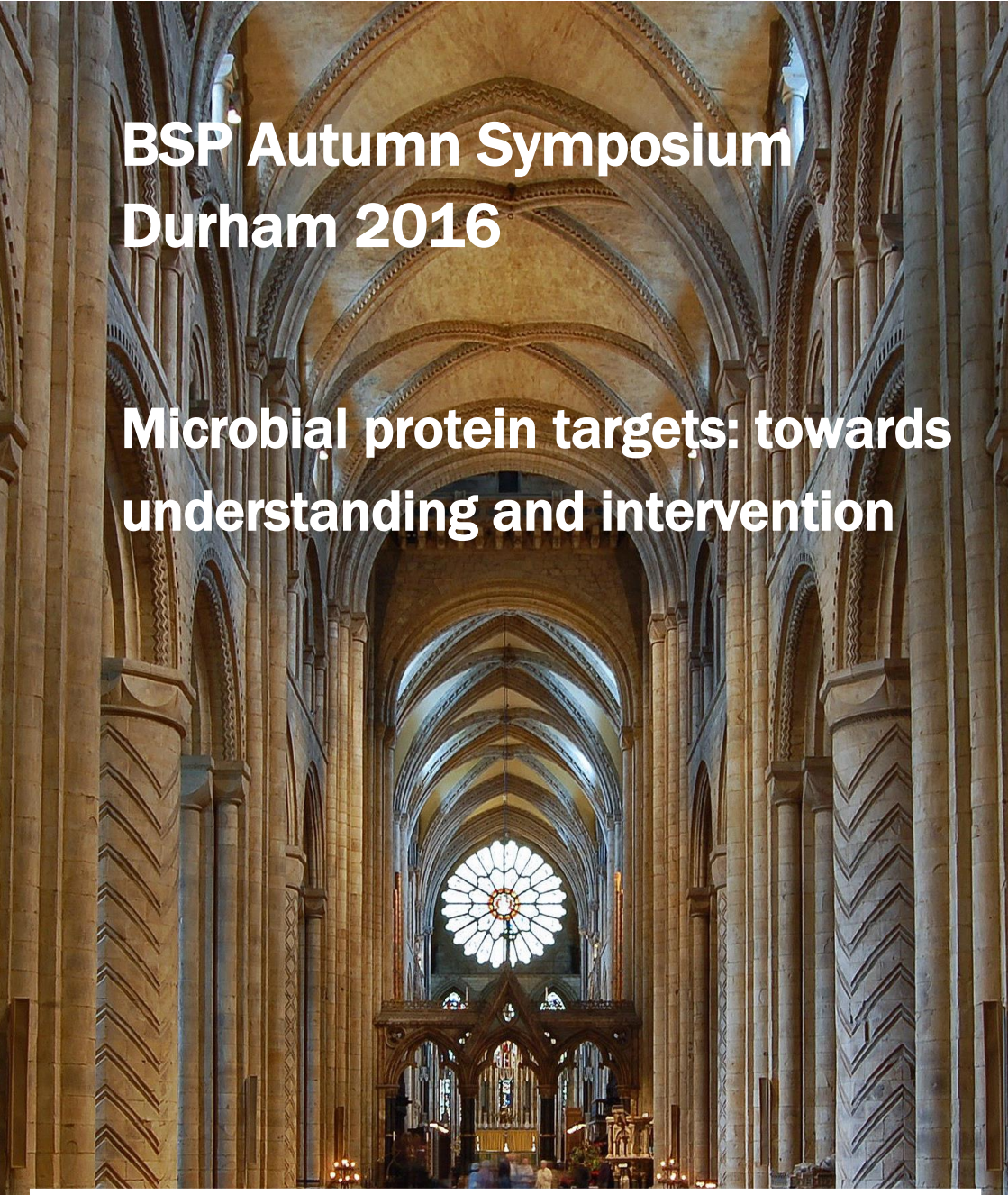
BSP Autumn Symposium Durham 2016

Microbial protein targets: towards understanding and intervention



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Event Schedule

BSP Autumn Symposium 2016, Durham - Microbial protein targets: towards understanding and intervention

