virulence factors are known to modulate macrophage signalling pathways to favour infection, the delivery mechanisms are largely unknown. During differentiation to amastigotes the promastigote flagellum shortens dramatically and the fate of the excess flagellar membrane is unknown. My research investigates the possibility that during *Leishmania* differentiation the flagellar membrane is shed as extracellular vesicles (EVs) which provide a virulence factor delivery mechanism. Ultracentrifugation was used to isolate EVs from differentiating parasite culture or a control promastigote parasite culture. Mass spectrometric analysis showed that known virulence factors and known EV-associated proteins were enriched in the differentiating sample in addition to many uncharacterised proteins. 34 candidate EV-associated proteins have been fluorescently tagged and localised, and work is ongoing to confirm whether these proteins are EV-associated. Isolated EVs induced changes in cytokine secretion by human macrophages, including reductions in chemokines and interferon gamma. To test whether this effect is independent of the known virulence factor LPG, ongoing research is comparing the effect of EVs from LPG1 knockout parasites with wild-type EVs.

Evidence of asynchronous replication of *Trypanosoma cruzi* amastigotes within single infected cells *in vivo* - Poster 46 : A11114
Presenter: **Dr Martin Taylor**, *Senior Lecturer, London School of Hygiene and Tropical Medicine*

Authors: **M C Taylor**[1]; A Fortes-Francisco[1]; S Jayawardhana[1]; J M Kelly[1];
[1] *London School of Hygiene and Tropical Medicine*

Studies on the pathogenesis and immunology of chronic murine Chagas disease are limited by the scarcity of parasites which complicates analysis of local responses to infected cells. We recently developed highly sensitive bioluminescent (BLI) *in vivo* imaging models for *Trypanosoma cruzi* using a red-shifted luciferase variant (PpyRE9h). However, the utility of such models is limited by the low resolution of BLI at the level of individual infected host cells. To circumvent this problem, we have generated *T. cruzi* expressing a fusion protein consisting of an N-terminal luciferase and a C-terminal green fluorescent domain. The bioluminescence of the new parasite line is unchanged by the fusion, the green fluorescent domain has no effect on the wavelength of bioluminescent emission, and the fusion protein is expressed throughout the life cycle. The modified parasites show identical infection characteristics to the original bioluminescent line. The

dual reporter system allows the sites of tissue-specific infection in mice to be visualised (based on bioluminescence), and the identification and isolation of infected cells (based on fluorescence). Intriguingly, approximately 50% of infected cells contained an uneven number of amastigotes suggesting that intracellular parasite replication is asynchronous. Extracellular amastigote-like forms with a short protruding flagellum and epimastigote&hypen;like forms could also be detected in infected mice. We are currently developing protocols to optimise the isolation and phenotyping of infected host cells. These techniques will provide new approaches for investigating the mechanisms of immune evasion and parasite persistence during the chronic phase of the disease.

FtsH protease of *Trypanosoma brucei*- Poster 48 **:** A11119
Presenter: **Dr Anton Horvath**, *Assoc. Prof., Comenius University, FNS, Dept. Biochemistry*

Authors: **A Horvath**[1,2]; B Kováová[1,2]; T Kovalinka[1,2]; ;
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Protozoan parasite *Trypanosoma brucei* (Euglenozoa, Kinetoplastea, Trypanosomatida) is the causative agent of sleeping sickness in human and disease Nagano in animals. Better knowledge of this organism is the key for successful fight against caused diseases. Our research focuses on mitochondrial FtsH protease, which was not being closely studied among trypanosomatids yet. FtsH protease is a representative of mitochondrial AAA proteases associated with various cell activities. In genome of *T. brucei* has been identified up to six homologs of FtsH subunits unlike S. cerevisiae and human which each possess only three different subunits. Subunits can form homo- or hetero- hexameric structures oriented into the mitochondrial matrix or intermembrane space, depending on transmembrane sequences on their N terminus. We have prepared cell lines with inducible RNAi of each subunit, and also V5 tagged cell lines. Influence of gene silencing on growth phenotype and activity of oxidative phosphorylation has been examined. We have also performed in silico analysis for the presence of transmembrane domains of these six genes, predicting their orientation.

Blocking VSG synthesis in bloodstream form *Trypanosoma brucei* triggers an ER stress response- Poster 50 : A11121
Presenter: **Dr Cher Pheng Ooi**, *Research Associate, Imperial College London*

Authors: **C P Ooi**[2]; S Ridewood[1]; N Vasileva[4]; S D'Archivio[3]; C Gadelha[3]; B Wickstead[3]; G Rudenko[2];
[1] Creek Institute; [2] Imperial College; [3] Nottingham University; [4] Porton Down

Variant surface glycoprotein (VSG) is the most highly expressed protein in bloodstream form trypanosomes (10% total). Blocking VSG221 synthesis using RNAi or morpholino oligos triggers a precise pre-cytokinesis arrest which is rescued by ectopic VSG117 expressed from the VSG221 expression site. However, we find that VSG117 expressed from the rDNA array does not rescue this arrest. High levels of VSG117 are expressed, but the precise cell-cycle checkpoint is not triggered. Here, *VSG221* RNAi results in multi-nucleated cells that do not divide but re-enter S-phase and re-initiate nuclear division. This less severe reduction in VSG levels results in cells that presumably do not have adequate VSG to form a cleavage furrow, but still reinitiate S-phase. This indicates that a precise cell-cycle checkpoint is only triggered when VSG synthesis drops below a critical level. We find that levels of the ER chaperone BiP increase when VSG synthesis is blocked and RNAseq analysis of these stalled cells has revealed additional up-regulated ER-associated genes following *VSG* RNAi. Epitope tagging has confirmed ER localisation of these genes and

we are currently characterising them to dissect the signalling pathway between the ER stress response induced by a VSG synthesis block and progression through the trypanosome cell-cycle.

Deciphering the role of *Leishmania* Casein kinase 1 in host subversion- Poster 51 : A11137
Presenter: **Mr Daniel Martel**, *PhD Student, Institut Pasteur*

Authors: **D Martel**[2]; D Loew[1]; F Dingli[1]; G F Späth[2]; N Rachidi[2];
[1] Institut Curie, Paris, France; [2] Institut Pasteur, Paris, France

L. donovani is the causative agent of visceral *Leishmania*sis, a major public health problem worldwide. We are interested in understanding how *Leishmania* interacts with its host, and particularly the impact of secreted parasitic proteins on intracellular survival. Casein Kinase 1 isoform 2 (CK1.2), a protein kinase involved in signaling, has been identified in exosomes by proteome analysis and is important for intracellular parasite survival. However very little is known about the functions it may carry outside of the parasite. One possibility would be to target host proteins to modulate host cell biology and evade the innate anti-*Leishmania*l immune response. We addressed this hypothesis by:
(i) Investigating whether CK1.2 is essential for intracellular parasite using a null mutant approach.
(ii) Determining the localization of CK1.2 in extra- and intracellular parasites and establishing its presence inside the host cell cytoplasm using cell imaging. Our preliminary results suggest that the localization of V5-tagged CK1.2 is vesicular in promastigotes and intracellular amastigotes, supporting the hypothesis of its presence in exosomes.
(iii) Identifying CK1.2 binding partners allowing it to carry out its functions, in particular to be secreted into the host cell by exosomes. Applying immunoprecipitation and mass spectrometry analyses on promastigote and axenic amastigote extracts allowed us to establish a list of potential binding partners. In promastigotes, we identified proteins involved mostly in vesicular processes, folding and transport, whereas in axenic amastigotes CK1.2 partners were involved in metabolism translation and transport. We are currently performing similar experiments to identify host partners

A genome editing toolkit for *Leishmania*- Poster 52 : A11143
Presenter: **Dr Eva Gluenz**, *Royal Society Research Fellow, University of Oxford*

Authors: T Beneke[1]; R Madden[1]; J Valli[1]; L Makin[1]; **E Gluenz**[1];
[1] University of Oxford, Sir William Dunn School of Pathology

The CRISPR-Cas9 system offers a powerful method for precise genome editing, which promises to revolutionise genetic manipulation of *Leishmania* spp., offering for the first time a simple method to generate loss-of-function mutants. We have generated a cloning-independent, PCR-only toolkit for rapid CRISPR-Cas9 genome editing with the following key components: (1) *L. mexicana* cell lines that constitutively express Cas9 and T7 RNA polymerase, (2) a set of plasmids with several resistance genes and tags to generate donor DNA, in a one-step PCR, for homology directed repair of Cas9-induced double strand breaks using 30nt homology arms, and (3) a new protocol for delivery of single-guide RNAs using PCR-generated DNA templates, which are transcribed by T7 RNA polymerase *in vivo*. Using simultaneous selection with different drugs, two or more alleles can be targeted at once; here we show examples of gene tagging and of knockout phenotypes produced in a single round of transfection. This method can allow for targeting of multi-copy gene families and can circumvent the technical challenges posed by the plasticity of the *Leishmania* genome. Our toolkit is rapid, facile and scalable to a 96-well plate format, allowing targeting of large cohorts of genes for localisation and mutant phenotype screening.

Nuclear factors involved in developmental differentiation of *Trypanosoma brucei* - Poster 53 : A11146
Presenter: **Nicole Eisenhuth**, *PhD student, University of Wuerzburg*

Authors: **N Eisenhuth**[2]; C Goos[2]; F Butter[1]; C J Janzen[2];
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During their complex life cycle, trypanosomes shuttle between an insect vector and a mammalian host. This process requires the tightly regulated coordination of several developmental pathways in order to survive in these extremely different environments. Changes to the nuclear architecture and chromatin structure have also been described during differentiation of the parasite. To learn more about these adaptation processes we analysed 4270 proteins during differentiation of the parasite using quantitative mass spectrometry. I want to use this dataset to find factors responsible for dynamic changes in chromatin structure during developmental differentiation. To this end, I combined the data from the quantitative proteomic study with an additional mass spectrometry analysis of the nuclear proteome. I am now focusing on the characterisation of nuclear proteins that are transiently upregulated during differentiation. These include hypothetical proteins with unknown functions, as well as the histone methyltransferase DOT1B and proteins of the nuclear pore complex. Further characterisation of these factors could provide more insight into the poorly-understood differentiation machinery of trypanosomes.

Nucleotide excision repair in trypanosomatids – streamlining and neofunctionalisation of the machinery due to multigenic transcription- Poster 55 : A11152
Presenter: **Miss Viviane Da Silva**, *PhD, University of Glasgow*

Authors: V G Da Silva[2]; C R Machado[1]; **R McCulloch**[2];
[1] Universidade Federal de Minas Gerais, Brazil; [2] University of Glasgow

DNA lesions that lead to helix distortions or block replication signalise to Nucleotide Excision Repair (NER) activation. Two sub-pathways act in the first step involving recognition of the DNA lesion: global genomic-NER (GG- NER) and transcription-coupled NER (TC-NER). The way the pathway works in *T. brucei* is still unclear as some components are missing. With the aim to identify the cellular components involved in TC-NER in *T. brucei* mutants for CSB, the protein that recognizes a stalled RNA Polymerase, were generated. CSB knockouts show increased susceptibility to UV treatment. This effect in more pronounced in the presence of Caffeine. The over-expression of CSB results in no difference in the susceptibility or resistance to UV treatment. To better understand the role of each component, myc-tagged CSB, XPC, XPG and XPBz variants were used for subcellular localization. They are nuclear localized and no change in the subcellular localization was observed after treatment with UV. Our results suggest a neofunctionalisation of the machinery in trymanosomatids.

Selecting for simultaneous activation of two VSG expression sites- Poster 56 : A11159
Presenter: **Mr. James Budzak**, *Research Assistant, Imperial College London*

Authors: **J Budzak**[1]; L Kerry[1]; K Witmer[1]; E Pegg[1]; B Hall[1]; J Wood[1]; M Kushwaha[1]; S D'Archivio[2]; B Wickstead[2]; G Rudenko[1];
[1] Imperial College; [2] University of Nottingham

Antigenic variation in *Trypanosoma brucei* is mediated by transcription of Variant Surface Glycoprotein (VSG) genes located in telomeric VSG expression sites (ES). Only one VSG is expressed at a time from 1 of 15 ESs. Despite the fact that monoallelic exclusion of VSG is critical for continued survival of the parasite, little is known about how this process is regulated mechanistically. To gain more insight into this, we generated three 'double-expressing' (DE) *T. brucei* lines with the VSG221 ES (marked with eGFP) and the VSGV02 ES (marked with mCherry) both simultaneously active. Generation of DE lines is rare (10 7 and they are highly unstable without selection. Flow cytometry quantitating expression of mCherry or eGFP has allowed us to characterise dynamics of ES expression in these three independently generated DE lines. These *T. brucei* DE lines show different degrees of stability of the DE phenotype, which is reflected in differences revealed through RNA-seq analysis. This indicates that different sets of events can occur to maintain a DE state. However some genes are universally up or downregulated in all three DE lines. These include the telomere binding protein TRF, which has earlier been shown to be key for ES control. We are currently analysing these differentially expressed candidate genes through knock-down experiments for their role in mono-allelic control of ESs

Amino acid transporters of the AAT7 family facilitate uptake of neutral amino acids- Poster 57 : A11161
Presenter: **Alexander Haindrich**, *Aminoacid Transportes in T. brucei, Institute of Plant Sciences - University of Bern*

Authors: **A C Haindrich**[1]; A F Dubois[1]; J P Macêdo[1]; M Suter Grotemeyer[1]; D Rentsch[1];
[1] Institute of Plant Sciences, Univeristy of Bern, Switzerland

Many proteinogenic amino acids are essential for cell viability in the kinetoplastid *Trypanosoma brucei*. These amino acids are not only required for protein synthesis, but are also substrates in metabolic pathways such as lipid synthesis or used for energy production. Here we characterized two members of the amino acid transporter (AAT) family. Both belong to the AAT7 locus that comprises nine close homologs in a tandem array. The characterized AAT7 members showed high affinity for transport of threonine (apparent K_m 298.96 and 74.41 µM), besides transporting other small and uncharged amino acids like serine and alanine. Depletion of these proteins by RNAi in bloodstream form *T. brucei* did not result in a growth defect in culture, showing that alternative import pathways (e.g. by other members of the AAT7 family) and/or *de novo* synthesis of threonine and serine are sufficient to sustain cell viability.

SI-2, a quinone derivative with potential sirtuin inhibitor activity decreases the replication of *T. brucei* procyclic forms and induces apoptosis-like cell death mechanism- Poster 58 : A11168
Presenter: **Dr Carla Cristi Del Campo Avila**, *Post-doctoral, USP*

Authors: **M Crispim**[3]; R Girard[3]; C G Baptista[3]; P Silva[2]; A M Silber[3]; F Emery[2]; G H Trossini[1]; C C Avila[1];
[1] Department of Clinical and Toxicologial Analysis Faculty of Pharmaceutical Sciences - University of São Paulo, São Paulo, SP, Brazil; [2] Faculty of Pharmaceutical Sciences at Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil; [3] Laboratory of Biochemistry of Tryps - LaBTryps - Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil

The therapy against *Trypanosoma brucei*, presents several limitations in terms of toxicity, drug administration and efficiency. Sirtuins were proposed as interesting potential drug targets. We designed and synthetized 46 new compounds based on known Sirtuin inhibitors and tested against *T. brucei* Lister 427 procyclic forms (PCF) proliferation. The Sirtuin Inhibitor 2 (SI-2) exhibited the most promising inhibition and

dose-dependent response (EC_{50} = 0.75 µM). We further investigated the cell death mechanism triggered by SI-2. A sub-population of these parasites were labeled by Annexin V but not propidium iodide, suggesting an apoptotic-like cell death. Moreover, parasites treated with SI-2 showed an increased production of reactive oxygen species (ROS) and intracellular calcium levels. Concomitant with this, a mitochondrial depolarization was observed. Taken together, these results show that SI-2 trigger programmed cell death, making of SI-2 a promising drug candidate against *T. brucei.* Further studies are being conducted to investigate in more detail the effect of SI-2.

Antigenic variation in *Trypanosoma brucei*: dissecting VEX1, a VSG allelic exclusion regulator- Poster 59 : A11169
Presenter: **Dr Catarina de Almeida Marques**, *Postdoctoral Research Assistant, University of Dundee*

Authors: **C A Marques**[1]; L Glover[2]; S Hutchinson[1]; D Horn[1];
[1] Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee; [2] Institut Pasteur, France

Trypanosoma brucei bloodstream form cells express a single variant surface glycoprotein (VSG) gene per cell at a given time. VSG Exclusion-1 (VEX1) is a large putative SWIM-type zinc-finger protein that has been recently shown to control this process. VEX1 associates with the active *VSG* and co-ordinates both positive and negative controls, with both over-expression and knockdown leading to allelic exclusion defects. To dissect VEX1 function, we have expressed VEX1-fragments and analysed their sub-cellular localisation and impact on allelic exclusion. Preliminary results suggest that a region involved in association with the active VSG resides towards the *N*-terminus, while a nuclear localisation signal appears to reside towards the *C*-terminus. VEX1 has also been shown to drive its own expression when under the control of a pol-I promoter so we are setting up strains that allow for VEX1 expression from a T7-promoter to determine whether this positive-feedback control is promoter-specific.

Mapping metabolism in the parasite *Trypanosoma brucei* using U-13C-labelled amino acids and LC-MS.- Poster 60 : A11171
Presenter: **Dr. Fiona Achcar**, *Leverhulme Fellow, University of Glasgow*

Authors: **K Johnston**[1]; D H Kim[2]; M P Barrett[1]; F Achcar[1];
[1] University of Glasgow; [2] University of Nottingham

The metabolism of *Trypanosoma brucei* has several unique features when compared to other eukaryotes. For example, it relies on a partly compartmentalised glycolytic pathway as its only energy supply. Gaining a more detailed knowledge of the parasite's metabolism can help design new drugs and understand the mechanisms of action and resistance of current drugs. LC-MS-based metabolomics using uniformly (U)-$_{13}$C-labelled glucose enabled Creek et al (PMID: 25775470) to explore *T. brucei* metabolism more extensively; they found that more pathways use glucose derived carbon than previously thought, including pathways essential to the parasite survival.
This study also raised many new questions. In order to answer them and complete our map of bloodstream form *T. brucei* metabolism, we used 5 U-$_{13}$C-labelled amino acids: cysteine, glutamine, methionine, arginine and proline. Parasites were grown in the presence of these labelled amino acids for 48 hours, prior to analysing their intracellular extracts using LC-MS to trace the fate of individual carbon atoms derived from these precursors inside the cells.

The results show the presence of metabolic pathways previously thought to be absent or present only in the insect stage of the parasite. We could also detect the presence of unexpected novel metabolites, likely to be inadvertent products of promiscuous enzymes.

Investigating the attachment plaque in Trypanosoma congolense epimatigotes- Poster 61 : A11176
Presenter: **Dr. Helen Farr**, *Post-doctoral Researcher, University of Oxford*

Authors: **H Farr**[1]; C McKechnie[1]; K Gull[1];
[1] *University of Oxford*

A common feature of the life cycles of many kinetoplastid parasites is attachment within the vector; this is important for differentiation and persistence of the infection. Epimastigote stage trypanosomes form attach to the epithelial cells of the Tsetse salivary glands or lining of the proboscis, depending on species. Attachment is mediated by hemidesmosomal structures in elaborations of the flagellar membrane, but molecular components of these have not been studied previously. The epimastigote stage itself has been relatively little studied as *Trypanosoma brucei* cells do not readily differentiate *in vitro*. However, by using *Trypanosoma congolense*, which differentiates *in vitro* from procyclic to epimastigote forms, we have been able to study the attachment plaque that forms between the flagellum membrane and the flask.We have used mass spectroscopy to identify proteins in isolated flagella from procyclic and epimastigote forms. The comparison between the two life cycle stages allowed us to filter out flagellar proteins common to both. Validation by localising a fluorescently-tagged candidate protein revealed a number of epimatigote-specific proteins.

A model to study the effect of *Leishmania* parasite virulence on interaction with host cells- Poster 62 : A11178
Presenter: **Mr. Salah Al-Hajri**, *PhD student, Nottingham Trent University*

Authors: S Al-Hajri[2]; A Faraj[1]; K S Ali[3]; S S Ali[2];
[1] *Hawler Medical University, Iraq;* [2] *Nottingham Trent University;* [3] *Sert University, Libya*

A model system has been developed to study the effect of *Leishmania mexicana* parasite virulence status on their interaction with host cells. Parasite freshly isolated from Balb/c mice skin lesions were grown *in vitro* (passage 1-highly virulent (P1)) and subsequently sub-cultured for 20 passages. Parasites lost their virulence with time and completely failed to infect Balb/c mice at passage 20 (P20). The *in vitro* growth characteristics of P1 and P20 parasite were similar. In this study, the interaction of P1&P20 with monocytic human cell line (U937) and Balb/c bone marrow macrophages has been assessed using parasite cell count, parasite morphology and the expression profile of IL-6 and IL1 b cytokines. The number of free extracellular P1 parasites was significantly lower than that of P20 whether in host cells of U937 cells or bone marrow macrophages. Significant morphological differences between P1 & P20 in infected cultures were also observed, P1 parasite cells were shorter, more rounded and with short or no flagella. Infection of host cells for 2 or 24 hours with P1 has significantly up-regulated the expression of IL1-b in comparison with the expression of IL-6 as measured by qPCR. In contrast infection with P20 has reversely up-regulated the expression of IL-6 cytokine in comparison with the expression of IL-1. In conclusion this model can be used further to analyse the interaction of *Leishmania* parasite with host cell.

An APOL1 variant that kills all *T. brucei* sub-species including T.b. gambiense- Poster 63 : A11183

Presenter: **Dr. Caroline Clucas**, *Lab Manager, Glasgow University*

Authors: A Cooper[1]; P Capewell[1]; **C Clucas**[1]; N Veitch[1]; W Weir[1]; R Thomson[2]; J Raper[2]; A Macleod[1];
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Humans are protected against infection from most African trypanosomes by lipoprotein complexes present in serum that contain the trypanolytic pore-forming protein, apolipoprotein L1 (APOL1). Two trypanosomes, *Trypanosoma brucei rhodesiense* and *T.b. gambiense* have evolved separate mechanisms that allow them to resist lysis by APOL1 and cause human African trypanosomiasis. Here we demonstrate the lytic ability of serum from a species of West African baboon, *Papio papio*, which is able to kill all sub-species of *T. brucei* including *T. b. gambiense*, the most common agent of human African trypanosomiasis. We show that this *in vitro* lytic ability is the result of a novel variant of APOL1, which is able to counteract the human serum resistance mechanisms of both *T. b. rhodesiense* and *T. b. gambiense*. The identification of a variant of APOL1 that can kill all *T. brucei* sub-species could form the basis of universal APOL1-based therapeutic strategies, effective against all pathogenic African trypanosomes.

Lifecycle-dependent localization of the signature sequence free potassium channel TbIRK in *T. brucei*- Poster 64 : A11187
Presenter: **Dr. Remo Schmidt**, *Postdoc, Swiss Tropical and Public Health Institute*

Authors: **M E Steinmann**[1]; R S Schmidt[2]; P Bütikofer[1]; P Mäser[2]; E Sigel[1];
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Not available at time of print awaiting results.

A 3D electron microscopy study of interactions between the *Leishmania* amastigote flagellum and the parasitophorous vacuole - Poster 65 : A11189
Presenter: **Ms Jessica Valli**, *PhD Student, University of OXford*

Authors: **J Valli**[1]; E Johnson[1]; E Gluenz[1];
[1] University of Oxford

The *Leishmania mexicana* promastigote flagellum has several well-characterised functions including motility and sensing environmental stimuli, but the role of this organelle in the amastigote life cycle stage remains elusive. Some insight has been provided by the similarity in axoneme structure to that of a typical mammalian primary cilium, and by the observation that the amastigote flagellum tip is often closely associated with the parasitophorous vacuole membrane, suggesting a potential role in parasite-host interactions. We used serial block face scanning electron microscopy and transmission electron tomography to explore the interaction between the amastigote flagellum and the parasitophorous vacuole of *in vitro* infected macrophages. The resulting 3D models showed that amastigotes were typically tucked into the parasitophorous vacuole membrane, with the main vacuole volume bulging away from them, and revealed invaginations of the vacuole membrane at flagellum contact points. These invaginations differ from previously observed indentations of the vacuole membrane at the point of contact with the flagellum, and appear to represent vesicles either budding from or fusing with the vacuole membrane. Ongoing work addresses molecular interactions through the analysis of flagellar trafficking mutants which are unable to infect macrophages, and the detection of proteins in the amastigote flagellar membrane using a proximity-dependent biotin labelling approach.

Two different cap binding proteins (eIF4Es) are associated with distinct mRNA populations in *Trypanosoma brucei*.- Poster 66 : A11191
Presenter: **Miss Maria Jose Ribeiro Bezerra** , *PhD student, FIOCRUZ - Pernambucco*

Authors: **M R Bezerra**[2]; E R Freire[2]; F B Holetz[1]; J Zamudio[3]; A M Rezende[2]; D M Moura[2]; N Sturm[3]; D Campbell[3]; O P de-Melo-Neto[2];
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It is well known that the control of gene expression in Trypanosomatids is primarily mediated by post-transcriptional processes that regulate, among other events, the recruitment of mRNAs for translation. In other eukaryotes, mRNA recruitment is mediated by the mRNA cap binding protein, eIF4E, part of the eIF4F complex. In trypanosomatids, six eIF4E homologues were identified, with two of those (EIF4E3 and EIF4E4) implicated during translation initiation. Their potential to selective recruit distinct mRNA populations for translation, however, remain undefined. The aim of this study then was to investigate which mRNA populations bind to EIF4E3 and EIF4E4 in *Trypanosoma brucei*. Independent experiments were carried out using the native or ectopically expressed tagged proteins in immunoprecipitation assays which were followed by RNA extraction and SOLiD or Illumina next generation sequencing, respectively. The Gene Ontology molecular function annotation was then used to identify the different mRNA populations. The results indicate that although there are mRNAs co-precipitating with both proteins, EIF4E4 seems to associate preferentially with mRNAs encoding abundant housekeeping proteins with structural roles, such as the ribosomal proteins. In contrast, mRNAs bound to EIF4E3 are enriched in those coding for proteins with catalytic and binding activities. A complementary profile can then be observed, consistent with different populations of mRNAs bound to each protein.

A molecular survey of cAMP response proteins (CARPs) reveals a novel, non-linear signaling network- Poster 67 :A11130
Presenter: **Dr Sabine Bachmaier**, *Postdoc, Ludwig - Maximilians - University Munchen*

Authors: **S Bachmaier**[1]; M K Gould[1]; R Omelianczyk[1]; A E Aristodemou[1]; D N Tagoe[3]; A E Brennand[1]; E Polatoglou[1]; J Van Den Abbeele[2]; H P de Koning[3]; M Boshart[1];
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By a recent whole genome RNAi library screen selecting for cells resistant to elevated cAMP levels, we identified several putative cAMP response proteins (CARPs) in *T. brucei*. The collection of CARPs verified by independent RNAi mediated knock down encompasses ten proteins that according to epistasis and interaction analyses represent components of a novel cAMP signaling network rather than a linear signaling pathway. CARP1, a protein unique to kinetoplastids, binds cAMP via three cAMP-binding like domains. Six of the verified CARPs are unique to kinetoplastids or trypanosomes. Phenotypic analysis of CARP knock down cell lines confirmed the previously described roles of cAMP signaling in normal growth and cell division in bloodstream forms. Furthermore, in procyclic forms one of the CARPs is directly involved in control of social motility, as evidenced by knockdown and knockout analyses.

Trypanosomes lacking the expression of ZC3H30 are hypersensitive to stress- Poster 68 : A11064
Presenter: **Chatitali Chakraborty**, *PhD student, Universitat Heidelberg*

Authors: **C Chakraborty**[1];
[1] ZMBH - Heidelberg University, Germany

Trypanosoma brucei, the causative agent of Human African Trypanosomiasis (HAT) commutes between two markedly different environments, the human host and the tsetse fly. In the blood and tissue fluids of mammals it experiences temperatures ranging from 36°C to 40°C . In the tsetse fly glands or gut, the trypanosomes may experience temperature fluctuations depending on the environment the fly is exposed to (25°C-42°C). In such hostile conditions the parasite experiences stress which it must overcome in order to survive. Trypanosomes like other kinetoplastids overcome such stress by post-transcriptional gene regulation. Transcription in these kinetoplastids are polycistronic; premature mRNAs are spliced to generate mature mRNA that undergo translation. Therefore, the regulation of gene expression almost exclusively relies on the trans-acting factors like RNA Binding Proteins (RBPs). The fate of a mRNA depends on the function of the partner RBP. Some RBPs bind to certain recognition motifs located on the 3'UTR, or sometimes within the open reading frame (ORF) of the target mRNA. If the RBP interacts with protein binding partners like PBP1, PABP1, or MKT1, it is likely to stabilise the mRNA for translation, and lower the chances of its degradation. On the other hand, RBPs which interact with deadenylases like CAF1, CCR4, ribonucleases,and with the exosome machinary, will lead to the destabilisation and dragradation of its target mRNA. The RBP ZC3H30 can bind to reporter gene constructs and decrease the expression by destabilising the transcript. The expression of ZC3H30 in not necessary for the survival of the pathogen under normal growth condition. But, when the cells are inflicted with stress (heat shock, arsenite and ethanol), the cells that lack the expression do not survive. It will be interesting to find the constituents of the transcriptome and proteome of the ZC3H30knockouts, which might illuminate the cause of its hypersensitivity to stress.

Effects of host-derived chemokines on the motility and viability of *Trypanosoma brucei* brucei- Poster 69 : A10632
Presenter: **Mr. Omar Alfituri**, *PhD student, The Roslin Institute*

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African trypanosomes are single-celled extracellular protozoan parasites that are transmitted via the tsetse fly vector across sub-Saharan Africa. Mammalian infection begins when the tsetse fly injects trypanosomes into the skin. The parasites invade the circulatory and lymphatic systems, reaching the draining lymph nodes and disseminate systemically. How this occurs is not known. Chemokines play important roles in attracting leukocytes towards the lymphatics and lymph nodes.To investigate how trypanosomes migrate from the bite site to the draining lymph nodes, we determined whether chemokines act as chemoattractants for trypanosomes. As certain chemokines also possess antimicrobial properties, including against the protozoan parasite *Leishmania* mexicana ,we tested their potential cytotoxic effects against T. b. brucei . Our data showed that these chemokines do not induce chemotaxis of T. b. brucei . The motility characteristics of the parasites were also not affected. Moreover, these chemokines do not exert any trypanostatic effects on trypanosomes. This data suggests trypanosomes use alternative cues to reach the lymphatics post-infection. Identifying the mechanisms involved in establishing African trypanosome infections in the skin and their systemic dissemination will aid the development of novel approaches to block disease transmission.

The proteome of trypanosome nuclear periphery granules- Poster 70 : A10680
Presenter: **Carina Goos**, *PhD student, Biozentrum der Universität Würzburg*

Authors: **C Goos**[1]; M Dejung[2]; M Engstler[1]; F Butter[2]; S Kramer[1];
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In trypanosomes, mRNAs are transcribed as long polycistrons and processed by trans-splicing. Inhibition of trans-splicing causes the formation of a novel type of RNP granule at the cytoplasmic site of the nucleus, nuclear periphery granules (NPGs). Here, we have determined the granule's proteome by co-purifying the granules with trypanosome nuclei followed by validation via eYFP tagging. We have identified 45 novel NPG granule proteins and a further 58 NPG candidate proteins. NPGs contain almost exclusively proteins with a known connection to mRNA metabolism. The absence of translation factors, together with the granule's perinuclear localisation, dependency on active transcription and insensitivity to cycloheximide indicates that NPGs are cytoplasmic RNP complexes prior to entry into translation: the presence or absence of a certain protein in the NPG proteome can therefore serve as a prediction tool for its entry-time into mRNA metabolism. Single mRNA FISH showed no enrichment of unspliced mRNAs at the nuclear periphery, but a large fraction of unspliced mRNA in the cytoplasm. This argues against a function of NPGs in mRNA quality control and we discuss alternative reasons for NPG formation. While the exact function and nature of NPGs still remains unknown, their formation must result from an interruption of the mRNA nuclear export pathway. The further analysis of such a unique 'frozen state'can contribute to a better understanding of nuclear export mechanisms.

Cargo selection and VSG transport in the early secretory pathway of *Trypanosoma brucei*
- Poster 71 : A10682
Presenter: **Dr Emilia Kruzel**, *Postdoctoral Fellow, University at Buffalo, SUNY*

Authors: E K Kruzel[1]; G P Zimmett[1]; **J D Bangs**[1];
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In African trypanosomes, trafficking of GPI-anchored Variant Surface Glycoprotein (VSG) to the cell surface is critical to parasite survival and pathogenesis. VSG is initially synthesized in the Endoplasmic Reticulum (ER), and is then trafficked to the Golgi apparatus in Coat Protein II (COPII)-coated vesicles. Efficient ER exit of VSG requires its GPI anchor and is mediated by specific COPII subunits, suggesting a sorting receptor that simultaneously recognizes the GPI anchor on VSG (lumenal-facing) and the assembling COPII subunits at ER exit sites (cytoplasm-facing). In other systems, GPI recognition during ER exit is carried out by members of the p24 family of transmembrane proteins. We have identified 8 putative p24s in the *T. brucei* genome: TbERP1-8 (EMP24-related protein). Of these, only TbERP1,2,3, and 8 are expressed during bloodstream-form growth, and RNAi silencing of each caused a significant delay in the trafficking of VSG to the cell surface. We confirmed TbERP localization to ERES, consistent with function during ER exit. TbERP1,2,3 and 8 show inter-dependence, and the protein stability of a given TbERP depends on the expression of the others. The shared RNAi phenotypes, co-localization, and coordinate expression suggest a TbERP complex with novel specificity. Experiments are underway to confirm direct physical interactions amongst the TbERPs and with VSG cargo.

The effect of genotypic diversity on the progression of *T. cruzi* infections- Poster 72 :
A10710
Presenter: **Ms Catherine Perez**, *PhD candidate, Murdoch University*

Authors: **C J Perez**[1]; A J Lymbery[1]; R C Thompson[1];
[1] Murdoch University, Australia

Models assessing the acute phase of *Trypanosoma cruzi* infection, where parasitemia may be observed, are widely utilised, yet it is the chronic phase of infection that causes the debilitating effects of Chagas Disease (CD). Progression of disease from the indeterminate phase into the chronic phase of infection does not always occur and its cause is not well understood. The development of models allowing for the study of the indeterminate or chronic phases of infection may therefore prove useful in improving our understanding of CD progression. Our studies investigating the effects of genetic diversity on *T. cruzi* mono-infections in a mouse model found differences between the 10R26 and C8 clone 1 isolates, in both consequences of immune suppression and reinfection. Disease progressions observed within these models appear to mimic the indeterminate phase of infection where clinical symptoms and host morbidity are absent but the parasite is present within host organs. For the two isolates, decreased overall body condition and increased host morbidity were induced by immune suppression and/or reinfection, suggestive of either disease progression into a ?chronic phase? of infection or reactivation of disease with an absence in detectable parasitemia. These findings are clinically relevant to increasing our understanding of of the factors affecting the disease progression and exacerbation of CD.

Generation of a *Trypanosoma brucei* whole-genome gain-of-function library- Poster 73 : A10715
Presenter: **Prof. Galadriel Hovel-Miner**, *Assistant Professor, George Washington University*

Authors: **G A Hovel-Miner**[1]; H S Kim[2]; D Schulz[2];
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African trypanosomes are tsetse vector transmitted unicellular parasites that result in devastating human and livestock infections throughout sub-Saharan Africa. In addition, *Trypanosoma brucei* has demonstrated its usefulness as a model organism toward elucidating fundamental principles in diverse research areas that include cell biology, molecular genetics, and epigenetics. In spite of decades of active discovery in all areas of trypanosome research, more than 60% of the *T. brucei* genome is annotated as hypothetical genes of unknown function. Further progress in understanding both the pathogenesis and basic biology of Trypanosomes requires the development of versatile approaches for genome-scale functional analysis. Strides have been made using an available RNAi based whole-genome loss-of-function library, yet a complementary gain-of-function library had not been produced. While individual gene studies have proven the usefulness of overexpression studies, whole-genome approaches had been hindered by the complexities of trypanosome gene expression. Using up-to-date *T. brucei* genome annotations and ribosomal profiling data we have addressed this challenge by generating a PCR amplicon-based overexpression library. The quality of both the plasmid libraries and bloodstream form *T. brucei* libraries are in the final stages of quality validation. Upon their completion, the libraries will be made broadly available to Trypanosome researchers.

PRMT or not a PRMT: The major protein arginine methyltransferase in *T. brucei* functions as an enzyme-prozyme complex.- Poster 74 : A10720
Presenter: **Lucie Kafkova**, *PhD candidate, University at Buffalo*

Authors: **L Kafkova**[2]; E W Debler[3]; J C Fisk[2]; K Jain[1]; S G Clarke[1]; L K Read[2];

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Arginine methylation is posttranslational modification catalyzed by protein arginine methyltransferases (PRMTs). In *Trypanosoma brucei*, over ten percent of the proteome bears arginine methylmarks. These include proteins in wide-ranging processes such as RNA processing, DNA repair, metabolism, and protein trafficking. The majority of asymmetrically dimethylated arginine is catalyzed *in vivo* by the *T. brucei* homolog of human PRMT1, previously termed TbPRMT1. Here, we show that TbPRMT1 functions as a heteromeric enzyme-prozyme pair. *in vivo* and *in vitro* studies demonstrate that active TbPRMT1 is a heterotetramer comprised of two subunits: $TbPRMT1^{ENZ}$ (previously TbPRMT1) and $TbPRMT1^{PRO}$, an apparently inactive paralog. Heterotetrameric TbPRMT1 catalyzes robust production of monomethylarginine and asymmetric dimethylarginine. Mutational analysis definitively demonstrates that $TbPRMT1^{ENZ}$ is the sole AdoMet binding and catalytic subunit. Conserved catalytic residues in $TbPRMT1^{PRO}$ are completely dispensable for complex function, but $TbPRMT1^{PRO}$ is essential for allosteric activation of $TbPRMT1^{ENZ}$. These results expand the prozyme paradigm in *T. brucei*, and present a novel mode of PRMT function that likely promotes unique types of PRMT regulation.

Epidemiological and Characterization studies on vector (Tabanidae) flies and their control measures for Trypnosomiasis (surra) in camels.- Poster 75 : A10771
Presenter: **Prof. Muhammad Fiaz Qamar**, *Chairman, University of Veterinary & Animal Sciences*

Authors: **M F Qamar**[1];
[1] *University of Veterinary & Animal Sciences, Lahore, Pakistan*

Trypanosoma evansi is a Trypanozoon parasite originated in Africa transmitted by tsetse by deletion of kinetoplastic maxicircles. It is principally a parasite of camels and horses in Pakistan and is transmitted mechanically by biting insects such as tabanids and stomoxes. Because of the high rate of healthy carriers, the parasite easily spreads in various directions. Trypanosomosis, caused by Trypanosoma evansi is one of the most important diseases of camels resulting in high morbidity (30%) and mortality (around 3%). Severe outbreaks, Natural and experimental infection with Trypanosoma evansi have been described in different parts of the world. The disease causes decrease in meat, milk production, transportation and draught power, as well as by products wool, hair, skin and hides. Currently, it is difficult to assess the impact of the disease in camels because there are no suitable diagnostic tools for determination of the extent of its prevalence, incidence and morbidity. Definitive diagnosis of a current infection with T. evansi relies on the demonstration of the parasites in the blood or tissue fluids of infected animals. However, in camel rearing areas the studies of vector has not been conducted. There is dire need of epidemiology of vector relating to the identification of parasite with techniques like ELISA, CATT (card agglutination test) and the detection of trypanosomal DNA by polymerase chain reaction.