14-Sep-2016 to 16-Sep-2016
Durham University, Durham, United Kingdom

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Omic approaches to target validation (Ken Wade)

Chairs - Paul Denny

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16:00 (40 mins**) Identification *Mycobacterium tuberculosis* cell wall hits and targets through modern omics based methods** (Gurdyal Besra)

16:50 (220 mins) **Reception and Poster session** (Kingsley & Derman)

17:00 (60 mins) **Outreach – Public understanding of Science** -Schools' event, with Prof Michael Barrett and Dr Richard Bellamy

Day 2

Genetic approaches to target validation (Ken Wade)

Chairs - Prof. Paul Horrocks

09:00 (40 mins) **Targeting pathways of host/parasite interaction for anti-leishmanial drug discovery** (Gerald Spaeth)

09:40 (40 mins) **Genome wide functional screen of yin and yang (reversible phosphorylation) and malaria parasite biology** (Rita Tewari)

Autumn Symposium 1 (selected talks) (Ken Wade)

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10:35 (15 mins) **Analysing the Trypanosoma brucei Flap Endonuclease** (Sarah Oates)

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11:50 (15 mins) **Targeting histone modifying enzymes in *Leishmania*: a new venue for chemotherapy?** (Lamotte Suzanne)

12:05 (15 mins) **Confirming the PanDZ complex as a cellular target for antimetabolites** (Michael Webb)

12:20 (100 mins) **Lunch Break and Poster session (**Kingsley & Derman)

Drug target structure and drug discovery (Ken Wade)

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Chairs - Ehmke Pohl

14:45 (15 mins) **Biochemical characterisation of heparan sulphate and its role in *Leishmania* infection of host macrophages** (Marissa Maciej-Hulme)

15:00 (15 mins) **Discovery of novel antimalarial agents by structure-based design and multi-parameter lead optimisation** (Martin McPhillie)

15:15 (15 mins) **Dissection and rational engineering of the biosynthetic pathway to enacyloxin, a promising anti-Gram-negative antibiotic** (Joleen Masschelein)

15:30 (15 mins) **Mediated polyvalent and cooperative interactions of drugs define mechanical force on microbial susceptibility** (Joseph Ndieyira)

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Fragment-based approaches to drug discovery (Ken Wade)

Chairs - Ehmke Pohl

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17:00 (30 mins**) BSP AGM** (Ken Wade) *note this is important for members to attend **as important changes are being voted on.***

18:30 **Conference dinner** (Durham Castle) *note: There is a drinks reception starting at 18:30 followed by dinner at 19:30-20:00.*

Day 3

Chemical approaches to target validation (Ken Wade)

Chairs - Patrick Steel

09:00 (40 mins) **Chemical proteomic target discovery and validation in infectious disease** (Ed Tate)

09:40 (40 mins) **Tres Cantos Open Lab: Present and future of this collaborative model for diseases of the developing world drug discovery** (Maria Marco-Martin)

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11:20 (15 mins) **Treatment of cutaneous leishmaniasis using sustained release delivery systems loaded with a novel chalcone** (Bartira Rossi- Bergmann)

11:35 (15 mins) **Use of photo-affinity labeling to determine the trypanosomatid protein target of analogues of the natural product chamuvarinin** (Stefanie Menzies)

Anti-parasitic drug discovery and the DDU (Ken Wade)

Chairs - Patrick Steel

12:00 (40 mins) **Translating the trypanosome surface** (Mike Ferguson)

12:40 (5 mins) **Closing Remarks**

12:45 (30 mins) Lunch (Kingsley & Derman)

Orals in time order

***Leishmania* and other intracellular pathogens: drug distribution, PK PD and selectivity-** A10537
Presenter: **Prof Simon Croft** , *London School of Hygiene and Tropical Medicine*

New drugs and treatments for intracellular pathogens, like *Leishmania*, have proved to be some of the most difficult to develop. The focus of much research has been on the identification of potent and selective compounds that inhibit target enzymes (or other essential molecules) or are cidal to the causative pathogen. Although this remains an essential part of the drug R & D pathway, over the past decade more emphasis (new approaches, new methodologies) has been given to the challenges to ensure that anti-infective drugs (administered by different routes) reach the target pathogen, within the host cell, with appropriate distribution to the infected tissue/organ. The presentation and review will focus on how these challenges are being met in relation to *Leishmania* and leishmaniasis.