Towards improving early diagnosis and surveillance of congenital Chagas disease in an endemic setting: lessons from the Bolivian Chaco - Poster 76 *:* A10796
Presenter: **Dr. Louisa Messenger**, *Post-doctoral research fellow, London School of Hygiene and Tropical Medicine*

Authors: **L A Messenger**[1]; C Bern[2];
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Following successful Chagas disease control programs across Latin America, congenital *Trypanosoma cruzi* transmission has become proportionally more important, accounting for 22% of new infections. Treatment during infancy is more efficacious and better tolerated than later, but current diagnostic methods fail to detect >50% of infected neonates and <20% complete follow-up. Pregnant women presenting for delivery in two urban hospitals in Santa Cruz, Bolivia, were recruited and their infants monitored at 1, 3, 6 and 9 months after birth to evaluate the performance of qPCR, IgM Western blots and micromethod for congenital Chagas disease screening. Of 518 infants from 507 seropositive women, unequivocal congenital transmission was identified in 32 infants of 29 mothers, including 3 sets of twins (5.7% transmission rate). Vertical transmission was more likely to occur in younger (23.5 vs. 26.9 years), first time mothers (7 vs. 52 mothers). Congenital *T. cruzi* infection was significantly associated with severe clinical outcomes including, premature birth and low birth weight and uninfected infants born to seropositive mothers were more likely to suffer from respiratory distress and premature rupture of the amniotic sac. We critically discuss the technical, logistical and economic obstacles of implementing routine molecular screening for congenital Chagas disease in resource-limited settings and describe the future prospects for improved disease management.

Genome analyses reveal unexpected levels of diversity and hybridization in natural populations of Trypanosoma congolense- Poster 77 : A10809
Presenter: **Dr. Frederik Van den Broeck**, *Postdoctoral researcher, Institute of Tropical Medicine*

Authors: E Tihon[1]; H Imamura[1]; J C Dujardin[1]; J Van Den Abbeele[1]; **F Van den Broeck**[1];
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Mating is not an obligatory part of the trypanosome life cycle with its frequency in natural populations depending on the species and the disease focus. Here we provide for the first time extensive genomic evidence for frequent mating in the putatively clonal *Trypanosoma congolense*, a parasite with detrimental effects on livestock productivity in sub-Saharan Africa. A genome-wide study in 54 isolates from across Africa revealed a large body of SNPs, indels and gene deletions that segregate in divergent *T. congolense* populations. We present unique data of a hybrid population of trypanosomes at a single focus in Zambia, resulting from outcrossing between phylogenetically distinct *T. congolense* parasites that led to a substantial increase in standing genomic variation. Subsequent cycles of sexual recombination produced a patchy genomic landscape varying in haplotypic ancestry and marker variability. We outline the genomic consequences of hybridization in trypanosomes and discuss potential associations with the spread of drug resistance.

Variation in Toll-like Receptor 2 and 4 Genes in the European Badger (Meles meles) in relation to infection with Trypanosoma (Megatrypanum) pestanai.- Poster 79 **:** A10816
Presenter: **Prof. Geoff Hide**, *Professor of Parasitology, School of Environment & Life Sciences*

Authors: **G Hide**[1];
[1] University of Salford

Wildlife are important sources and reservoirs for pathogens but little is known of the genetics of host-pathogen interactions. The badger (*Meles meles*) is well known for its role in the spread of bovine tuberculosis. *Trypanosoma pestanai* is the major trypanosome species in badgers. Toll-like receptors (TLRs) are key components of the innate immune system and studies have shown that variation in the TLR genes can affect susceptibility to infection in some pathogens. Little is known about variation in TLRs from badgers. TLR2 and TLR4 have been shown to be important in the recognition of trypanosome infections. This study

aims to investigate the sequence diversity of TLR2 and TLR4 in badgers in relation to infection with *T. pestanai*. In a population of badgers studied (n=82), 35.4% (25.9% - 46.2%; 95%CI) were infected with *T. pestanai* using detection with nested ITS-PCR. The DNA sequence for exon 3 of TLR4 from badgers (n=59) showed no variation. The complete TLR2 sequence (n=61) showed three amino acid haplotype variants in regions that predicted changes in receptor recognition motifs (leucine-rich repeats). Ninety-five percent of badgers were homozygous for a predominant haplotype (H1). This surprising lack of diversity in badger TLR genes sequence meant that it was not possible to derive any meaningful association between TLR diversity and trypanosome infection.

The early intradermal life of *Trypanosoma brucei* after natural transmission by the tsetse fly.- Poster 80 : A10822
Presenter: **Dr. Jan Van Den Abbeele**, *Professor, Institute of Tropical Medicine Antwerp*

Authors: G Caljon[3]; N Van Reet[1]; C De Trez[4]; M Vermeersch[2]; D Perez-Morga[2]; **J Van Den Abbeele**[1];
[1] Institute of Tropical Medicine Antwerp, Belgium; [2] Université Libre de Bruxelles, Belgium; [3] University of Antwerp, Belgium; [4] Vrije Universiteit Brussel, Belgium

Metacyclic *Trypanosoma brucei* parasites are naturally transmitted by tsetse bite into the mammalian host skin. Parasite emigration from this dermal site resulted in detectable trypanosome levels in the draining lymph nodes within 18 hours and in the peripheral blood within 42 h. A subset of parasites remained in the skin and actively proliferated. Scanning electron microscopy suggested that this retention was linked to interactions with adipocytes in the connective tissue, entanglement by reticular fibers of the periadipocytic baskets and embedment between collagen bundles. These skin-residing trypanosomes can be re-acquired by tsetse immediately after the initial transmission.

The genome of Euglena gracilis: Annotation, function and expression- Poster 81 : A10825
Presenter: **Mr. ThankGod Ebenezer**, *PhD Student, University of Cambridge*

Authors: **T E Ebenezer**[3]; M Zoltner[4]; V Hampl[1]; M Ginger[7]; A Jackson[8]; H de-Koning[6]; J Lukes[10]; J Dacks[2]; M Lebert[5]; M Carrington[3]; S Kelly[9]; M C Field[4];
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Euglena gracilis is a major component of the aquatic ecosystem and together with closely related species, is ubiquitous. Euglenoids are an important group of protists, possessing a secondarily acquired plastid and relatives to the Kinetoplastidae which themselves are global impacts as disease agents. To understand the biology of *E. gracilis*, and to provide further insight into the evolution and origins of Kinetoplastidae, we embarked on sequencing the nuclear genome. Earlier studies suggested an extensive nuclear DNA content, with likely a high degree of repetitive sequence, together with significance extrachromosomal elements. To produce a dataset of coding sequences we combined transcriptome data from both published and new data, as well as embarked on de novo genome sequencing using combination of 454, Illumina paired end libraries and PacBio platforms as well as transcriptomics and proteomics analysis under light and dark adaptations. Preliminary analysis suggests a surprising large genome approaching 2 Gbp, with highly fragmented architecture and extensive repeat composition. Over 80 % of the transcriptome maps to the genome, at par with *T. brucei* and *T. cruzi* . As a view of genome architecture, we have analyzed the tubulin and calmodulin genes together with several large genome contigs and which highlight potential novel splicing mechanisms. Analysis of the transcriptome and genome revealed a repertoire of *E. gracilis* genes conserved across all

eukaryotes (~ 55 %) and others shared with specific eukaryotic lineages and evidence of LGT. Functional annotation via a web interface http://euglenadb.org suggests that of 36,526 predicted proteins, 23,833 (~ 46 %) have homology to NCBI-NR entries, while 23,866 (~ 65 %) have hits against the Interpro database. Overall, our data suggest that the Euglena genome is a chimera with highly significant proteome changes.

The mRNAs that are increased after heat shock of procyclic-form *Trypanosoma brucei* include many that are induced during differentiation to mammalian-infective forms- Poster 82 : A10833
Presenter: **Prof. Christine Elizabeth Clayton**, *PI, ZMBH*

Authors: **I Minia**[1]; C Merce[1]; M Terrao[1]; C Clayton[1];
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African trypanosome procyclic forms are routinely cultured at 27°C. Heat shock at 37°C and above inhibits translation, and 1h at 41°C causes sequestration of mRNA in granules. By transcriptome analysis we found that mRNAs that bind to the heat shock regulator ZC3H11 remained in polysomes at 39°C and were protected from sequestration in granules at 41°C. The mRNAs that encode ribosomal proteins were excluded from heat-shock granules. 70 mRNAs moved towards the polysomal fraction at 39°C; many of these are also increased when trypanosomes migrate to the Tsetse salivary glands.

Discovery and lead-optimisation of a promising new anti*leishmania*l compound series within an academic-industry partnership.- Poster 83 : A10842
Presenter: **Dr Manu De Rycker**, *Project Leader, University of Dundee*

Authors: **M De Rycker**[2]; M Thomas[2]; T Miles[1]; DDU Kinetoplastids Team[2]; P Wyatt[2]; J Fiandor-Roman[2]; D Gray[2]; K Read[2]; I Gilbert[2];
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The Drug Discovery Unit at the University of Dundee and the GlaxoSmithKline Kinetoplastid Discovery Performance Unit, with support from the Wellcome Trust, have formed a five year partnership to conduct drug discovery within kinetoplastid diseases. This collaboration has made significant progress, highlighted by the identification of a lead optimisation series for visceral *Leishmania*sis (VL). Here we describe the initial identification of the series through transitioning a *Trypanosoma brucei* active series into a VL active series. The series was profiled extensively in a panel of *in vitro Leishmania* assays including promastigote, axenic amastigote and intracellular assays as well as rate-of-kill and clinical isolate assays, an effort which helped us develop our *Leishmania* screening cascade. An overview of the lead-optimisation campaign will demonstrate how a focus on balancing potency and solubility eventually delivered a compound with candidate-level properties. Current standard of care for VL suffers from multiple issues (lack of efficacy, safety, drug resistance, stability, cost, parenteral administration only) and as a community there is a limited pipeline for VL. The new chemical series presented here is a significant step forward towards the development of a new oral drug for visceral *Leishmania*sis. This work illustrates the substantial benefits that working in an academic-industry partnership brings for the development of new drugs for neglected diseases.

Flagellum Attachment Zone and the control of *Leishmania* flagellar pocket shape - Poster 85 : A10845
Presenter: **Dr Jack Daniel Sunter**, *Research Fellow, University of Oxford*

Authors: **J D Sunter**[2]; R Yanase[1]; K Gull[2];
[1] University of Hyogo, Japan; [2] University of Oxford

The flagellar pocket (FP) of *Leishmania* is an essential conduit through which the cell interacts with its extracellular environment and is likely to be critical for pathogenicity. In *Leishmania* promastigotes the flagellum is often described as "free", extending from the FP at the cell anterior; yet these parasites have homologs of many flagellum attachment zone (FAZ) proteins, which in trypanosomes are used to attach the flagellum laterally to the cell body. We have previously shown by electron tomography and endogenous gene tagging that *Leishmania* have a FAZ structure, which is intrinsically linked to the morphogenesis of the FP, implicating the FAZ in *Leishmania* pathogenicity. Here, using specific FAZ gene knockout cells we investigated the function of the FAZ in *Leishmania*. LmFAZ5 knockout causes a reduction in the length of the FAZ structure and a concomitant reduction in cell body length and volume. Electron microscopy shows the disruption of the FAZ structure leads to a loss of attachment between the flagellum and the cell body resulting in changes to the shape and size of the flagellar pocket and neck region. These data show that the FAZ in *Leishmania* is critical for determining cell shape and size and crucially for maintaining the shape of the FP and neck regions, potentially impacting on cell infectivity.

Enzymatic activation of the aminomethylphenoxy benzoxaboroles through hosts and pathogens- Poster 86 : A11202
Presenter: **Prof Mark C. Field**, *university of Dundee*

Authors: **N Zhang**[1]; M Zoltner[1]; P Scullion[1]; K Leung[2]; S Hutchinson[1]; R Canavate del Pino[1]; M Barret[3]; K Read[1]; D Horn[1]; M Field[1];
*[1] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee; [2] Department of Pathology, University of Cambridge; [3] Institute of Infection, Immunity and Inflammation, University of Glasgow***.**

The rapid spread of antimicrobial resistance is a global urgency in public health. To take on this tremendous challenge, it requires collective efforts towards understanding the mode of actions (MoAs) of antimicrobial medicines both in current use and under development. As part of these efforts, we looked into the MoAs of benzoxaboroles, a group of emerging novel antimicrobials, in *Trypanosoma brucei* first with a genomic RNAi library screening. The result led us to unravel the potential biological functions involved in determining the efficacy of the benzoxaboroles in the parasites. Especially, we discovered a mechanism underlying the activation of the aminomethylphenoxy derivatives. It is dependent on an enzymatic cascade that consists of an amine oxidase (AO) from the host and an aldehyde dehydrogenase (ALDH) from the parasite. Both enzymatic activities are indispensable for metabolizing the aminomethyphenoxy benzoxaboroles as pro-drugs first into the aldehyde metabolites, and further into the carboxyl acid products as the active form. Overall, our work reveals a novel drug metabolic pathway through the hosts and the pathogens, and addresses the potential risk of resistance development in future application in treating trypanosomiasis with one group of benzoxaborole derivatives.

Analysis of heparan sulphate and its role in the invasion of host macrophages by *Leishmania* parasites - Poster 87 : A11165
Presenter: **Dr. Marissa Maciej-Hulme**, *Post-doc, Keele University*

Authors: **M L Maciej-Hulme**[1]; M A Skidmore[1]; H Price[1];
[1] Keele University

*Leishmania*sis is caused by infection with the protozoan parasite *Leishmania* and is endemic in 98 countries, with approximately 2 million new cases annually. With resistant strains emerging to the few drugs available, more detailed information about mechanisms of infection is required to fuel new approaches to therapeutics. The role of carbohydrates in *Leishmania* infection remain largely unexplored. Previous work in the field has shown that the glycosaminoglycan, heparan sulphate (HS), can modify the adhesion of various *Leishmania* species to cells, possibly mediating parasite pathogenicity. HS is a linear polysaccharide comprised of repeating backbone structure of glucuronic acid and N-acetylglucosamine disaccharides. During synthesis, the backbone is modified by a plethora of enzymes, resulting in fine chemical patterning of the chain. This primary structure is thought to bind target biomolecules and mediate their function. Compositional analysis of human macrophage HS shows a large quantity of 2-O-sulphation of the chain. The identification of specific HS epitopes on macrophages will enable targeted therapies to be developed to reduce parasitic invasion of macrophage cells. Indeed, using a novel small soluble inhibitor of HS, the expression of HS at the cell surface can be reduced and limits the parasite burden, thus potentiating an alternative therapeutic approach for *Leishmania*sis.

Molecular detection of *Leishmania* RNA virus 2 in *Leishmania* infantum- Poster 88 : A11116
Presenter: **Mr Ahmad Garziz**, *PhD, Bangor University*

Authors: **A A Garziz**[1]; C Antunes[1]; H R Braig[1];
[1] Bangor University, School of Biological Sciences

Unsegmented dsRNA viruses of the family *Todiviridae* are known from such divers hosts as fungi, arthropods, and parasites. In some plant-pathogenic fungi, the viruses reduce the virulence of the fungal host, while in mammalian parasites like *Leishmania* and Trichomonas; the viruses are associated with an increase in virulence. The impact of the viruses on the virulence of Giardia and Eimeria species is still unresolved. With the exception of Giardia and a few mycoviruses, the viruses are assumed to be only transmitted vertically without ever leaving the host cell, meaning they are not infectious. *Leishmania*viruses from the New World like, *L. (Viannia) brazilliensis* and *L. (Viannia) guyanensis* are known as *Leishmania RNA virus 1*, viruses from the Old World like *L. major* and *L. aethiopica* as *Leishmania RNA virus 2*. Here we report the molecular detection of a new *Leishmania RNA virus 2* in an isolate of *L. (Leishmania) infantum* from a dog with *Leishmania*sis in Portugal. The genome codes for two major proteins, a capsid protein and an RNA-dependent RNA polymerase. Within the species *Leishmania RNA virus 2*, the RNA-dependent RNA polymerase evolves so fast that it is only conserved for 60 % at the amino acid level, making it very difficult to find long-enough regions on the nucleotide level to design (degenerate) primers for screening purposes.

Correlative RNAseq and proteomics analyses of hamster-isolated *L. donovani* amastigotes and derived promastigotes reveal stage-specific regulation of protein abundance independent of transcript levels. - Poster 89 : A11135
Presenter: **Mrs Pascale Pescher** , *Engineer, Institut Pasteur*

Authors: **P Pescher**[2]; F Guerfali[4]; R Friedman[2]; J Kovarova[5]; M A Dillies[2]; C Proux[3]; H Varet[2]; T Chaze[2]; T Douche[2]; V Hourdel[2]; C Bécavin[2]; P Prieto-Barja[1]; I Erb[1]; J Y Coppée[2]; M P Barrett[4]; B Schwikowski[2]; M Matondo[2]; C Notredame[1]; G F Späth[2];
[1] Centre de Regulacio Genomica, Spain; [2] Institut Pasteur, France; [4] Institut Pasteur, Tunis, Tunisia; [3] WTCMP University of Glasgow

We applied systems-wide analyses at genomic, transcriptomic, proteomic and metabolomic levels to compare bona fide tissue-purified amastigotes with culture-derived virulent promastigotes shortly after *in*

vitro conversion with the aim to reveal *L. donovani* stage-specific expression profiles and gain new insight into post-transcriptional and -translational regulatory mechanisms. RNAseq and Gene Set Enrichment Analyses identified significant increased transcript abundance for 387 genes in amastigotes with significant enrichment in RNA binding and RNA processing functions, and for 991 genes in promastigotes showing enrichment in proteolysis and glycolysis activities. Using label-free quantitative proteomics, a total of 3805 proteins were identified. Significant increases in stage-specific abundance were observed in splenic amastigotes and promastigotes for 561 and 1252 proteins, respectively. The comparison of transcript and protein abundance allowed us to distinguish three regulatory groups, including (i) genes with a stage-specific increase in both transcript and protein abundance, suggesting regulation by RNA stability, (ii) genes with unchanged or reduced RNA abundance but increased protein abundance in one of the stages, suggesting regulation on the level of translational efficiency or protein stability, and (iii) genes with stage-specific increase in transcript abundance but no change or reduction in protein abundance, suggesting regulation by protein degradation. This first systems-wide comparison of *L. donovani* amastigote and promastigote under physiological conditions shed new light on stage-specific mechanisms regulating *Leishmania* gene expression at transcript and protein levels.

***Leishmania* amazonensis amastigotes reprogram the host macrophage transcriptome to inhibit NF-kB and inflammasome activation** - Poster 90 : A11043
Presenter: **Mr Herve Serge Lecoeur** , *Engineer, Institut Pasteur*

Authors: **H Lecoeur**[1]; E Prin- A[1]; T Rosazza[1]; N Aulner[1]; X Yue[2]; E Laplantine[1]; R Weil[1]; G Milon[1]; G X Meng[2]; G F Späth[1];
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*Leishmania*sis results from the capacity of intracellular *Leishmania* amastigotes to subvert host cell immune functions and hijack macrophage metabolic pathways. We observed that infection of primary macrophages with virulent *Leishmania amazonensis* amastigotes isolated from lesions of nude mice does not trigger inflammasome activation as judged by the lack of ASC speck formation, caspase-1 activation and secretion of IL-1beta / IL-18. This absence of inflammasome activation was sustained up to 30 days post infection, and was associated with a coordinated reprogramming of the host macrophage functions at the transcription level as revealed by Affymetrix GeneChip and real time quantitative PCR technologies. More specifically, these experiments revealed a down-modulation of NF-kB activators (TLRs, signaling kinases, NF-kB members) and an up-modulation of inhibitors of this pathway (de-ubiquitinating enzymes). These modulations were associated with a decreased transcription of genes whose products define different inflammasomes (NLRP3, NLRC4, AIM2, RIG1) and pro-inflammatory cytokines (IL-1beta / IL-18). Significantly, these subversions were even maintained after LPS / ATP stimulation, resulting in a strong decrease in IL-1beta and IL-18 secretion. Our study describes a new subversion mechanism deployed by amastigotes that target transcription of key regulators of the NF-kB and inflammasome activation pathways to dampen the macrophage pro-inflammatory response and favor parasite survival.

Phylogenetic Analyses Including a Novel Australian Trypanosomatid Supports the Supercontinent Hypothesis of *Leishmania* Origins- Poster 92 : A11074
Presenter: **Dr Joel Barratt**, *Chancellors Postdoctoral Fellow, University of Technology Sydney*

Authors: **J Barratt**[2]; A Kaufer[2]; D Stark[1]; J Ellis[2];
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The genus *Leishmania* includes ~53 species, 20 of which cause human *Leishmania*is, a significant tropical disease that has afflicted humans for millennia. But how ancient is *Leishmania* and where did it arise? Some suggests it originated in the Palearctic. Others suggest it appeared in the Neotropics. The Multiple Origins theory proposes that separation of Old World and Neotropical species occurred following the opening of the Atlantic. The Supercontinents hypothesis suggests *Leishmania* evolved on Gondwana 140-90 million years ago (MYA). We performed a detailed molecular and morphological characterisation of a novel Australian trypanosomatid. It was confirmed as a sister to the Neotropical *Leptomonas costaricensis*, and designated as *Leptomonas australis* sp. nov. Assuming *L. costaricensis* and *L. australis* split when Australia and South America separated, their divergence occurred 41-36 MYA. Using this event as a calibration point for a phylogenetic time tree, *Leishmania* was estimated to have appeared ~92 MYA. Ultimately, this study contributes to our understanding of *Leishmania* origins by independently supporting the Supercontinent hypothesis.

Quantitative Proteomic responses of macrophages to *Leishmania* infection using pulse-chase SILAC approach.- Poster 93 : A11113
Presenter: **Mrs. Najad Zamirah Zaki**, *Phd student, University of Glasgow*

Authors: **N Z Zaki[1]** ; J Mottram[2]; R Burchmore[1];
[1] University of Glasgow ; [2] University of York

The intricate interaction between human host and *Leishmania* is thought to be insightful in understanding the relationship as well as towards chemotherapeutic development. Employing high-resolution mass spectrometry coupled with pulse-chase stable isotope labeling by amino acid in cell culture (pcSILAC) technique, we delved into the investigation of proteome changes in *L. mexicana*-infected THP-1. Cells were pre-labelled with L-Arg-$^{13}C_6$ and L-Lys-$^{13}C_6$ until isotope incorporation of ≥98% was achieved. Media was then replaced with light Arg and Lys where they are pulsed into cells for 24 and 48 hour. In other words, protein synthesis is 'chased' with unlabelled amino acids. At each pulse, the cells were infected with *L. mexicana*. 2-D-LCMS/MS data revealed a total of 1998 proteins detected in both control and 24 hour infected cells while 2113 proteins detected in 48 hour infected cells. 34% of the quantified proteins were shown to be considerably upregulated in infected cells. These included Ras-related protein, cytoskeleton proteins such as tubulin and myosin, proteins known to be associated with infection such as annexin, carbonic anhydrase and galectin-9. Conversely, proteins that were significantly downregulated after infection included purine nucleoside phosphorylase, superoxide dismutase (Mn), gelsolin, vimentin, Pyruvate kinase PKM, moesin, Calreticulin, ribosomal proteins and actin. Furthermore, we have identified 405 newly-synthesized proteins after 48 hour infection including Toll-like receptor 2 and Interleukin-1. Some of the proteins we have found to be modulated upon *Leishmania* infection have previously been implicated in this process, while others have not previously been shown to be involved in macrophage:*Leishmania* interactions.

Translesion DNA Polymerases and genome maintenance in *Trypanosoma brucei* - Poster 94 : A11061
Presenter: **Miss Andrea Zurita**, *PhD student, University of Glasgow*

Authors: **A C Zurita-Leal**[1]; M Prorocic[1]; R McCulloch[1];
[1] University of Glasgow

The genome of every organism is subject to damage, which is tackled either by repair or tolerance. Many DNA repair pathways have been documented in*Trypanosoma brucei* but less attention has been paid to

damage tolerance, a reaction in which lesion bypass is needed, in particular to ensure continued genome replication. Such bypass is promoted by translesion DNA polymerases (TLS Pols), of which five putative examples can be found in *T. brucei.* We have used RNAi to examine the function of three proteins in bloodstream forms of *T. brucei*. Loss of PolN (Nu) was shown to be severely detrimental to growth, with accumulation of cells showing aberrant nuclei, suggesting a critical role in nuclear genome maintenance. RNAi of PolZ (zeta) did not impair growth, but resulted in increased sensitivity to methyl methanesulphonate (MMS) damage, suggesting a role in the response to alkylation. The sequence of PolQ (theta) suggests that the predicted protein may not be a joint polymerase-helicase like in other eukaryotes, but only a helicase. RNAi revealed that loss of the factor did not affect growth, nor did it result in increased MMS sensitivity. Taken together, these data reveal widespread and variant functions for three of five putative TLS DNA polymerases in *T. brucei* genome biology.

Loss of ATR in the Kinetoplastid parasite *Trypanosoma brucei* de-regulates repression of the silent VSG archive- Poster 95 **:** A11112
Presenter: **Mrs Jennifer Stortz**, *PhD Student, University of Glasgow*

Authors: **J A Stortz**[1]; N Dickens[1]; J C Mottram[2]; R McCulloch[1];
[1] *University of Glasgow;* [2] *University of York*

Genotoxic stress is a constant threat to the genome of any organism and if left unresolved, genome integrity becomes compromised. To this effect, cells have evolved numerous pathways to monitor, assess and ultimately control the outcome of genome lesions collectively referred to as the DNA Damage Response (DDR). Central to the DDR is the atypical protein kinase ATR which is predominantly activated in response to replicative lesions such as stalled replication forks. To date, the initiation of VSG switching has been linked to the generation of a direct double stranded break lesion within the active expression site to facilitate switching by recombination however evidence is accumulating that VSG switching may be linked to the generation of replicative lesions. Here, we show that loss of ATR in bloodstream form *T.brucei* parasites not only compromises cell proliferation and de-regulates the cell cycle but additionally results in the de-repression of silent VSG genes as confirmed by RNAseq analysis thus supporting a role for ATR during the regulation of VSG switching and the subsequent likely generation of replicative lesions as an initiation factor of VSG switching by recombination.

Dissecting the kinome of *T. brucei*: RIT-seq of cell cycle sorted *T. brucei* identifies kinases involved in the regulation of nuclear DNA replication- Poster 97 **:** A11060
Presenter: **Dr Daniel Paape**, *Research Associate, University of Glasgow*

Authors: **D Paape**[3]; C A Marques[1]; F Fernandez-Cortes[3]; J M Wilkes[3]; J C Mottram[2]; R McCulloch[3];
[1] *University of Dundee;* [2] *University of York;* [3] *WTCMP University of Glasgow*

The coordinated replication and segregation of the genome to daughter cells is an integral cellular process to ensure inheritance and the preservation of life. Several protein kinases (PKs) have been documented to regulate multiple steps in nuclear DNA replication in yeast and other eukaryotes. However, though information is emerging about the machinery and coordination of *T. brucei* nuclear replication, nothing is known about the putative PK regulation of the reaction. Identification of how nuclear replication is regulated would not only provide insight into the evolution of this essential process, but may open up new avenues for the therapeutic intervention of the diseases caused by *T. brucei* and other kinetoplasts. To address this, we pooled all bloodstream form (BSF) *T. brucei* cell lines that individually target every PK (183 in total) by inducible RNAi. The pool was then sorted, with and without RNAi induction, according to their cell cycle

stage based on DNA content (G1, S-phase and G2/M) and relative read depth mapped (RITseq) over time and per cell cycle stage. This screen revealed PKs already known to be involved in cell cycle progression (e.g. transition from G1 to S: CRK1 and CRK2), as well as several novel PKs. Functional characterisation of two of the PK revealed that their kinase activity is presumably obsolete. Perhaps, rather a structural role is implicated in the replication of the nuclear DNA.

Quantitative proteomics uncovers novel factors involved in developmental differentiation of *Trypanosoma brucei*- Poster 98 : A11034
Presenter: **Dr Christian Janzen** , *PI, Lehrstuhl für Zell- und Entwicklungsbiologie, Biozentrum der Universität Würzburg*

Authors: M Dejung[2]; I Subota[3]; F Bucerius[1]; G Dindar[3]; A Freiwald[2]; M Engstler[3]; M Boshart[1]; F Butter[2]; **C J Janzen**[3];
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Developmental differentiation is a universal biological process that allows cells to adapt to different environments to perform specific functions. African trypanosomes progress through a tightly regulated life cycle in order to survive in different host environments when they shuttle between an insect vector and a vertebrate host. To learn more about this process, we quantified 4270 protein groups during stage differentiation from the mammalian-infective to the insect form and provide classification for their expression profiles during development. Our study revealed previously unknown components of the differentiation machinery that are involved in essential biological processes such as signaling, posttranslational protein modifications, trafficking and nuclear transport. Furthermore, guided by our proteomic survey, we identified the cause of the previously observed differentiation impairment in the histone methyltransferase DOT1B knock-out strain as it is required for accurate karyokinesis in the first cell division during differentiation. This epigenetic regulator is likely involved in essential chromatin restructuring during developmental differentiation of trypanosomes. Our proteome data set will serve as a resource for detailed investigations of cell differentiation to shed more light on the molecular mechanisms of this process in trypanosomes and other eukaryotes.

Does RNA incorporation into the DNA genome of *Trypanosoma brucei* drive DNA replication and antigenic variation?- Poster 99 : A10998
Presenter: **Miss Emma Briggs** , *PhD student, University of Glasgow*

Authors: **E M Briggs**[2]; L Lemgruber[2]; C A Marques[1]; R McCulloch[2];
[1] University of Dundee; [2] University of Glagow

During transcription, nascent mRNA can bind to the template strand of unwound DNA, forming thermodynamically stable R loops. Although threatening to stability, R loops are powerful regulators of DNA replication, gene expression and recombination. In humans, the most efficient origins of replication (ORIs) localise to CpG island promotors. These sites form R loops upon transcription and bind the origin recognition complex, ORC. *T. brucei* co-transcribes its nuclear genes from multigene clusters, with adjacent clusters separated by poorly characterised promoters and terminators at so called strand switch regions (SSRs). Transcription and replication appear to be linked as ORIs localise to select SSRs and ORC binds to potentially all SSRs. Given this, we are investigating whether R loops in *T. brucei* sequester ORC and direct DNA replication. In order to preserve R loops, we have generated mutants deficient in two nuclear RNase H

genes that digest hybridised RNA and will describe the impact on growth and DNA synthesis. We are also employing ChIP-seq technology map R loops across the genome. In addition, R loops facilitate class switching in activated B lymphocytes to generate antibody diversity, a system which may be analogous to variant surface glycoprotein switching in *T. brucei*. Hence, we will describe the effect of RNase H mutation on antigenic variation. Finally, we will describe a third apparently catalytic RNase H, localised to the antipodal sites of the kinetoplast DNA.

Metabolic signals and glycosomal isocitrate dehydrogenase (IDHg) control procyclic to metacyclic development of *Trypanosoma brucei* fly stages- Poster 100 : A11123
Presenter: **Prof. Michael Boshart**, *Prof, University of Munich LMU*

Authors: N Ziebart[1]; S Allmann[1]; J Van Den Abbeele[2]; C Huber[3]; W Eisenreich[3]; J W Dupuy[4]; M Bonneau[4]; F Bringaud[5]; **M Boshart**[1];
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Metabolic adaptation of trypanosomes to changing host environments and carbon source availability is essential for progression in the parasitic life cycle. Using a culture model for fly stage development, we have investigated the impact of carbon source availability on procyclic to metacyclic development. Glucose withdrawal significantly accelerated and glycerol inhibited stage development, suggesting that these carbon sources act as metabolic signals for development in the fly. Global proteome analysis has identified a set of enzymes of citrate metabolism (citrate synthase, CS; aconitase, ACO; glycosomal isocitrate dehydrogenase, IDHg) that are induced by glucose withdrawal conditions and repressed by glycerol. Metabolite analysis of knock out lines deficient in these enzymes provides evidence for a novel pathway feeding glycosomal NAD(P)H production by IDHg. When a KO mutant of IDHg was tested, development arrested in the epimastigote stage and no metacyclic forms were detected. In agreement, fly passage of the same mutant revealed a dramatic and rescueable maturation phenotype with normal midgut infections, but sterile salivary glands. Hence, IDHg plays an essential role for development in the tsetse. In contrast, the complete tricarboxylic acid cycle (TCA) is not essential in the procyclic stage, with and without glucose. The biochemical role in development of a uniquely bi-specific NAD(P)H-dependent IDH (see X. Wang et al. this meeting) will be discussed.

Regulation of *Trypanosoma brucei* Acetyl-CoA Carboxylase by Environmental Lipids-
Poster 101 : A10975
Presenter: **Kimberly Paul**, *Assoc. Prof., Clemson University*

Authors: S S Ray[1]; **K S Paul**[1];
[1] Clemson University, United States

T. brucei relies on uptake of host fatty acids and *de novo* synthesis to meet its fatty acid needs. We hypothesized *T. brucei* modulates Acetyl-CoA Carboxylase (TbACC), the first step in fatty acid synthesis, in response to environmental lipids. To test our hypothesis, bloodstream (BF) and procyclic forms (PF) were grown in low, normal, or high lipid media (prepared by modulating media serum) and the effect on TbACC mRNA, protein, and enzymatic activity was examined. qRT-PCR analysis of TbACC mRNA levels showed no significant difference, indicating little transcriptional regulation in response to environmental lipids. In BFs,

media lipids had no effect on TbACC protein levels or activity, but in PFs we observed 2.7-fold lower TbACC protein levels and 37% lower TbACC activity in high lipid compared to low lipid media. Supplementation of low lipid media with fatty acids mimicked the effect of high lipids on TbACC activity, suggesting this effect was due to the lipid component of the media. In PFs TbACC phosphorylation increased by 3.9-fold in high lipid compared to low lipid media, and phosphorylation was shown to inactivate TbACC. These results demonstrated that PFs possess an environmental response pathway that enables PFs to monitor the host lipid supply and modulate TbACC and fatty acid synthesis accordingly.

Molecular analysis of Tsetse from Hurungwe, Zimbabwe- Poster 102 : A11133
Presenter: **Dr Ewan Macleod**, *Lecturer, University Of Edinburgh*

Authors: **E Macleod**[2]; M R Goodwin[2]; L C Hamill[2]; N E Anderson[3]; W Nyakupinda[4]; L Gwenhure[1];
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Tsetse representing two species (G. m. morsitans and G. pallidipes) were sampled from Hurungwe, Zimbabwe. Following DNA extraction, PCR was used to examine the tsetse for presence of trypanosome DNA and the secondary endosymbiont S. glossinidius. T. vivax (31.6%), T. b. brucei (14.8%) and T. godfreyi (9.6%) were the most commonly detected trypanosome species. The prevalence of S. glossinidius was similar in the two tsetse species sampled.

Cytoskeletal Morphogenesis in *Trypanosoma brucei* - Poster 103 : A10929
Presenter: **Prof. Christopher de Graffenried**, *Assistant Professor, Brown University*

Authors: **C L de Graffenried**[1]; M R McAllaster[1]; J A Perry[1]; A N Sinclair-Davis[1]; N A Hilton[1];
[1] Brown University, United States

The *T. brucei* homolog of polo-like kinase (TbPLK) is an essential regulator of cell morphogenesis. We performed proteomic screens to identify potential TbPLK interactors to better understand the molecular mechanisms of kinase function. We found a cohort of uncharacterized proteins that are essential components of morphogenic cytoskeletal organelles. Domain analysis and depletion experiments on cohort members have identified their functions and mode of TbPLK interaction. This work provides the foundation for establishing the molecular networks through which TbPLK directs cell division in *T. brucei*.

Dissecting the Interstrand Crosslink Repair Pathways of *T. brucei*- Poster 104 : A11076
Presenter: **Miss Ambika Kumar**, *PhD Student, Queen Mary University of London*

Authors: **A Kumar**[1]; S Wilkinson[1];
[1] Queen Mary University of London

Genomes are constantly challenged by agents that promote DNA damage, with interstrand crosslinks (ICLs) representing a particularly dangerous lesion. Formed when the two 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. In many

organisms, zinc-dependent nucleases belonging to the SNM1/PSO2 family play a specific and key role in repair of these lesions, with the *T. brucei* homologue (TbSNM1) recently characterised. To further understand the complexity of ICL repair pathways within this parasite a series of cell lines lacking TbEXO1, TbCSB, TbMRE11, TbMPH1 or TbCHL1 were generated in wildtype or Tb*snm1*-deficient genetic backgrounds: these repair enzymes are reported to function in ICL repair in other organisms. Phenotypic screens using a variety of DNA damaging agents were performed, revealing that TbSNM1, TbMRE11, TbEXO1 and TbCSB all help to fix ICLs. TbMPH1 and TbCHL1 played no role in repairing these lesions. In the case of TbCSB and TbEXO1, their activities are epistatic with TbSNM1. In contrast, the ICL repairing activity of TbMRE11 occurs independently of TbSNM1. By unravelling how *T. brucei* can repair ICLs, specific inhibitors against key components of these pathways could be developed and used to target trypanosomal and *Leishmania*l infections.

Investigations into a Novel Alternative Oxidase in Trypanosomatids- Poster 105 : A10853
Presenter: **Ms Stefanie Menzies**, *PhD Candidate, University of St Andrews*

Authors: **S K Menzies**[1]; A L Fraser[1]; E R Gould[1]; E F King[1]; L T Tulloch[1]; M K Zacharova[1]; G J Florence[1]; T K Smith[1];
[1] *University of St Andrews*

We have confirmed the expression of the secondary alternative oxidase (AOX2) in all three human-infective trypanosomatids. Our protein, unlike the trypanosome alternative oxidase TAO, has conserved orthologues in *T. cruzi* and *Leishmania* spp., and importantly is not present in mammalian cells. We have shown that AOX2 is essential in all three of these parasites, making it an attractive multi-trypanosomatid drug target. We have confirmed the protein to be localized to the mitochondrion and are investigating the effects of over- and under-expression on mitochondrial respiration. We have successfully solubilized and purified recombinant AOX2 for determination of enzymatic activity and identification of potential inhibitors using our in-house focused natural product-like library and a fragment-based screen.

A model for vaccine testing against *Trypanosoma cruzi* infection that exploits highly sensitive bioluminescence imaging- Poster 106 : A11023
Presenter: **Mr. Gurdip Singh Mann**, *PhD Student, London School of Hygiene and Tropical Medicine*

Authors: **G S Mann**[1]; M C Taylor[1]; A F Francisco[1]; S Jayawardhana[1]; M Caroline[1]; J M Kelly[1];
[1] *London School Of Hygiene and Tropical Medicine*

Chagas disease, caused by *Trypanosoma cruzi* , affects 6-8 million people. Those who develop chronic stage pathology often suffer severe, life-threatening cardiac and/or gastrointestinal symptoms. The current drugs can have severe side effects and treatment failures are frequently reported. Pre-clinical testing of vaccine candidates has been limited by the complex nature of the disease and difficulties in detecting the extremely low parasite burden during chronic infections. Here, we describe a new murine model for vaccine testing, based on highly sensitive bioluminescence imaging, which circumvents these problems and provides new insights into conferred immunity. In preliminary experiments, mice were infected with bioluminescent *T. cruzi* , cured by benznidazole treatment, and then subjected to re-infection. The mice were found to be highly resistant to re-infection with the same strain, whereas cross-strain immunity, although effective at reducing the parasite burden, was insufficient to confer sterile protection. We next assessed if the level of protection was influenced by the route of inoculation or the length of the primary infections. The data demonstrate that this predictive model can be used to establish the correlates of

protection, and that it can have a central role in studying the efficacy of recombinant vaccines, and the relative merits of prophylactic and therapeutic vaccines as strategies for combatting this complex parasitic infection.