Metabolomics in understanding anti-protozoal drug mode of action and resistance- A10535
Presenter: **Professor Mike Barrett**, *University of Glasgow*

Many drugs exert their activity by interfering with cellular metabolism. Moreover, alterations to metabolism can underpin drug resistance. Untargeted metabolomics enables analysis of metabolism in any biological system and is capable of identifying areas of metabolism which are perturbed in an unbiased manner. We have applied metabolomics to understand more about the modes of action of numerous antimicrobial agents. In trypanosomes, for example, the inhibition of ornithine decarboxylase by eflornithine was readily visible and resistance to this drug was shown to relate to loss of drug uptake due to deletion of the gene encoding a transporter responsible for uptake. In *Leishmania*, combining metabolomics analysis with genome sequencing has identified a gene responsible for resistance to amphotericin B. We have also shown how a number of other drugs exert their activity against protozoa and bacteria. The untargeted metabolomics platform has also been shown to be capable of identifying biomarkers of infection and the stage of disease in African trypanosomiasis.

Identification *Mycobacterium tuberculosis* cell wall hits and targets through modern omics based methods- A10536
Presenter: **Professor Gurdyal Besra**, *Bardrick Professor of Microbial Physiology & Chemistry, University of Birmingham*

Tuberculosis (TB) continues to cause more deaths and human suffering than any other infectious disease. In addition to peptidoglycan (PG), arabinogalactan (AG) and lipoarabinomannan (LAM) are two other carbohydrate-based macromolecules which are essential components of the

Mycobacterium tuberculosis cell wall. Fully matured AG is esterified at its non-reducing terminus with long chain mycolic acids, thus acting as a core molecular scaffold connecting PG to the outer mycolate layer. This huge cell wall complex ultimately provides the TB-bacilli with structural support, rigidity and a primary defensive barrier against toxic insult. By adopting a range of biochemical and molecular genetic approaches, we have made significant advances in delineating the genetic and molecular basis of AG and LAM biosynthesis. Mycobacterial cell wall assembly is currently back in vogue. This is, perhaps, partly due to recent findings that describes benzothiazinones (BTZ) as a new class of molecules targeting the early stages of AG biosynthesis. This, in addition to the discovery that a potent combination of meropenem and clavulanate exhibits inhibitory activity against a range of M. tuberculosis and Multi-Drug Resistant tuberculosis (MDR-TB) strains, provides corroborating evidence supporting the notion that targeting critical steps of Mycobacterial cell wall assembly is a valid and worthwhile approach to the discovery of novel drug targets. This talk aims to cover various aspects of Mycobacterial PG, AG and LAM biosynthesis, specifically focusing on our laboratories multi-disciplinary experimental approaches, contextualized with a summary our recent findings relating to the overall assembly of the Mycobacterial cell wall and how we are looking to develop an anti-TB drug discovery program at Birmingham.

Targeting pathways of host/parasite interaction for anti-leishmanial drug discovery- A10548
Presenter: **Prof Gerald Spaeth** , *Institut Pasteur*

The eukaryotic biology of *Leishmania* differs considerably from its hosts thus providing ample opportunity for parasite-specific chemotherapeutical intervention. We have previously identified *Leishmania*-specific mechanisms of stress signaling, validated various stress kinases as novel drug targets, and discovered a series of hit compounds through target-based and phenotypic screening approaches using kinase-biased inhibitor libraries. However, all strategies that directly target parasites for chemotherapy are prone to select rapidly for drug resistance through largely unknown mechanisms of *Leishmania* evolutionary adaptation. Applying HTseq analysis on *L. donovani* clinical isolates and lab strains we uncovered chromosomal amplification coupled with haplotype selection as a main mechanisms for parasite adaptation that needs to be considered in future drug discovery efforts. To limit the emergence of drug resistance I will discuss novel strategies developed in our consortia to kill intracellular parasites in an indirect fashion by targeting pathways of host/parasite interaction that are essential for *Leishmania* survival.

Genome wide functional screen of yin and yang (reversible phosphorylation) and malaria parasite biology- A10786
Presenter: **Prof Rita Tewari**, *University of Nottingham*

Reversible protein phosphorylation is catalysed by protein kinases (PKs) and protein phosphatases (PPs) controlling many signalling and cellular pathways in most eukaryotes. In the malaria parasite to unravel these signalling molecules are of great interest, for both better understanding of signalling pathways during complex parasite life cycle and identification of novel drug targets for

intervention at different stages of life cycle. We have performed genome wide functional analysis of both the kinase and phosphatase gene family in *Plasmodium* to unravel their role in parasite developmental pathway. These studies have revealed the functional clusters of kinases/phosphatases that are unique to *Plasmodium*. In addition we have identified molecules that are required for sexual development and sporogony in mosquito vector which can inform targets of intervention for parasite transmission. Overall, our two major studies identifies how kinase and phosphatases regulate parasite development and differentiation, provides a systematic functional analysis for all PKs/PPs in *Plasmodium*, and can inform identification of novel drug targets in malaria.

A chemical tool to characterise essential catabolic functions within the trypanosomatid lysosome.- A10812
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.

Analysing the *Trypanosoma brucei* Flap Endonuclease- A10795
Presenter: **Miss Sarah Oates** , *Research Assistant, School of Life Sciences*

Authors: **S L Oates**[2]; J R Sayers[2]; H Price[1]
[1] *Keele University;* [2] *The University of Sheffield*

Emerging drug resistance in kinetoplastid parasites is increasing the burden on the global health system. It is therefore essential to identify and characterise novel therapeutic targets. Flap endonucleases are involved in DNA replication and repair. These enzymes have been shown to be essential in other organisms including mice and bacteria. The human homologue of the enzyme is

up-regulated in certain cancers, and has potential as a target for anti-cancer therapies. In the current study we analysed the potential of the *Trypanosoma brucei* flap endonuclease as a therapeutic target. A wild-type and a mutated variant of the flap endonuclease were over-expressed in *E. coli*. A comparative analysis was performed on the recombinant proteins using a DNA cleavage assay, based on Förster resonance energy transfer (FRET), a bio-layer interferometry-based DNA binding assay and circular dichroism. These analyses showed that the mutation rendered the enzyme catalytically inert, whereas structural integrity and DNA binding were similar to the wild-type. Over-expression of both the wild-type and variant genes were analysed in the *T. brucei* parasite. The catalytically inert protein had a significantly detrimental effect on cell growth, and morphological changes were observed 72 hours post-induction. However, no effect on cell cycle progression was observed after 72 hours. A known flap endonuclease inhibitor, myricetin, was assessed for its ability to target the *T. brucei* flap endonuclease.The ability of myricetin to interact with the *T. brucei* flap endonuclease was assessed *in silico*, and inhibitory effects analysed *in vitro*. Inhibition of the *T. brucei* FEN correlated with cell death *in vivo*. Further work is in progress to determine if cell death is due to FEN1: myricetin interaction.