Insight into a C11 peptidase essential for *Trypanosoma brucei* kinetoplast maintenance and viability of *Leishmania* mexicana - Poster 107 **:** A10943
Presenter: **Dr Jaspreet Singh Grewal** , *Research Associate, University of York*

Authors: **J S Grewal**[1]; C C Preta[1]; J C Mottram[1];
[1] *University of York*

A cysteine peptidase of the Clostripain family (Clan CD, family C11) has been identified and characterized in *Trypanosoma brucei* [i] PNT1 (PMID: 26940875). Phylogenetic analysis showed that kinetoplastid PNT1 proteins are very divergent from orthologues of other organisms. Knockdown of PNT1 was lethal in the bloodstream form by RNAi and the induced population accumulated akinetoplastic (1N0K) cells. Electron microscopy data confirmed that PNT1 is localized on the kinetoplast. Downregulation of PNT1 in an akinetoplastic cell line resulted in viable parasites, suggesting an essential function in the mitochondrion. Mislocated kinetoplasts were observed in cells overexpressing PNT1. Data from the recoded wild type and mutated add backs of PNT1 confirm that cysteine peptidase activity is essential for PNT1 function. Moreover, preliminary data in *Leishmania mexicana* [i] using the DiCRE and CRISPR CAS9 mediated knockdown of the PNT1 orthologue suggests that PNT1 is essential for the survival of this parasite. In summary, our data suggest that PNT1 cysteine peptidase is essential for cell viability in both *Trypanosoma brucei* [i] and *Leishmania mexicana* [i]. Importantly, as there are no homologs of PNT1 in mammalian cells, PNT1 is a potential drug target across the trypanosomatids. This work is supported by the MRC.

Reciprocal SHAM-hypersensitivity in aquaglyceroporin-null and melarsoprol-resistant trypanosomes- Poster 108 : A11022
Presenter: **Dr Laura Louise Jeacock**, *PDRA, University of Dundee*

Authors: **L Jeacock**[3]; N Baker[2]; N Wiedemar[1]; P Mäser[1]; D Horn[3];
[1] *Swiss Tropical and Public Health Institute, Switzerland;* [2] *University of Canterbury;* [3] *University of Dundee*

Aquaglyceroporins (AQPs) transport water and glycerol and play important roles in drug-uptake in pathogenic trypanosomatids. For example, AQP2 in the human-infectious African trypanosome, *Trypanosoma brucei gambiense*, is responsible for melarsoprol and pentamidine-uptake, and AQP2-defects are responsible for melarsoprol treatment-failure. To further probe the roles of these transporters, we assembled a *T. b. brucei* strain lacking all three AQP-genes. Triple-null *aqp1-2-3 T. b. brucei* displayed only a very moderate growth defect *in vitro*, established infections in mice and recovered effectively from hypotonic-shock. The triple-null trypanosomes did, however, display glycerol uptake and efflux defects; they failed to utilise glycerol as a carbon-source and displayed increased sensitivity to salicylhydroxamic acid; an inhibitor of trypanosome alternative oxidase (TAO) that increases intracellular glycerol. Notably, disruption of AQP2 alone also generated cells with glycerol transport defects. As predicted by our findings, re-expression of AQP2 and reversal of melarsoprol-resistance in a clinical isolate reduced SHAM-sensitivity, consistent with repair of a glycerol-efflux defect. Thus, African trypanosome AQPs are not required for viability or osmoregulation but do make important contributions to drug-uptake and glycerol-flux. This improved understanding of AQP-function, and AQP2-mediated drug-uptake and glycerol-efflux in particular, could be exploited. For example, therapies alternating TAO-inhibitors with pentamidine or using TAO-inhibitors and

melarsoprol sequentially could be more effective than previously anticipated and could mitigate the further emergence and spread of melarsoprol-resistance.

Does TbPIP39 target all, or only the newly synthesised glycosomes upon differentiation? - Poster 110 : A11140
Presenter: **Dr Balazs Szoor**, *Senior Postdoctoral Fellow, University of Edinburgh*

Authors: **B Szoor**[2]; D Simon[2]; D R Robinson[1]; K R Matthews[2];
[1] Laboratoire de Microbiologie Fondamentale et Pathogénicité UMR-CNRS 5234, France; [2] University of Edinburgh

Trypanosoma brucei is held poised for transmission to it's tsetse vector by the activity of a tyrosine phosphatase, TbPTP1. We have discovered that the substrate of TbPTP1 is a glycosomal phosphatase, TbPIP39, whose enzymatic activity is repressed by PTP1-mediated dephosphorylation. Interestingly, a feedback regulation operates whereby inactive PIP39 stimulates PTP1, reinforcing its own repression. The composition of trypanosome glycosome is very different in bloodstream (BSF) and procyclic-forms (PCF). Moreover, during differentiation there is rapid pexophagy, which quickly turns over existing glycosomes, promoting metabolic adaptation. We envisaged that PIP39 might (a) be directed to all glycosomes during differentiation, or (b) selectively target only the newly-synthesised PCF-type glycosomes, with BSF-type glycosomes remaining PIP39-negative and being rapidly degraded. We used PIP39-specific antibody to stain stumpy cells undergoing differentiation for the relative colocalisation of PIP39 with constitutive glycosomal proteins present in both BSF and PCF-type glycosomes. Cell samples were assayed by fluorescence and dual-label confocal microscopy. We found that the PIP39 localisation changes as stumpy cells differentiate to procyclic forms, moving from the proximity of the flagellar pocket to a glycosomal location. This rapid PIP39 relocalisation is visible within 1 hour of cis-Aconitate exposure and provides a novel early differentiation marker.

***Trypanosoma brucei* glycosomal isocitrate dehydrogenase reveals dual coenzyme specificity towards $NADP^+$ and NAD^+**- Poster 111 : A10980
Presenter: **Miss Xinying Wang**, *PhD student, the University of Tokyo, Nagasaki University*

Authors: **X Wang**[4]; D K Inaoka[4]; E O Balogun[3]; N Ziebart[2]; S Allmann[5]; M Boshart[2]; F Bringaud[5]; T Shiba[1]; S Harada[1]; K Kiyoshi[4];
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Isocitrate dehydrogenase (IDH) is an ubiquitous enzyme catalyzing the decarboxylation of isocitrate to a-ketoglutarate (a-KG) with reduction of a coenzyme. Depending on the types of coenzymes, IDHs are classified as NAD^+ or $NADP^+$-specific IDHs. The parasite *Trypanosoma brucei* lacks NAD-IDH in its genome, while two NADP-IDHs are localized in mitochondria and glycosomes. The latter (TbIDHg) is essential for differentiation within the insect stage (see
Ziebart et al., this meeting), linking glycosomal metabolism and parasite differentiation. In order to obtain insights into the function of TbIDHg, we have purified recombinant TbIDHg and characterized its biochemical properties. Different from other known IDHs, we provided the first evidence that TbIDHg is capable to reduce both $NADP^+$ and NAD^+ at similar catalytic efficiency. Surface Plasmon Resonance analysis confirmed the direct binding of NADPH and NADH with TbIDHg, showed high dissociation constant (K_D) consistent with

low affinity and high catalytic efficiency towards both coenzymes. Furthermore, crystal structures of TbIDHg revealed a completely closed state of the active site for the ternary complexes with NAD(P)H/Ca^{2+}/a-KG. Interestingly, TbIDHg does not induce a half-closed state of the binary complex with $NAD(P)^{+}$/Ca^{2+}, but a completely open state was observed for the first time among all structures of the IDH family, indicating a distinct reaction mechanism from those proposed for other NADP-IDHs.

Benznidazole causes genome-wide mutagenesis in *Trypanosoma cruzi* and can confer multi-drug resistance- Poster 112 : A11000
Presenter: **Dr Monica Campos**, *PostDoc, London School of Hygiene and Tropical Medicine*

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Benznidazole (BNZ) is the front-line drug for Chagas disease. However, treatment failures are widely reported. To assess mechanisms of action and resistance, we sequenced the genomes of *T. cruzi* Y strain and three BNZ-resistant clones derived from a single drug-selected population. The genomes (35.5 Mb) contain 8,289 predicted protein-coding genes, 5119 where function can be inferred. Surprisingly, we identified a total of 26,495 point mutations in the drug-resistant clones. In coding regions, 49% of these were non-synonymous, many linked to radical amino acid changes. Analysis of copy number also revealed widespread DNA amplifications. Mutations were identified in genes of all functional categories, including DNA repair. This was associated with increased susceptibility to DNA alkylating and/or interstrand cross-linking agents. Resistance to benznidazole could be partially explained by stop-codon generating mutations in the drug-activating nitroreductase, a phenomenon which conferred widespread cross-resistance to other nitroheterocyclic compounds. Unexpectedly, we found that each clone was also resistant to posaconazole (6-22 fold), a drug which has been proposed for use against *T. cruzi* infections, in combination with benznidazole. Our findings therefore identify the highly mutagenic activity of benznidazole metabolites in *T. cruzi* , demonstrate how these might promote the development of multi-drug resistance, and indicate the need for vigilance when benznidazole is used in combination therapy.

An *in vivo*, focused RNAi screen identifies *Trypanosoma brucei* stress response protein kinases required for survival in mammals- Poster 113 : A11045
Presenter: **Mr Fernando Fernandez-Cortes**, *PhD student, University of Glasgow*

Authors: **F Fernandez-Cortes**[1]; T D Serafim[1]; J Wilkes[1]; N G Jones[1]; R Ritchie[1]; R McCulloch[1]; J C Mottram[1];
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A modified RNAi target sequencing approach, pooling 176 individual cell lines covering all predicted *Trypanosoma brucei* protein kinases (PKs), assessed essentially for bloodstream form proliferation *in vivo*. 48h after RNAi induction, 49 lines had a significant loss of fitness *in vivo* in two independent experiments, finding strong correlation between *in vitro* and *in vivo* results for the majority. Nine PKs were required for growth *in vivo* long before they were needed *in vitro*. Amongst these PKs were two MAP3Ks, involved osmotic shock resistance; several PKs with putative functions in stress response through PI3K/TOR, including BUD32-like, involved in translational regulation; VPS15, component of the PI3K complex with roles in autophagosome formation and vesicular trafficking; and CK2A2, a promiscuous PK capable of stress-induced mobilization. Three of the *in vivo* PKs have been implicated in repair of alkylation-induced cellular damage: SRPK1, a stress-response RNA splicing regulator; AUK2, a controller of entry in mitosis; and

CAMKL, putatively involved in metabolic regulation. Finally, a parasite-specific pseudokinase which localizes to the flagellum attachment zone, FAZ20, was also found in that group. Identification of novel virulence-associated PKs provides new insights on parasite-host interaction with potential as drug targets. This strategy can be a valuable research tool to evaluate protein phosphorylation involvement in many biological processes.

Development of a computational model for visceral *Leishmania*sis drug development using transcriptomic and immunological data- Poster 114 : A11014
Presenter: **Dr Sarah Forrester**, *Postdoc, University of York*

Authors: **S J Forrester[1]**; H Ashwin[1]; E Clark[3]; K Seifert[2]; N Brown[1]; P Andrews[3]; S L Croft[2]; J Timmis[3]; J Mottram[1]; P Kaye[1];
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Viscerotropic strains of *Leishmania donovani* and *Leishmania* infantum are responsible for a significant health burden worldwide, with 200,000 to 400,000 new cases reported annually; however many aspects of the host-parasite interplay during infection have yet to be elucidated. CRACK-IT is a interdisciplinary project that brings together immunological, pharmacological and transcriptomic data in an attempt to understand host-parasite dynamics during infection and following treatment. Based on a published Petri net model of the host response to infection (Albergante et al PLoS Comp. Biol. 9(11):e1003334), we are constructing a multi scale model of infection that will significantly reduce the numbers of animals required for pre-clinical evaluation of drug combination therapies (including immuno-chemotherapy). Here, we discuss issues pertinent to understanding the interplay between host immunity, drug pharmokinetics and parasite genomic plasticity, and how these can be brought together by the use of transcriptome-based analysis. We will describe tissue and disease-stage specific transcriptomic profiles in the blood, liver and spleen, and discuss the approaches we are taking to tease apart the transcriptomic responses observed during therapy and that are due to direct drug-immune interactions from those that represent a confounding effect due to changes in parasite load.

Switch or become stumpy!- Poster 115 : A11057
Presenter: **Ms Henriette Zimmermann**, *PhD student, Biozentrum der Universität Würzburg*

Authors: **H Zimmermann**[1]; I Subota[1]; C Batram[1]; S Kramer[1]; C J Janzen[1]; N G Jones[1]; M Engstler[1];
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The proliferative slender bloodstream form of *Trypanosoma brucei* stochastically switches the expressed variant surface glycoprotein (VSG) and, thus, escapes the immune attack of its mammalian host (antigenic variation). While parasitemia rises, the trypanosome-secreted stumpy induction factor (SIF) accumulates and upon reaching a threshold the slender cells differentiate to the G1/0 arrested fly-infective stumpy stage. Previously, we have shown that overexpression of an ectopic VSG leads to the attenuation of the entire active VSG expression site (ES), followed by growth retardation. This was reminiscent of stumpy stage formation and, thus, indicated that ES-attenuation could act as a trigger for stumpy development. We now have tested this possibility by VSG overexpression in a pleomorphic strain, which possesses full developmental competence. This revealed a marked phenotypic plasticity. Upon VSG overexpression the endogenous VSG was silenced in all clones, but surprisingly, the entire ES was not equally attenuated. We show that if the transcriptional activity of the ES falls below a specific threshold, the almost immediate

development of fly-infective stumpy cells is induced. Our results also indicate that ES-attenuation triggers stumpy differentiation in a cell density-independent manner, downstream of the quorum sensing factor SIF. We suggest that the activity status of the ES represents the interface between antigenic variation and transmissibility.

Different roads to Rome: Targeting of *T. brucei* Arl13b to the flagellum- Poster 116 : A11075
Presenter: **Mr Yiliu Zhang**, *Graduate Student, National University of Singapore*

Authors: **Y L Zhang**[1]; C Y He[1];
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The small GTPase Arl13b is associated with Joubert's Syndrome – a severe genetic disease caused by defective cilia. Previous studies using animal models have shown that Arl13b localizes to the cilium and is important for various cilia-related processes. Molecular mechanisms behind its plethora of phenotypes however is only at the very early stage. We have utilized the versatile *T. brucei* model to study the Arl13b functions in an early diverged, single-flagellated organism. Our results show that TbArl13b is a flagellar protein crucial for cell motility, flagellum attachment/FAZ assembly, flagellum biogenesis and cell survival. Distinctively among other eukaryotic organisms, TbArl13b associates with the axoneme rather than the flagellar membrane. A Dimerization/Docking domain is necessary and sufficient for this association and localization. Cytoplasmic TbArl13b lacking the D/D domain fail to restore cell growth under TbArl13b RNAi. However, substituting the D/D domain with a flagellar membrane targeting domain creates a viable alternative to WT TbArl13b, suggesting that enrichment in the flagellar compartment rather than the specific sub-flagellum localization is critical for TbArl13b functions.

Activation of bicyclic nitro-drugs by a novel nitroreductase (NTR2) in *Leishmania*- Poster 117 : A11032
Presenter: **Dr Susan Wyllie**, *Team leader, University of Dundee*

Authors: **S Wyllie**[1]; S Norval[1]; S Patterson[1]; A H Fairlamb[1];
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Recently, the bicyclic nitro-compounds (*R*)-PA-824, DNDI-VL-2098 and delamanid have been identified as potential candidates for the treatment of visceral *Leishmania*sis. Using a combination of quantitative proteomics and whole genome sequencing of susceptible and drug-resistant parasites we identified a putative NAD(P)H oxidase as the activating nitroreductase (NTR2). Whole genome sequencing revealed that deletion of a single cytosine in the gene for NTR2 resulted in expression of a non-functional truncated protein. Susceptibility of *Leishmania* was restored by reintroduction of the WT gene into the resistant line, which was accompanied by the ability to metabolise these compounds. Overexpression of NTR2 in WT parasites rendered cells hyper-sensitive to bicyclic nitro-compounds, but only marginally to the monocyclic nitro-drugs, nifurtimox and fexinidazole, known to be activated by a mitochondrial oxygen-insensitive nitroreductase (NTR1). Conversely, a double knockout NTR2 null cell line was completely resistant to bicyclic nitro-compounds. Recombinant NTR2 was capable of reducing bicyclic nitro-compounds in the same rank order as drug sensitivity *in vitro*. Thus, NTR2 is necessary and sufficient for activation of these bicyclic nitro-drugs. These findings may aid the future development of better, novel anti-*Leishmania*l drugs.

The African trypanosome *Trypanosoma brucei* is susceptible to therapeutic concentrations of RNA polymerase I inhibitors- Poster 118 : A11041
Presenter: **Miss Louise Kerry**, *PhD Student, Imperial College London*

Authors: **L E Kerry**[1]; E E Pegg[1]; D Cameron[2]; J Budzak[1]; R D Hannan[2]; G Rudenko[1];
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Trypanosoma brucei relies on a dense Variant Surface Glycoprotein (VSG) coat for survival in the host bloodstream. High VSG expression within an expression site body (ESB) is mediated by RNA polymerase I (Pol I), which normally exclusively transcribes rDNA. Pol I inhibitors are currently in clinical trials against cancer, as they target cells that are reliant upon high levels of Pol I transcription. As *T. brucei* also relies on Pol I transcription of a single *VSG* gene for survival within the host, we investigated Pol I inhibitors quarfloxin, CX-5461, and BMH-21 for selective efficacy. Cytotoxicity assays showed all three Pol I inhibitors have IC50 concentrations in the nanomolar range in *T. brucei*, and selective toxicity compared with mammalian cells. Parasite growth inhibition was due to rapid and specific inhibition of Pol I transcription, as rRNA precursor transcript was reduced to 2-3% and VSG precursor transcript to 6-9% normal levels within 15 minutes incubation with these substances. Incubation with Pol I inhibitors also resulted in rapid loss of the ESB and disintegration of the nucleolus within one hour. As ESB loss followed the rapid reduction in Pol I precursor transcripts, this suggests the ESB is a ‘transcription-nucleated’ subnuclear structure. In addition to providing insight into Pol I transcription and ES control, Pol I inhibitors potentially also provide new leads against trypanosomiasis.

Genomic adaptation of *Leishmania* mexicana in serial passage conditions- Poster 119 **:** A11054
Presenter: **Miss Samantha Campbell**, *PhD Student, University of Glasgow*

Authors: **S J Campbell**[2]; N J Dickens[2]; S M Duncan[1]; C A Marques[1]; R McCulloch[2];
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DNA replication is an essential process in all eukaryotes initiated from sites termed origins of replication. Recent studies in the kinetoplastid species *Leishmania* and *Trypanosoma brucei* have revealed striking differences in the process of DNA replication between the largely syntenic genomes. The genome of *Leishmania* parasites is able to adapt efficiently in different life cycle stages and in response to changing environmental factors. Genome plasticity and tolerance of pervasive mosaic aneuploidy are established features of the *Leishmania* genome, although the underlying mechanism allowing this behaviour is not clear. We have used genomics approaches to investigate the adaptation of *Leishmania* mexicana from a mouse infection in serial passage conditions by sequencing DNA samples at regular intervals. We assess chromosome and gene copy number variation, the presence and absence of SNPs and structural variants and the potential relationship between aneuploidy and DNA replication from a single origin. We observe a correlation between chromosome length and relative fold change throughout passage indicating a potential limit to the extent of replication completed by a single origin in fast-replicating cells in serial passage conditions.

The RNA binding protein-RBP10 defines bloodstream form trypanosome identity- Poster 120 : A11058
Presenter: **Mr Elisha Mugo**, *PhD student, University of Heidelberg*

Authors: **E Mugo**[1]; C Clayton[1];
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Transitions towards trypanosomes differentiation are complex and require well coordinated gene expression programs. Unlike other eukaryotes, transcription in trypanosome is polycistronic; many basal transcription factors are missing. In absence of RNA polymerase II dependent transcription regulation, what are the regulatory factors that triggers changes in trypanosome gene expression and ensures irreversibility after the initial differentiation signal? We here show that a single *T. brucei* RNA binding protein-RBP10 acts as an on/off switch which defines the trypanosome differentiation state. RBP10 is a cytosolic RNA-binding protein which is expressed only in multiplying bloodstream forms. The bloodstream forms depleted of RBP10 can survive only as procyclic forms. More remarkably, expression of RBP10 in procyclic forms results in their direct conversion to bloodstream forms within 2 days. RBP10 binds to procyclic-specific mRNAs containing the sequence UAUUUUUU, targeting them for translation repression and destruction. The products of RBP10 target mRNAs include not only the major procyclic-specific surface protein and various enzymes of energy metabolism, but also signaling and RNA-binding proteins required for procyclic-form survival. RBP10 is therefore a paradigm for the definition and maintenance of a eukaryotic cell differentiation state by a post-transcriptional regulatory cascade.

Transspecies surface coats of African trypanosomes: What can they teach us about VSG functionality? - Poster 121 : A11079
Presenter: **Mr. Erick Aroko**, *PhD Student, Biozentrun der Universität Würzburg*

Authors: **E Aroko**[2]; N G Jones[2]; M Carrington[1]; M Engstler[2];
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African trypanosomes protect themselves from destruction by host defences by covering their surface with a dense coat of a single protein, the variant surface glycoprotein (VSG). The VSG and the coat it forms have been best characterized in *Trypanosoma brucei*. In this species the protein consists of a larger, elongated N-terminal domain that is exposed and a shorter, more secluded C-terminal domain consisting of one or two structured regions. Sequence variability in the N-terminal domain is fundamental for antigenic variation; how the C-terminal domain supports VSG functionality is however not clear.
Other species such as *T. congolense* and *T. vivax* also possess a VSG surface coat which presumably has the same protective function. Though similar, VSGs have evolved to differ from species to species, for instance *T. congolense* VSGs lack structured regions in their C-terminal domain and this is probably also the case for the *T. vivax* VSGs.
In order to address the compositional differences in terms of functionality we attempted to generate transgenic *T. brucei* cells expressing VSGs of either of the other species on their cell surface. Whereas a *T. congolense* VSG could readily be expressed as the only VSG on *T. brucei* cells, this was not the case for the chosen *T. vivax* VSG. Supporting mutagenesis studies on *T. brucei* VSGs shed new light on the structure-function relationship in VSGs.

Evidence towards a role for the GTPase Ran in mRNA export in trypanosomes.- Poster 122 : A11082
Presenter: **Dr Samson Obado**, *Research Associate, The Rockefeller University*

Authors: **S O Obado**[1]; M Brillantes[1]; W Zhang[1]; N E Ketaren[1]; B T Chait[1]; M C Field[2]; M P Rout[1];
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The nuclear pore complex (NPC) is responsible for multiple processes, including nucleocytoplasmic transport, interactions with the nuclear lamina and mRNA processing, and thus constitutes a hub for the

control of gene expression. Remarkably, despite retaining similar protein compositions, there are exceptional architectural dissimilarities between yeast and vertebrate and trypanosome NPCs. Whilst elements of the inner core are conserved, numerous peripheral structures are highly divergent, perhaps reflecting requirements to interface with divergent nuclear and cytoplasmic functions. Moreover, the trypanosome NPC has almost complete nucleocytoplasmic symmetry, in contrast to the fungal and animal NPC and a remarkable absence of components involved in mRNA export in fungi and animals. Concomitant with this, we find evidence supporting Ran-dependent mRNA export in trypanosomes, similar to protein transport, whilst in animals and fungi mRNA export is Ran-independent. Identification of these lineage-specific features suggests that despite an overall well conserved structure, several aspects that differentiate the trypanosome NPC and suggest significant divergence in mRNA export mechanics.

Opposing associations of APOL1 genetic variants with African trypanosomiasis resistance in East and West Africa- Poster 123 : A11085
Presenter: **Dr. Anneli Cooper**, *PostDoc, University of Glasgow*

Authors: **A C Cooper**[4]; H Ilboudo[1]; V Jamonneau[1]; V P Alibu[2]; J Enyaru[2]; W Weir[4]; E Matovu[2]; B Bucheton[3]; A Macleod[4];
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Pathogen-mediated selection can drive an increase in the frequency of alleles that reduce susceptibility to infectious disease, but maybe otherwise deleterious. It is hypothesised that reduced susceptibility to Human African Trypanosomiasis underlies the high allele frequency of two APOL1 renal risk variants, G1 and G2 in populations with recent African ancestry. In a case-control study we report opposing dominant associations for the G2 variant with protection against T.b. rhodesiense infection, but with more severe disease for T.b. gambiense. Conversely, the G1 variant is not associated with infection resistance for either parasite sub-species but with asymptomatic carriage in T.b. gambiense infection. This analysis suggests a more complex relationship between APOL1 variants and trypanosomiasis than has previously been proposed with opposing evolutionary selective forces at play.

A genetic basis for asymptomatic human African trypanosomiasis - Poster 125 : A11087
Presenter: **Dr. Paul Capewell**, *Research Associate, University of Glasgow*

Authors: **P Capewell**[3]; H Ilboudo[1]; C Clucas[3]; A Cooper[3]; T A Gorman[3]; A Patakas[3]; W Weir[3]; P Garside[3]; B Bucheton[2]; V Jamonneau[2]; A MacLeod[3];
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Asymptomatic carriers of pathogens are an often overlooked impediment to the control and elimination of many diseases. One such disease is Human African trypanosomiasis (HAT), an economically important disease of sub-Saharan Africa in which a serologically positive but asymptomatic reservoir population has recently been identified. Our study aimed to understand the genetic underpinnings of the asymptomatic phenotype by analysing the transcriptomes of immune cells from both symptomatic HAT patients and asymptomatic individuals to identify functional groups, pathways, local expression quantitative trait loci (eQTL) and polymorphisms associating with HAT susceptibility. This analysis implicated T cell activation and the major histocompatibility (MHC) locus as factors affecting HAT disease outcome. We verified this in a mouse model for trypanosomiasis, demonstrating that blocking T cell activation reduces parasitaemia and

improves animal condition, mimicking the human asymptomatic phenotype and demonstrating a clear link from genetics to disease outcome. Understanding how asymptomatic individuals control infection and the role T cell activation plays in the phenomenon will lead to the development of interventions that alleviate symptoms in symptomatic HAT patients and also allow individuals more likely to develop disease to be identified and pre-emptively treated.

The Small Tim Homologues in the Mitochondrion of *Trypanosoma brucei* are Critical for Redox Homeostasis and Stability of TbTim17- Poster 126 : A11115
Presenter: **Minu Chaudhuri***, Meharry Medical College*

Authors: **M Chaudhuri**[1]; J Smith[1]; U Singha[1]; S Misra[1];
[1] Meharry Medical College, United States

Small Tims (translocase of the mitochondrial inner membrane) make up a group of mitochondrial intermembrane space chaperones. *Trypanosoma brucei*, the protozoan parasite that causes African trypanosomiasis, possesses at least 3 small Tim proteins that include the homologs of Tim9 (TbTim9), Tim10 (TbTim10), and a third one that is homologous to both Tim8 and Tim13 (TbTim8/13). Each possesses a pair of CX3C motifs, a conserved feature required for oxidative folding of this protein family. We found that TbTim9 and TbTim10 are required for optimum cell growth and TbTim8/13 is essential for *T. brucei* survival. TbTim8/13 interacts with TbTim9 as well as TbTim10, however, TbTim9 and TbTim10 do not interact with each other, which may explain the essentiality of TbTim8/13. Knockdown of the small TbTims reduces the steady state levels and assembly of mitochondrial carrier protein, MCP5, but do not have any effect on the import of the presequence-containing proteins. Interestingly, small TbTim knockdown increases the levels of reactive oxygen species up to 12 fold and reduces the stability of TbTim17, the major component of the TIM complex in *T. brucei*, while import of TbTim17 is minimally affected. The stability of TbTim17 is similarly reduced when *T. brucei* is treated with paraquat. Together, our results show that small TbTims with differential interaction pattern than higher eukaryotes are critical for cellular redox homeostasis and TbTim17 acts as a redox-sensor in *T. brucei*.

Towards the identification of the stumpy induction factor (SIF)- Poster 127 **:** A11122
Presenter: **Dr Ines Subota**, *Postdoctoral fellow, Universität Würzburg*

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The parasite *Trypanosoma brucei* has evolved mechanisms to guarantee persistent infection and tsetse fly transmissibility. A key in this interplay is the up to now elusive stumpy induction factor (SIF), which is parasite-derived and accumulates in a cell-density dependant manner. Once a threshold is reached, SIF triggers differentiation of the proliferating slender form to the cell-cycle arrested stumpy stage, which is preadapted for transmission by the vector.
Our study aims at identifying SIF by combining liquid chromatography (LC), untargeted metabolomics and activity bioassays using a pleomorphic reporter cell line.
In a first purification step conditioned cell culture medium containing SIF was subjected to methanolic protein precipitation extraction. The resulting deproteinated concentrate was further purified by reversed-phase solid phase extraction eluted with 10% methanol in water, which demonstrated the highly polar nature of SIF. We thus opted for hydrophilic interaction LC using an amide column in order to separate polar metabolites. Surprisingly, the active fractions contained pyruvate as a possible candidate. In fact, pyruvate can mimic SIF

action, however, only at concentrations that appear unphysiological. We present data that rule out that pyruvate is SIF, but that also suggest that it might be a related compound.

N-glycosylation enables high lateral mobility of GPI-anchored proteins at a molecular crowding threshold- Poster 128 : A11128
Presenter: **Mr. Marius Glogger**, *PhD student, Biozentrum der Universität Würzburg*

Authors: **M Glogger**[3]; **A Hartel**[3]; N Jones[3]; W Abuillan[1]; C Batram[3]; A Hermann[3]; S Fenz[3]; M Tanaka[2]; M Engstler[3];
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The protein density in biological membranes can be extraordinarily high, but the impact of molecular crowding on the diffusion of membrane proteins has not been studied systematically in a natural system. The diversity of the membrane proteome of most cells may preclude systematic studies. African trypanosomes, however, feature a uniform surface coat that is dominated by a single type of variant surface glycoprotein (VSG). Here we study the density-dependence of the diffusion of different GPI-anchored VSG-types on living cells and in artificial membranes. Our results suggest that a specific molecular crowding threshold (MCT) limits diffusion and hence affects protein function. Obstacles in the form of heterologous proteins compromise the diffusion coefficient and the MCT. The trypanosome VSG-coat operates very close to its MCT. Importantly, our experiments show that N-linked glycans act as molecular insulators that reduce retarding intermolecular interactions allowing membrane proteins to function correctly even when densely packed.

Intracellular trafficking of GFP expressing *Leishmania* aethiopica in terminally differentiated THP-1 cells.- Poster 129 **:** A11144
Presenter: **Medhavi Ranatunga**, *PhD Research Student, University of Greenwich*

Authors: **R M Ranatunga**[1]; G Getti[1];
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Leishmania parasites establish themselves inside mammalian host following a carrier sandfly bit. Once inside the macrophages parasites establish themselves within membrane bound organelle known as parasitophorous vacuoles (PV). Established amastigotes replicate and spread to neighbouring cells causing a range of diseases that affect 20million people worldwide. Even though the mechanism of parasites bind and enter has been investigated, very little is known about *Leishmania's* movement through the endocytic pathway and no data is available defining *L.aethiopica* trafficking. In this research, GFP expressing *L.aethiopica* were used to investigate parasite trafficking inside the host macrophages. Terminally differentiated THP-1 cells infected with metacyclic promastigotes, newly developed & validated axenic amastigotes and infected cells were analysed for early and late endosomal colocalization up to 72h from infection. Colocalization with EEA-1 was only detectable from 10min to 4h after infection with axenic amastigotes. Interestingly EEA-1 colocalization following promastigotes and cell-mediated amastigote infection was not detectable. When infected cells were used to start infection, parasites colocalized with LAMP-1 as early as 10min after co-culture. Axenic amastigotes colocalized with LAMP-1 at 1h after infection and promastigotes at 4h after infection. These data showed that *L.aethiopica* trafficking is depend on the parasite stage and this is the first study to compare trafficking of those three stages. The data clearly

showed differences in internalization which relate to the type of infection and indicate that intracellular parasites might spread to uninfected osts within the PV of previously infected cells.

Nitroheterocyclic drugs cure experimental *Trypanosoma cruzi* infections more effectively in the chronic stage than in the acute stage- Poster 130 : A10858
Presenter: **Dr Amanda Francisco**, *Researcher, London School of Hygiene and Tropical Medicine*

Authors: **A F Francisco**[2]; S Jayawardhana[2]; M D Lewis[2]; K L White[1]; D M Shackleford[1]; G Chen[1]; J Saunders[1]; M Osuna-Cabello[3]; K D Read[3]; S A Charman[1]; E Chatelain[4]; J M Kelly[2];
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Chagas disease is caused by the insect-transmitted protozoan *Trypanosoma cruzi* and is the most important parasitic infection in Latin America, affecting 5-8 million people. The disease is characterised by an acute phase, which is partially resolved by the immune system, but then develops as a chronic life-long infection. The nitroheterocyclic compounds benznidazole (BZ) and nifurtimox (NF) are the front-line drugs. Unfortunately, they display a range of side-effects, that impact on patient compliance. Furthermore, both require bioactivation by the same parasite nitroreductase, a source of cross-resistance. There is consensus that BZ and NF are more effective against the acute stage. However, confirmative studies have been restricted by difficulties in demonstrating sterile cure. Here, we describe a systematic study of nitroheterocyclic drug efficacy using highly sensitive bioluminescence imaging of murine infections. We find both drugs are more effective at curing chronic infections, judged by treatment duration and therapeutic dose. This was not associated with factors that influence plasma drug concentrations in the two disease stages. We also observed that fexinidazole and fexinidazole sulfone are more effective than BZ and NF as curative treatments, particularly for acute stage infection, and that this is associated with higher and more prolonged exposure of the sulfone derivative.

VSG mRNA promotes S-phase in the bloodstream African trypanosome- Poster 131 : A11170
Presenter: **Mr Sebastian Hutchinson**, *PhD Student, University of Dundee*

Authors: S Hutchinson[1]; A Trenaman[1]; J E Wright[1]; **D Horn**[1];
[1] University of Dundee

The bloodstream African trypanosome surface is protected from immune attack by a super-abundant variant surface glycoprotein (VSG) coat that is required for cytokinesis. These protozoa also display other unusual cell-cycle controls and readily uncouple S-phase and cytokinesis, producing multinucleated ‘monster’ cells when cellular architecture is disrupted. We find that VSG mRNA promotes S-phase and, in the absence of (VSG)-protein, the production of multinucleated cells. We used MS2 coat protein (MS2-cp) to block translation of a transgenic VSG mRNA containing a cognate hairpin in the 5´-untranslated-region; VSG mRNA abundance increased under these conditions. Like VSG mRNA knockdown, a VSG translation block triggered global translation arrest and cytokinesis arrest. In contrast to VSG mRNA knockdown, however, S-phase and mitosis continued in the presence of blocked VSG mRNA. Indeed, these cells re-entered S-phase and mitosis, yielding multi-nucleated trypanosomes. S-phase and cytokinesis uncoupling, triggered by

defects in VSG-translation or trafficking, may explain why perturbations of cellular architecture often produce multi-nucleated cells. Our results reveal a bloodstream stage-specific VSG expression sensing mechanism that controls key cell-cycle phases, with the mRNA and protein coat promoting DNA replication and cytokinesis, respectively.

Metabolomics-driven development of a minimal essential medium for the animal trypanosomiases- Poster 132 : A11190
Presenter: **Dr Pieter Steketee**, *Postdoctoral Researcher, The Roslin Institute*

Authors: **P C Steketee**[2]; E Paxton[2]; F Achcar[3]; F Giordani[3]; H de Koning[3]; T Rowan[1]; M P Barrett[3]; L J Morrison[2];
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The trypanosome subspecies *Trypanosoma congolense* and *T. vivax* are the primary causative agents of animal African trypanosomiasis (AAT). In contrast to *T. brucei*, our biological knowledge of these parasites remains limited and as a result, there is a severe lack of novel therapeutics to counter diseases which affect tens of millions of livestock annually. Moreover, there have been no new drugs for >50 years and resistance has been reported to the few veterinary trypanocides available. One of the key issues in studying AAT remains our limited ability to culture both *T. congolense* and *T. vivax*. The primary aim of this project is the use of omics-driven approaches to further our understanding of both *T. congolense* and *T. vivax*, with a view to developing optimised *in vitro* culturing media that will enable the culture of field isolates, as well as high-throughput *in vitro* drug screens. Using a metabolomics-driven approach, we have identified key differences in the metabolic usage and output of bloodstream forms of a laboratory-adapted *T. congolense* strain (IL3000). 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*, where pyruvate is a primary output. These data suggest bloodstream form *T. congolense* is not solely reliant upon glycolysis, but also utilises other metabolic pathways for central carbon metabolism, highlighting significant metabolic differences between *T. congolense* and *T. brucei*.

Identifying and Characterising developmentally regulated RNA-binding proteins (RBPs) in *Leishmania* mexicana.- Poster 133 : A11179
Presenter: **Dr Luis Miguel De Pablos Torro**, *PDRA, University of York*

Authors: L M De Pablos Torro[1]; **P B Walrad**[1];
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Leishmania spp. parasites must rapidly respond to environmental shifts within the insect vector and the mammalian host for successful progression toward human infective forms. Due to negligible trascriptional regulation in these parasites, trans-acting factors drive *Leishmania* spp. lifecycle progression. These trans-acting RNA binding proteins (RBPs) coordinate the location, translation and decay of RNAs within messenger ribonucleoprotein complexes (mRNPs) in response to shifting environmental cues. To isolate key trans-regulators implicit in virulent parasite stages, we pursued developmentally-regulated mRNA-associating proteins in the three most- characterised parasite lifecycle stages; procyclic promastigote (P), metacyclic promastigote (M) and amastigote (A) forms of *Leishmania* mexicana. These lifecycle stages were isolated and purified by optimised axenic culturing (P,M) and macrophage infections (A). Each stage was verified by molecular markers, FACS analysis and human complement susceptibility prior to an enhanced system-wide mRNA interactome capture approach. Preliminary data from this has confirmed known RBP expression from previous literature and identified novel RNA interactors. To examine candidate trans-

regulator expression, we endogenously tagged specific RBPs whose RNAs exhibit developmental regulation. Distinctions in relative protein/RNA expression were confirmed, indicating stage-specific control of transcript stability and translation and supporting the role for these RBPs in developmental progression and parasite virulence. We are currently quantifying the L.mexicana lifecycle's mRNA-bound proteome while confirming RNA-binding activity and transcript target identity for select trans-regulator candidates. This work will provide novel insight into trans-regulatory mRNP complexes which drive the *Leishmania* spp. lifecycle. To examine candidate trans-regulator expression,we endogenously tagged specific RBPs

The Application of Irradiation Technology as a Tool for Vaccine Development in Trypanosomosis- Poster 134 : A11181
Presenter: **Richard Kangethe**, *Post Doc, IAEA*

Authors: **R T Kangethe**[1]; E M Winger[1]; T B Settypalli[1]; V M Wijewardana[1]; H Unger[1]; A Diallo[1];
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Recent advances using irradiation in vaccine development against parasitic diseases such as malaria have reignited studies in related diseases such as trypanosomosis in livestock. The objective of this work is to utilise irradiation to produce living but non-infectious trypanosomes so as to understand mechanisms that are important for establishing an infection. *In vitro* experiments with irradiated parasites receiving irradiation doses above 200 Gy do not recover. However, inoculating Balb/c mice with parasites irradiated with doses below 200 Gy leads to an infection that is less virulent when compared to non-irradiated parasites. The transcriptional profiles of parasites irradiated using different doses at different time points was compared to non-irradited parasites using microarray. Parasites irradiated using 100Gy and analysed 20 hours post exposure show 68 genes with known function up-regulated and 18 genes down-regulated. This is in contrast to the up-regulation of 21 genes and the down-regulation of 267 genes when using a dose of 200Gy. Genes that are consistently down-regulated when using both doses include those coding for Metallo-peptidases and Ubiquitin. Further analysis of the data derived from these experiments will help elucidate how irradiation affects processes that are important for establishing disease in the mammalian host.