Identifying and exploiting deubiquitinating cysteine peptidase (DUBs) of *Leishmania*- A10802
Presenter: **Mr. Andreas Damianou**, *PhD student , University of York*

Authors: **A Damianou**[1]; P H Celie[3]; R Burchmore[2]; B Rodenko[4]; J C Mottram[1]
[1] Department of Biology, University of York; [2] Institute of Infection and Inflammation, University of Glasgow; [3] Netherlands Cancer Institute; [4] UbiQ Bio BV, Amsterdam

DUBs are a class of peptidases whose function is to cleave the post-translational modifier ubiquitin from proteins or ubiquitin-conjugates. DUBs play crucial roles in many biological processes such as protein degradation and gene regulation. *Leishmania* also has a ubiquitin system and its genome suggest the presence of 20 DUB orthologues. Still, the identity, function and essentiality of DUBs in *Leishmania* remain to be revealed. In this study, a chemical proteomics approach using a fluorescent ubiquitin-based probe was used for activity-based protein profiling, revealing the presence of many active DUBs in *L.mexicana*. A number of stage-specific DUBs have been identified, including some that have amastigote-specific activity. The DiCRe inducible gene knockout system is being used to evaluate *L.mexicana* DUB1, with preliminary data suggesting that DUB1 is essential. Furthermore, active recombinant DUB1 protein has been expressed and purified using a baculovirus expression system and an HTS-compatible fluorescence polarisation assay developed based on the proteolysis of tetramethylrhodamine-labelled Lys(Ub)Gly. Our approach combines chemical and genetic screening to identify essential *Leishmania* DUBs as a starting point for drug discovery activities.

***Leishmania infantum* Dual Specificity Tyrosine (Y) Regulated Kinase 1 in the development of infective promastigotes and in stage differentiation-** A10803
Presenter: **Dr Despina Smirlis** , *Visiting Scientist (Paris), Pasteur Institute/Inserm U1201*

Authors: V P Costa Rocha[1]; M Dacher[3]; F Kolokousi[2]; A Efstathiou[2]; G F Späth[3]; M B Soares[1]; D Smirlis[3];
[1] Fundação Oswaldo Cruz, Brazil; [2] Hellenic Pasteur Institute, Greece; [3] Pasteur Institute & INSERM U1201, France

DYRKs comprise a family of pro-survival eukaryotic protein kinases against stress and differentiation stimuli. For combating leishmaniasis, a serious disease caused by the protozoan parasite *Leishmania*, it is essential to increase the validated drug target repertoire. To this end we have selected a member of the *Leishmania* DYRK family, *L. infantum* (Lin) DYRK1, for probing its druggability potential. We employed a facilitated null mutant analysis in promastigotes that ectopically express DYRK1 from a plasmid that carries a negative selection marker, and a GFP reporter. Episomal persistence in logarithmic promastigotes after 15 passages under negative selection was used as a readout of essentiality. Our results showed that episome loss was tolerated in logarithmic parasites, suggesting that LinDYRK1 deletion could be compensated. However, early stationary phase LinDYRK1-/- promastigotes displayed a round morphology, mitotic arrest, and subsequent parasite death. Moreover, LinDYRK1-/- parasites showed both reduced thermotolerance to heat-shock, a signal required for stage differentiation, and reduced intracellular survival. Over-expression of LinDYRK1 resulted in G1 cell-cycle phase prolongation. Overall our results suggest that LinDYRK1 is an important pro-survival kinase in stationary growth phase and parasite differentiation, and thus a potential drug target for the prophylaxis against leishmaniasis.

Targeting histone modifying enzymes in *Leishmania*: a new venue for chemotherapy?- A10810
Presenter: **Miss Suzanne Lamotte**, *PhD student, Institut Pasteur*

Authors: **S Lamotte**[1]; K Cheeseman[3]; N Aulner[1]; A Mai[2]; C Muchardt[1]; J B Weitzman[3]; E Prina[1]; G F Spaëth[1]
[1] Institut Pasteur Paris, France; [2] Sapienza Università di Roma, Italy; [3] Université Paris Diderot, France