Identification and functional characterization of ornithine transporters in *Trypanosoma brucei* and their involvement in drug action- Poster 135 : A11192
Presenter: **Juan Pereira de Macêdo**, *Postdoc, University of Bern*

Authors: **J P de Macêdo**[1]; R Currier[2]; C Wirdnam[1]; D Horn[3]; S Alsford[2]; D Rentsch[1];
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Ornithine is an essential precursor for the synthesis of polyamines in *Trypanosoma brucei*. Ornithine decarboxylase, an enzyme that converts ornithine into putrescine, is the target of eflornithine, an approved drug for therapy against sleeping sickness. To date, no ornithine carrier has been identified in *T. brucei*. Complementation of Saccharomyces cerevisiae amino acid transport mutants with putative amino acid transporters (AAT) from *T. brucei* identified TbAAT2 and TbAAT10 as potential histidine transporters. Interestingly, TbAAT2-mediated histidine uptake was inhibited by ornithine, suggesting that ornithine can also be transported. Systematic investigations using [^{3}H]ornithine revealed that TbAAT10 and TbAAT2 mediate high-affinity ornithine transport (apparent K_m 4.3 and 4.0 μM), however, with an obvious difference in substrate specificity. While TbAAT10 was selective for ornithine, uptake of ornithine by TbAAT2 was significantly inhibited by histidine, suggesting that TbAAT2-mediated ornithine uptake is influenced by the extracellular histidine concentration. Moreover, RNAi knockdown of TbAAT10 caused a small but significant

growth defect in *T. brucei* bloodstream forms, whereas TbAAT2 down-regulation did not affect cell proliferation in culture. Interestingly, drug sensitivity assays revealed opposing effects on suramin and eflornithine toxicity. RNAi against TbAAT10 caused a 2-fold increase in resistance to suramin and, in contrast, a 10-fold higher sensitivity to eflornithine. Together, our data provide insights on ornithine uptake and metabolism in *T. brucei* and its relevance for therapeutics against sleeping sickness.

Trypanosome flagellum transition zone proteome reveals compartmentalisation and differential dynamics of ciliopathy complexes- Poster 136 : A11175
Presenter: **Dr Samuel Dean**, *Scientist, University of Oxford*

Authors: **S Dean**[2]; F Moreira-Leite[2]; V Varga[1]; K Gull[2];
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The biology of the trypanosome flagellum provides opportunities for insights into trypanosome pathology and can be generalised to other eukaryotic flagella.
The flagellum transition zone (TZ) is positioned between the distal end of the basal bodies and the proximal end of the 9+2 axoneme. It is increasingly recognised as having critical roles in flagellum growth and function, acting as a “gate” that controls the composition of the flagellum. The importance of the TZ is reflected in the many human diseases (termed ciliopathies) that are caused by mutations in TZ complexes. Here, we present the most systematic analysis of the TZ in any organism to date. We use a new proteomics technique to find trypanosome TZ proteins and then leverage this proteome to uncover basic TZ biology. We show that TZ proteins, including members of ciliopathy complexes, localize to different TZ sub-domains. Analysis using RNAi and HaloTag fusion protein approaches reveals that most TZ proteins (including the Meckel Syndrome ciliopathy complex) show long-term stable association with the TZ, whereas the Bardet-Biedl syndrome complex is dynamic.
RNAi depletion of these proteins causes a range of phenotypes, ranging from no cytological perturbation to absence of the central pair of microtubules and complete absence of the flagellum. This highlights the importance and variety of functions of this flagellum sub-compartment.

Functional annotation of Euglena gracilis mitochondrial proteome. - Poster 137 : A11243
Presenter: **Anna Nenarokova**, *University of South Bohemia*

Authors:**A Nenarokova**[4,3]; E Dobáková [4]; P Flegontov[4]; T Ebenezer[1]; M Zolner[1]; M Field[1]; J Lukeš[4,2,3]; P Flegontov[5];
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Euglena gracilis is a secondary green alga belonging to the phylum Euglenozoa, which also contains kinetoplastid parasites Trypanosoma and Leishmania. Despite the fact that E. gracilis is a well-studied model organism, little is known about its single reticulated mitochondrion. Few previous studies have focused primarily on individual functional groups of mitochondrial proteins, making no attempt to explore mitochondrial proteome as a whole. In this study, we have examined entire E. gracilis mitoproteome using de novo whole-transcriptome sequencing and tandem mass spectrometry of organelle fractions, as a part of the Euglena genome project. We made functional annotation of the detected mitochondrial proteins by

similarity to proteins with known function (BlastP, Blast2GO and KAAS), and assigned them into several metabolic pathways and fuctional modules, using KEGG database. E. gracilis mitochondrial proteome contains near 1400 proteins, including subunits of respiratory chain complexes I-V, other components of aerobic and anaerobic energy metabolism, mitoribosomal proteins, iron-sulfur cluster assembly machinery, components of heme biosynthesis pathway, proteins of lipid and aminoacid metabolism, and others. Components of sulfate assimilation pathway were 3 to 10 times more abundant in the mitochondrial fraction than in the chloroplast fraction, providing evidence of its unusual localization.

***L. donovani* from the Indian Subcontinent is pre-adapted for a rapid development of antimonial resistance, driven by aneuploidy**- Poster 138 : A10813
Presenter: **Prof. Jean-Claude Dujardin**, *Dept of Biomedical Sciences, Head, Institute of Tropical Medicine*

Authors:**J C Dujardin**[2]; F Dumetz[2]; H Imamura[2]; B Cuypers[2]; M Domagalska[2]; S Rijal[1]; G De Muylder[2];
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In a previous phylogenomic study of *L. donovani* in the Indian subcontinent (ISC), we identified molecular events likely involved in SSG resistance, like an intra-chromosomal amplification (ICA) of the H- and MAPK1-loci and a 2-nt indel in AQP1 gene. We experimentally selected here Sb3-resistance in 3 ISC reference strains (A, ICA-neg, B and C, ICA-pos and respectively SSG-resistant and -sensitive). Initial Sb3 sensitivity of B-C promastigotes was 10 times lower than A. Time-to-resistance was shorter for B-C (4 cycles) than A (7 cycles). All quadruplicates of B-C reached the last cycle but only 1 replicate of A was recovered at the final concentration. At genomic level there were no local CNV, no indel and very few SNPs (only in A); in contrast, chromosomal copy number increased (noteworthy in chr 23, carrying H-locus), aneuploidy being more pronounced in A. Metabolomic results are currently integrated and will be presented. B-C parasites (main ISC population) are likely pre-adapted to antimonials and the gene dosage effect of ICA is multiplied by aneuploidy, while resistance seems harder to acquire for A (highland genotype).

Developing a genetic modification system of marine protist *Diplonema papillatum* - Poster 139 : A11244
Presenter: **Binnypreet Kaur**, *University of South Bohemia*

Authors:**B Kaur**[1]; D Faktorová[1]; P Peña-Diaz[1]; P J Lukeš[1]
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Diplonemids (Diplonemea, Euglenozoa) are a sister group of euglenids - important in freshwater ecosystems - and kinetoplastid flagellates, which include highly pathogenic Trypanosoma and Leishmania spp. Both kinetoplastids and euglenids have long been recognized as virtually omnipresent and very species-rich, whereas diplonemids have remained largely unnoticed. They came into the spotlight only recently thanks to the Tara Oceans expedition, which revealed their global presence and extreme abundance in the world ocean. Indeed, diplonemids may comprise the 6th most abundant and 3rd most species-rich group of marine eukaryotes. However, until now, only few diplonemid species have been formally described. Goal of our study is to fill the enormous gap in our knowledge about this major player in the oceanic ecosystem and turn (at least) one strain into a genetically tractable system, which is a critical step towards functional studies of its genes. Our model species is *Diplonema papillatum* that can be easily cultivated axenically in the laboratory, reaches high density and grows in large volumes. We have already measured the cell viability and tested that D. papillatum is sensitive to multiple drugs that can be used as selectable markers. Recently,

we are preparing a range of constructs that will be used to deliver DNA into this protist, so far not subjected to genetic manipulations.

TbPH1: a trypanosomatid-specific pleckstrin homology domain containing kinesin-like protein- Poster 140 : A11245
Presenter: **Sabine Kaltenbrunner**, *, University of South Bohemia*

Authors:**S Kaltenbrunner**[1]; H Hashimi[1,2]; J Lukeš[1,2]
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The kinesin and kinesin-like protein superfamily is among one of the largest in *Trypanosoma brucei*, with almost 100 encoding genes dispersed throughout the genome. Among these is a gene encoding a protein we call TbPH1 (Tb927.3.2490), which contains a pleckstrin homology (PH) domain that inspired its name. The 110 kDa multidomain protein is made up of an N-terminal kinesin domain whose Walker A motif is ablated by a single substitution, an intervening coiled-coil region, followed by the PH and helix-turn-helix domains. While its role as a microtubule motor is suspect, it bears other motifs that suggest interactions with other proteins, lipids and even double-stranded nucleic acids. RNAi-silencing of TbPH1 in procyclic (PCF) and long slender bloodstream (BSF) forms compromises parasite fitness, likely due to a cell cycle defect resulting in an accumulation of 1N2K cells. In situ N- and C-terminal epitope-tagging reveals an interesting, somewhat punctate localization pattern that is distributed throughout the cell but often enriched between the nucleus and kinetoplast. Its localization predominantly excludes such organelles as the mitochondrion, endoplasmic reticulum and acidocalcisomes. Fractionation of *T. brucei* into cytoskeleton and soluble fractions does not support TbPH1 being a component of the former. However, TbPH1 is trapped by microtubule sieving and released when the corset is depolymerized in high salt conditions.

Exploring the bioenergetics of the bloodstream *T. brucei* mitochondrion- Poster 141 **:** A11246
Presenter: **Gergana Taleva**, *, Biology Centre ASCR, Institute of Parasitology*

Authors:**G Taleva**[1,2]; B Panicucci[2]; A Zíková[1,2];
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It is currently believed that in bloodstream form (BF) *T. brucei*, the ATP/ADP carrier (TbAAC) transports cytosolic ATP generated from glycolysis into the mitochondrion, where it is hydrolyzed by FoF1-ATPase to maintain the essential mitochondrial (mt) membrane potential. Interestingly, BF parasites tolerate high doses of carboxyatractyloside (CATR), a specific inhibitor of AAC. Therefore, we explored the necessity of this transporter by generating BF TbAAC double knockout (DKO) cell lines, which display only a slight growth phenotype compared to their parental cells. While we further characterize these genetically modified trypansomes, we explored if the source of mt ATP doesn't come from within the organelle by mt substrate phosphorylation, since a subunit of succinyl CoA synthetase (TbSCoAS) has already been established to be essential in BF parasites (Zhang X, Nucleic Acids Res (38) 2010). However, we again were able to generate TbSCoAS DKO cells that also demonstrate only a mild growth phenotype. We have determined that these cells have increased TbAAC expression and are now very sensitive to CATR. This suggests that these two proteins can compensate for each other in rich media, so we are characterizing the depletion of TbAAC in TbSCoAS DKO cell lines. Furthermore, it will be interesting to determine if the loss of fitness measured in culture for both DKO cell lines will be amplified in a mouse model.

Posttranscriptional regulation of mitochondrial ATP synthase inhibitor TbIF1 during the life cycle of *Trypanosoma brucei* - Poster 142 : A11247
Presenter: **Ondřej Gahura**, , *University of South Bohemia*

Authors:**O Gahura**[2]; B Panicucci[2]; A Zíková[1,2]
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The mitochondrial ATP synthase is a bi-directional nanomotor, which in majority of eukaryotes works in its forward mode to synthesize ATP. The activity of this enzyme is negatively regulated by highly conserved inhibitory protein IF1. In *Trypanosoma brucei*, the mitochondrial ATP synthase switches its activity from the ATP production to ATP hydrolysis as the parasite differentiates from the insect procyclic stage (PF) to the mammalian bloodstream stage (BF). We identified *T. brucei* homolog of IF1 (TbIF1) and showed that on the protein level it is present only in PF cells and its expression is transiently upregulated during the differentiation from PF to metacyclic stage, the BF precursor. Upregulation of IF1 and ATP synthase inhibition is also observed in cancer cells, which undergo a metabolic switch from oxidative phosphorylation to glycolysis. Thus, TbIF1 can be involved in similar metabolic rewiring during the parasite's differentiation. To comprehensively understand PF to BF metabolic transition, we study mechanisms underlying TbIF1 differential expression during the *T. brucei* life cycle. We showed that both steady state level and half-life of TbIF1 mRNA are dramatically increased in PF compared to BF, which in the context of absence of transcriptional regulation in *T. brucei*, indicates regulation by mRNA stability. Inhibition of translation results in TbIF1 mRNA accumulation in BF, which suggest existence of short-lived destabilizing RNA-binding protein. Using CAT-based reporters, we dissect TbIF1 UTRs to identify sequential and/or structural elements directing TbIF1 mRNA stability. The factors, which interact with the identified motifs, are being purified using Csy4, an inducible RNA hairpin-binding nuclease. Together, the data reveal mRNA-controlled mechanisms contributing to metabolic progression during the development of the infectious form of this parasite.

The role of inhibitory factor IF1 during the life cycle differentiation of the human pathogen *T. brucei* - Poster 143 : A11248
Presenter: **Eva Doleželová**, , *University of South Bohemia*

Authors:**E Doleželová**[2]; B Panicucci[2]; A Zíková[1,2];
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Trypanosoma brucei is a lethal human parasite with a complex life cycle that alternates between a mammalian host and the blood-feeding insect vector, a tsetse fly. Exposed to vastly different nutritional environments throughout their distinct life stages, they must adapt their energy metabolism accordingly. With access to abundant glucose, the intercellular bloodstream stage is able to produce sufficient ATP by aerobic glycolysis, while the insect stage depends on amino acid catabolism and mitochondrial oxidative phosphorylation for efficient ATP synthesis. Therefore, during the complex development of the parasite in the tsetse fly, the single mitochondrion undergoes dramatic structural and metabolic changes. Until recently, it was challenging to work with the various intermediate life stages, but now each of the insect vector developmental cell types can be created in vitro by simply overexpressing a single RNA binding protein. Our preliminary data with this cell line suggests that the molecular mechanisms responsible for the metabolic rewiring may be similar to what is described in cancer cells. We observe increased expression of TbIF1, the *T. brucei* inhibitory factor of the FoF1-ATPase, and the subsequent increase in ROS production that

potentially signals a switch to aerobic glycolysis. The putative role of TbIF1 in mitochondrial remodeling during *T. brucei* differentiation will be discussed.

Differential binding of trypanosome mitochondrial transcripts by MRB8170 and MRB4160 regulates distinct RNA processing fates- Poster 145 : A11250
Presenter: **Sameer Dixit**, *, Institute of Parasitology, Biology Center, Czech Academy of Sciences*

Authors:**S Dixit**[4,6]; M Müller-McNicoll[5]; V David[6]; K Zarnack[1]; J Ule[3]; H Hashimi[4,6]; J Lukeš[2,4,6]
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A dozen mRNAs are edited by multiple insertions and/or deletions of uridine residues in the mitochondrion of *Trypanosoma brucei*. Several protein complexes have been implicated to perform this type of RNA editing, including the mitochondrial RNA binding complex 1 (MRB1). Two protein components of MRB1 loosely associated with its core are MRB8170 and MRB4160, which represent novel RNA binding proteins. However, information related to both the protein's role in RNA editing and its effect on target mRNAs is limited. Quantitative data obtained in this study revealed preferential binding of these proteins to mitochondrial mRNAs, positively correlating with their editing extent. Integrating in vivo and in vitro data, we propose a model in which differential binding of MRB8170 and/or MRB4160 onto pre-edited mRNA marks initiation of editing, which helps to recruit other components of MRB1, ensuring efficient editing.

African trypanosome genes specifically required for fitness in vivo - Poster 146
Presenter**: James Shrimpton**

Authors:**James Shrimpton**[1], Lucy Glover[1,3], Nicola Baker[1], Axel Martinelli[2], Matthew Berriman[2] and David Horn[1]
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Analyses of African trypanosome biology are often carried out in HMI culture medium which is rich in nutrients and may not necessarily be representative of the environments encountered in vivo. We reasoned that parasites in vivo are likely to display increased dependence upon some nutritional pathways as well as defenses against oxidative stress and innate immune attack. In order to address this we ran a genome-scale RNAi fitness-profiling screen in rats. Among genes that displayed a specific loss-of-fitness in vivo were two genes previously linked to phosphatidylinositol metabolism, providing validation for our screen. Other hits included genes linked to Folate metabolism and DNA repair. We have begun validation of the hits from the screen and are developing systems for more detailed follow-up. One particularly promising approach is the growth of T. brucei in "100% calf-serum", which likely effectively mimics aspects of the in vivo environment.

Index

A 3D electron microscopy study of interactions between the *Leishmania* amastigote flagellum and the parasitophorous vacuole - Poster 65 63
A cAMP response protein necessary for *Trypanosoma brucei* social motility- Poster 24 45
A chemical tool to characterise essential catabolic functions within the trypanosomatid lysosome.- Poster 30 48
A genetic basis for asymptomatic human African trypanosomiasis - Poster 125 90
A genome editing toolkit for *Leishmania*- Poster 52 57
A leucine aminopeptidase is involved in kinetoplast DNA segregation in *Trypanosoma brucei* 25
A model for vaccine testing against *Trypanosoma cruzi* infection that exploits highly sensitive bioluminescence imaging- Poster 106 81
A model to study the effect of *Leishmania* parasite virulence on interaction with host cells- Poster 62 61
A molecular survey of cAMP response proteins (CARPs) reveals a novel, non-linear signaling network- Poster 67 64
A Network of Cap-Binding Proteins in The Service of Stress Physiology in Trypanosomatids 15
A receptor's tale: structural and functional diversification of the trypanosome haptoglobin-haemoglobin receptor 17
Achcar 61
Activation of bicyclic nitro-drugs by a novel nitroreductase (NTR2) in *Leishmania*- Poster 117 87
Adaptation of *Leishmania* parasite shape to different hosts 32
Akiyoshi 28
Al Kufi 42
Al-Hajri 61
Alzahrani 54
Amegatcher 35
Amino acid transporters of the AAT7 family facilitate uptake of neutral amino acids- Poster 57 59
An APOL1 variant that kills all *T. brucei* sub-species including T.b. gambiense- Poster 63 62
An improved *Leishmania donovani* reference genome after PacBio SMRT sequencing: the road to Whole Genome Sequence of the parasite- Poster 14 41
An *in vivo*, focused RNAi screen identifies *Trypanosoma brucei* stress response protein kinases required for survival in mammals- Poster 113 85
An outer membrane protein complex mediates mitochondrial genome inheritance 33
Analysis of heparan sulphate and its role in the invasion of host macrophages by *Leishmania* parasites - Poster 87 73
Antigenic variation in *Trypanosoma brucei*: dissecting VEX1, a VSG allelic exclusion regulator- Poster 59 60
Are vesicles from the *Leishmania* flagellum a source of virulence factors?- Poster 45 55
ARF regulating proteins as novel drug targets against kinetoplastids- Poster 38 52
Aristodemou 45
Aroko 89
Asexual maintenance of genetic diversity in the protozoan pathogen *Leishmania donovani*- 12
Avila 60
Bachmaier 64
Bajak 49
Barratt 75
Bartossek 19
Basal body connections in *Trypanosoma brucei* 33

Beneke ... 23
Benz ... 54
Benznidazole causes genome-wide mutagenesis in *Trypanosoma cruzi* and can confer multi-drug resistance- Poster 112 ... 84
Bezerra ... 63
Blocking VSG synthesis in bloodstream form *Trypanosoma brucei* triggers an ER stress response- Poster 50 ... 56
Bogacz ... 46
Boshart ... 79
Briggs ... 78
Budzak ... 59
Campbell ... 88
Campos ... 84
Can folate/pteridine transporters transport pyrimidine in protozoa?- Poster 44 ... 54
Capewell ... 90
Cargo selection and VSG transport in the early secretory pathway of *Trypanosoma brucei* - Poster 71 ... 66
CARP3 is a plasma-membrane and adenylate-cyclase associated regulator of cyclic AMP signalling in *Trypanosoma brucei* ... 29
Catta-Preta ... 33
Cep164 genes in the transition zone of the mature basal body- Poster 40 ... 53
Chakraborty ... 64
Chandrathas ... 52
Characterisation of the hook complex protein TbSmee1 (Tb927.10.8820)- Poster 29 ... 48
Characterizing genes involved in function and biogenesis of mitochondrial DNA in *Trypanosoma brucei*- Poster 8 ... 38
Chaudhuri ... 91
Chemical V-ATPase inhibition fully compensates for ablation of mitochondrial RNA editing ligase 1 in *Trypanosoma brucei*- Poster 9 ... 38
Clayton ... 71
Clucas ... 62
Codon usage links translation and mRNA decay in *Trypanosoma brucei* ... 28
Comparative genomics of trypanosomatids: identification of novel virulence factors and new insights into evolution of *Leishmania* ... 12
Cooper ... 36, 90
Correlative RNAseq and proteomics analyses of hamster-isolated *L. donovani* amastigotes and derived promastigotes reveal stage-specific regulation of protein abundance independent of transcript levels. - Poster 89 ... 74
Cren ... 40
Crouch ... 44
Cultured bloodstream *Trypanosoma brucei* adapt to life without their mitochondrial translation release factor ... 22
Cytoskeletal Morphogenesis in *Trypanosoma brucei* - Poster 103 ... 80
Da Silva ... 58
Data mining using TriTrypDB resources- Poster 20 ... 44
de Graffenried ... 80
De Koning ... 29
De la Torre Olvera ... 39
de Macêdo ... 96

De Pablos Torro 95
De Rycker 71
Dean 96
Deciphering the role of *Leishmania* Casein kinase 1 in host subversion- Poster 51 57
Decoding antitrypanosomal drug action and resistance 15
Developing a genetic modification system of marine protist *Diplonema papillatum* - Poster 139 98
Development of a computational model for visceral *Leishmania*sis drug development using transcriptomic and immunological data- Poster 114 85
Dewar 14
Different roads to Rome: Targeting of *T. brucei* Arl13b to the flagellum- Poster 116 87
Differential binding of trypanosome mitochondrial transcripts by MRB8170 and MRB4160 regulates distinct RNA processing fates- Poster 145 101
Discovery And Lead-Optimisation Of A Promising New Anti*Leishmania*l Compound Series Within An Academic-Industry Partnership.- Poster 83 71
Discovery of new *Leishmania* motility mutants in a CRISPR-Cas9 knockout screen of the flagellar proteome 23
Dissecting the Interstrand Crosslink Repair Pathways of *T. brucei*- Poster 104 81
Dissecting the kinome of *T. brucei*: RIT-seq of cell cycle sorted *T. brucei* identifies kinases involved in the regulation of nuclear DNA replication- Poster 97 77
Dixit 101
Does RNA incorporation into the DNA genome of *Trypanosoma brucei* drive DNA replication and antigenic variation?- Poster 99 78
Does TbPIP39 target all, or only the newly synthesised glycosomes upon differentiation? - Poster 110 83
Doleželová 100
Domagalska 13
Drugs and trafficking in trypanosomes 20
Dujardin 41, 98
Duncan 37
Dynamic regulation of the *Trypanosoma brucei* transferrin receptor is mediated via the 3'UTR- Poster 34 50
Ebenezer 70
Effects of host-derived chemokines on the motility and viability of *Trypanosoma brucei* brucei- Poster 69 65
Eisenhuth 58
Elutriation of PCF and BSF *Trypanosoma brucei* allows separation of cell cycle stages- Poster 43 54
Enzymatic activation of the aminomethylphenoxy benzoxaboroles through hosts and pathogens- Poster 86 72
Epidemiological and Characterization studies on vector (Tabanidae) flies and their control measures for Trypnosomiasis (surra) in camels.- Poster 75 68
Erben 47
Evidence for a cullin RING ligase ubiquitylation switch modulating the surface proteome in African trypanosomes- Poster 28 47
Evidence of asynchronous replication of *Trypanosoma cruzi* amastigotes within single infected cells *in vivo* - Poster 46 55
Evidence towards a role for the GTPase Ran in mRNA export in trypanosomes.- Poster 122 89
Evolutionary diversification of the trypanosome haptoglobin-haemoglobin receptor from an ancestral haemoglobin receptor- Poster 13 40
Exploitation of receptor-mediated ligand uptake for the targeted delivery of antibody- toxin conjugates into *Trypanosoma brucei* 23
Exploring the bioenergetics of the bloodstream *T. brucei* mitochondrion- Poster 141 99

Farr 61
Fernandes 49
Fernandez-Cortes 85
Ferroptosis in African trypanosomes- Poster 26 46
Field 20, 72
Flagellum Attachment Zone and the control of *Leishmania* flagellar pocket shape - Poster 85 72
FoF1-ATP synthase subunit α – A tale of two fragments- Poster 5 36
Forrester 85
Forward Genetics of Suramin Resistance in African Trypanosomes- Poster 22 45
Francisco 93
From solitary swimmers to swarms and back – trypanosomes on their journey through the tsetse fly 30
FtsH protease of *Trypanosoma brucei*- Poster 48 56
Functional analysis of TOEFAZ1 uncovers protein domains essential for cytokinesis in *Trypanosoma brucei*- Poster 23 45
Functional annotation of Euglena gracilis mitochondrial proteome. - Poster 137 97
Functional characterisation of six essential protein kinases in *Trypanosoma brucei* reveals 3 that are required for cytokinesis- Poster 11 39
Gahura 100
Garziz 73
Gene expression regulatory networks in *Trypanosoma brucei*: the RBPome awakens- Poster 27 47
Generation of a *Trypanosoma brucei* whole-genome gain-of-function library- Poster 73 67
Genome analyses reveal unexpected levels of diversity and hybridization in natural populations of Trypanosoma congolense- Poster 77 69
Genome and transcriptome dynamics throughout the life cycle of *Leishmania donovani* 13
Genomic adaptation of *Leishmania* mexicana in serial passage conditions- Poster 119 88
Germline Transmission of Targeted Baboon Apolipoprotein L-I in Mice Protects Against African Trypanosomes 18
Glogger 92
Glover 21
Gluenz 57
Goos 65
GPI-dependent trafficking of the Transferrin Receptor (TfR) in African trypanosomes 31
Grano 37
Grewal 82
GT-rich promoters drive RNA pol II transcription and deposition of H2A.Z in African trypanosomes 34
Gwira 52
Haindrich 59
Hamilton 41
Hernandez 51
Hide 69
Higgins 17
Horn 15
Horvath 56
Hovel-Miner 67
Hutchinson 94
Identification and functional characterization of ornithine transporters in *Trypanosoma brucei* and their involvement in drug action- Poster 135 96

Identifying and Characterising developmentally regulated RNA-binding proteins (RBPs) in *Leishmania* mexicana.- Poster 133 ... 95
Identifying highly divergent glycosyltransferases in the African trypanosome- Poster 6 ... 37
Importance of trypanosome motility in the early infection- Poster 12 ... 40
Insight into a C11 peptidase essential for *Trypanosoma brucei* kinetoplast maintenance and viability of *Leishmania* mexicana - Poster 107 ... 82
Intracellular trafficking of GFP expressing *Leishmania* aethiopica in terminally differentiated THP-1 cells.- Poster 129 ... 92
Investigating the attachment plaque in Trypanosoma congolense epimatigotes- Poster 61 ... 61
Investigations into a Novel Alternative Oxidase in Trypanosomatids- Poster 105 ... 81
Involvement of an RNA binding protein containing Alba domain in the stage-specific regulation of beta-amastin expression in *Trypanosoma cruzi* - Poster 2 ... 35
Iron Uptake in *Trypanosoma brucei* ... 16
Janzen ... 78
Jeacock ... 83
Kafkova ... 67
Kaltenbrunner ... 99
Kangethe ... 95
Käser ... 33
Kaur ... 98
Kerry ... 88
Kinetic of detection of *Leishmania donovani* DNA in the brain during experimental infection in mice. - Poster 7 ... 37
Kramer ... 16
Kruzel ... 66
Kumar ... 81
L. donovani from the Indian Subcontinent is pre-adapted for a rapid development of antimonial resistance, driven by aneuploidy- Poster 138 ... 98
Lane-Serff ... 40
Lecoeur ... 75
Leishmania amazonensis amastigotes reprogram the host macrophage transcriptome to inhibit NF-kB and inflammasome activation - Poster 90 ... 75
Leishmania Strain Panel Development for Phenotypic Screening- Poster 37 ... 51
Lifecycle-dependent localization of the signature sequence free potassium channel TbIRK in *T. brucei*- Poster 64 ... 62
Loss of ATR in the Kinetoplastid parasite *Trypanosoma brucei* de-regulates repression of the silent VSG archive- Poster 95 ... 77
MacGregor ... 23
Maciej-Hulme ... 73
Maclean ... 51
Macleod, E ... 80
Macleod, O ... 22
MacLeod,A ... 30
Magnetic Hyperthermia as a Novel Approach for Treatment of Cutaneous *Leishmania*sis- Poster 42 ... 53
Makin ... 55
Mann ... 82
Mapping metabolism in the parasite *Trypanosoma brucei* using U-13C-labelled amino acids and LC-MS.- Poster 60 ... 60

Marques........60
Martel........57
Matthews........26
Menzies........81
Messenger........14, 68
Metabolic signals and glycosomal isocitrate dehydrogenase (IDHg) control procyclic to metacyclic development of *Trypanosoma brucei* fly stages- Poster 100........79
Metabolomics-driven development of a minimal essential medium for the animal trypanosomiases- Poster 132........94
Miles........29
Miller........46
'Mind the gap': the relevance of genetic exchange and comparative genomics to combatting Chagas disease and visceral *Leishmania*sis.........29
Miskinyte........38
Mitochondrial organelle biogenesis during the cell cycle in *T. brucei*........24
Molecular analysis of Tsetse from Hurungwe, Zimbabwe- Poster 102........80
Molecular Characterization of Lifetime Infections with Trypanosomes in Individual Cattle in Ghana - Poster 35........50
Molecular characterization of the complement factor H receptor in the bloodstream and procyclic forms of *Trypanosoma brucei*........22
Molecular detection of *Leishmania* RNA virus 2 in *Leishmania* infantum- Poster 88........73
Morriswood........21
mRNAs encoding ribosomal proteins might be regulated by a novel RNA-binding protein- Poster 32........49
Mugo........19, 88
Multiple Mechanisms of kDNA Maintenance by Polymerase IC in *Trypanosoma brucei*.- Poster 25........46
Mutational analysis of the phosphorylation sites of the *Leishmania* mexicana kinesin homologue LmxKin29- Poster 16........42
Nascimento........28
Natural compounds: A potential source of novel chemical entities to kill *Leishmania*?- Poster 33........49
Nenarokova........97
N-glycosylation enables high lateral mobility of GPI-anchored proteins at a molecular crowding threshold- Poster 128........92
Nitroheterocyclic drugs cure experimental *Trypanosoma cruzi* infections more effectively in the chronic stage than in the acute stage- Poster 130........93
Nuclear factors involved in developmental differentiation of *Trypanosoma brucei* - Poster 53........58
Nucleotide excision repair in trypanosomatids – streamlining and neofunctionalisation of the machinery due to multigenic transcription- Poster 55........58
Oates........53
Obado........89
Ochsenreiter........24
Ofori........50
Ooi........56
Opposing associations of APOL1 genetic variants with African trypanosomiasis resistance in East and West Africa- Poster 123........90
Paape........77
Pan-American migration promotes the spread of pathogenic *Trypanosoma cruzi* hybrid strains........14
Parasite Secretomics: Investigating the secretome of *Leishmania* mexicana to elucidate mechanisms of intracellular survival- Poster 15........41

Paris 25
Paul 79
Peña -Diaz....25
Perez 66
Perez-Diaz....35
Pérez-Victoria....43
Perry 44
Pescher 74
Phenotypic and functional genetic analysis of virulence attenuation in *Leishmania donovani*- Poster 10 39
Phylogenetic Analyses Including a Novel Australian Trypanosomatid Supports the Supercontinent Hypothesis of *Leishmania* Origins- Poster 92....75
Piel....39
Population genomics of New World *Leishmania* parasites along the Andes reveal species diversity and patterns of allopatric speciation 31
Posttranscriptional regulation of mitochondrial ATP synthase inhibitor TbIF1 during the life cycle of *Trypanosoma brucei*- Poster 142....100
Price 43
PRMT or not a PRMT: The major protein arginine methyltransferase in *T. brucei* functions as an enzyme-prozyme complex.- Poster 74 67
Protein kinases that regulate *Leishmania* differentiation....33
Qamar....68
Quantitative Proteomic responses of macrophages to *Leishmania* infection using pulse-chase SILAC approach.- Poster 93 76
Quantitative proteomics uncovers novel factors involved in developmental differentiation of *Trypanosoma brucei* 78
Queuosine: The role of an essential tRNA modification in parasitic protist *Trypanosoma brucei*- 25
Ranatunga....93
Raper....18
Reciprocal SHAM-hypersensitivity in aquaglyceroporin-null and melarsoprol-resistant trypanosomes- Poster 108 83
Regulating the regulators: LmjPRMT7 in *Leishmania* parasite virulence and trans-regulator methylation 20
Regulation of *Trypanosoma brucei* Acetyl-CoA Carboxylase by Environmental Lipids- Poster 101 79
Reis 17
Remote control of reaction-diffusion processes with chemical microrobots 11
Replication protein A DNA repair foci persist through the cell-cycle and reflect a 'divide and repair later' strategy in trypanosomes....21
Sajko....42
Schaffner 38
Schichler....48
Schmidt....62
Schneider 27
Schuster 30
Screening for antiparasitic leads from a library of natural products from temperate zone plants- Poster 18 .43
Selecting for simultaneous activation of two VSG expression sites- Poster 56 59
Serum Biochemical Parameters and Cytokine Profiles Associated with Animal African Trypanosomiasis in Naturally Infected Cattle in Ghana- Poster 39 52
Shapira 15

SI-2, a quinone derivative with potential sirtuin inhibitor activity decreases the replication of *T. brucei* procyclic forms and induces apoptosis-like cell death mechanism- Poster 58 59
Sinclair-Davis 45
Spaeth 12
Stage-specific reporter gene expression in *Trypanosoma cruzi* - Poster 31 48
Steketee 94
Štěpánek 11
Stortz 77
Structural and functional studies of *Trypanosoma brucei* TbMORN1 protein- Poster 17 42
Subota 91
Subrtova 36
Sunter 27, 72
Switch or become stumpy!- Poster 115 86
Szoor 83
Taleva 99
Taylor 16, 55
TbPH1: a trypanosomatid-specific pleckstrin homology domain containing kinesin-like protein- Poster 140 99
TbSmee1 regulates hook complex biogenesis in procyclic *Trypanosoma brucei*- Poster 21 44
The African trypanosome *Trypanosoma brucei* is susceptible to therapeutic concentrations of RNA polymerase I inhibitors- Poster 118 87
The Application of Irradiation Technology as a Tool for Vaccine Development in Trypanosomosis- Poster 134 95
The complete structures of VSGs MITat1.1 and ILTat1.24: combining density and mobility through flexibility 19
The early intradermal life of *Trypanosoma brucei* after natural transmission by the tsetse fly.- Poster 80 70
The effect of genotypic diversity on the progression of *T. cruzi* infections- Poster 72 66
The flagella connector of *Trypanosoma brucei* is a kinesin-powered junction distinct from the axonemal capping structure 26
The genome of Euglena gracilis: Annotation, function and expression- Poster 81 70
The mitochondrial genome and gRNA-ome of pleomorphic *Trypanosoma brucei*- Poster 3 36
The mRNAs that are increased after heat shock of procyclic-form *Trypanosoma brucei* include many that are induced during differentiation to mammalian-infective forms- Poster 82 71
The non-canonical mitochondrial inner membrane protein translocase of trypanosomatids contains two essential rhomboid-like proteins 27
The proteome of trypanosome nuclear periphery granules- Poster 70 65
The real flux capacitor? A putative molecular valve in trypanosomes 21
The RNA binding protein-RBP10 defines bloodstream form trypanosome identity- Poster 120 88
The role of inhibitory factor IF1 during the life cycle differentiation of the human pathogen *T. brucei*- Poster 143 100
The significance of respiratory complex I and mitochondrial retrograde signalling in the transition of *Trypanosoma brucei* from bloodstream to insect form- Poster 1 35
The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes 30
The Small Tim Homologues in the Mitochondrion of *Trypanosoma brucei* are Critical for Redox Homeostasis and Stability of TbTim17- Poster 126 91
The ZC3H39/40 RNA-binding complex and the control of electron transport chain expression in African trypanosomes 24
Tiengwe 31

Towards improving early diagnosis and surveillance of congenital Chagas disease in an endemic setting: lessons from the Bolivian Chaco - Poster 76 68
Towards the identification of the stumpy induction factor (SIF)- Poster 127 91
Translesion DNA Polymerases and genome maintenance in *Trypanosoma brucei* - Poster 94 76
Transspecies surface coats of African trypanosomes: What can they teach us about VSG functionality? - Poster 121 89
Trenaman 24
Trypanosoma brucei glycosomal isocitrate dehydrogenase reveals dual coenzyme specificity towards $NADP^+$ and NAD^+- Poster 111 84
Trypanosoma cruzi RNA polymerase I: The trypanosomatid specific subunit TcRPA31 bears a functional nuclear localization signal.- Poster 36 51
Trypanosomatid parasites rescue heme from endocytosed hemoglobin through lysosomal HRG transporters- Poster 19 43
Trypanosome flagellum transition zone proteome reveals compartmentalisation and differential dynamics of ciliopathy complexes- Poster 136 96
Trypanosomes lacking the expression of ZC3H30 are hypersensitive to stress- Poster 68 64
TrypTag: Genome-wide protein localisation in the trypanosome 27
Two different cap binding proteins (eIF4Es) are associated with distinct mRNA populations in *Trypanosoma brucei*.- Poster 66 63
Tyc 53
Understanding the signalling pathway controlling African trypanosome quorum sensing and life-cycle differentiation 26
Understanding unconventional kinetoplastid kinetochores 28
Urbaniak 50
Valli 63
Van Den Abbeele 70
Van den Broeck 31, 69
Varga 26
Variation in Toll-like Receptor 2 and 4 Genes in the European Badger (Meles meles) in relation to infection with Trypanosoma (Megatrypanum) pestanai.- Poster 79 69
Vaughan 33
VSG mRNA promotes S-phase in the bloodstream African trypanosome- Poster 131 94
Walrad 20
Wang 84
Wedel 34
What do kinetoplastids need a kinetoplast for? Life cycle progression of *Trypanosoma brucei* in the presence and absence of mitochondrial DNA 14
Wheeler 32
Where is the trypanosome decapping enzyme?- 16
Wiedemar 45
Wyllie 87
Your genes are what you eat: The metabolic basis of genome composition and codon bias 18
Yurchenko 12
Zaki 76
Zhang 87
Zíková 22
Zimmermann 86
Zoltner 47

Zurita 76

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