Conservation of all classes of histone modifying enzymes (HME) in the parasite *Leishmania* suggests epigenetic regulation as an interesting target for chemotherapy. We screened 480 epigenetic inhibitors against extracellular *L. amazonensis* using a viability assay, and intracellular amastigotes using a high content phenotypic assay. We identified 25 hits that kill extra- or intracellular parasites at 10 µM or 1 µM targeting all major classes of HMEs, suggesting a potential essential role of epigenetic regulation in *Leishmania*. Interestingly, 10 hits exclusively killed intracellular parasites, revealing a host cell-dependent mechanism of action likely through modulation of macrophage epigenetic regulation. Current studies are focusing on hits potentially targeting histone methylation enzymes. Applying antibodies against epigenetic marks of histone H3 showed (i) nuclear localization for H3K4me3, H3K9me3, and H3K27me3 marks using

immunofluorescence analysis in *Leishmania*, and (ii) a decrease of H3K4me3 (activation mark) and an increase of H3K27me3 (repression mark) in infected macrophages by Western blot analysis. Our data reveal a surprising complexity of epigenetic host/parasite interactions that opens interesting new venues for anti-leishmanial intervention.

Confirming the PanDZ complex as a cellular target for antimetabolites- A10789
Presenter: **Dr. Michael Webb**, *Associate Professor, University of Leeds*

Authors: S Nozaki[3]; Z L Arnott[1]; D C Monteiro[1]; C P Bartlett[1]; H Niki[3]; A R Pearson[4]; **M E Webb**[1]; V Patel[1]; M D Balmforth[1]; P M Morrison[1]; S W Ness[1]; M D Rugen[1]; R Al-Zahrani[1]; T D Grant[2]; E H Snell[2];
[1] Astbury Centre for Structural Molecular Biology, University of Leeds, Japan; [2] Hauptmann-Woodward Institute, Buffalo, United States; [3] National Institute of Genetics, Japan; [4] University of Hamburg, Germany

Pantothenate biosynthesis in bacteria has long been thought not to be regulated. This is surprising since almost all bacterial metabolic pathways are highly regulated. We have recently reported the crystal structure of the PanDZ complex from Escherichia coli. PanZ is required for the post-translational activation of PanD to form catalytically-active aspartate decarboxylase; this is required for formation of beta-alanine and therefore pantothenate. In all organisms, pantothenate is metabolized to form coenzyme A. Somewhat surprisingly, the interaction between PanZ and PanD is dependent upon coenzyme A which suggested the presence of a positive feedback loop. This observation was subsequently rationalized by the observation of inhibition of catalytic activity of the activated protein - PanZ is bifunctional and mediates both activation of the enzyme and regulation of its activity. Subsequent investigations have focused on demonstrating this regulation in a cellular context. We have identified site-directed mutations and substitutions which dysregulate the pathway and have determined that the known antimetabolite pentyl pantothenamide acts on this complex to inhibit cell growth - cells in which the pathway is dysregulated are insensitive to the compound. Identification of this new regulatory complex has therefore provided a new, already validated target for antimicrobial drug development.

A structure-guided approach to the design Trypanosomatid tRNA synthetase inhibitors- A10538
Presenter: **Prof Wim Hol**, *University of Washington*

Wim G. J. Hol[1], Ximena Barros-Alvarez[1,2], Cho Yeow Koh[1,*], Zhongsheng Zhang[1], Ranae M. Ranade[3], Keshia M. Kerchner[1], Sayaka Shibata[1], Wenlin Huang[1], J. Robert Gillespie[3], Stewart Turley[1], Christophe L. M. Verlinde[1], Frederick S. Buckner[3] and Erkang Fan[1].
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Protein synthesis depends on the arrival of charged tRNAs at the ribosome. The attachment of the proper amino acid to a specific tRNA is carried out by tRNA synthetases in an ATP dependent fashion. Since protein synthesis is crucial for all organisms including parasites, the reaction catalyzed by tRNA synthetases is essential for life. Inhibiting this process is therefore in principle an interesting avenue in arriving at compounds which can stop parasite growth. Here we describe approaches taken in a project Authors: which aims to arrive at inhibitors of tyrosyl tRNA synthetase from *Leishmania donovani*, histidyl-tRNA synthetase (HisRS) from *Trypanosoma cruzi*, and the methionyl-tRNA synthetases from *T. brucei* and *T. cruzi*. Results with fragment cocktail crystallography of *T. cruzi* HisRS show a remarkably tendency of different ring systems to bind at a pocket near the active site, after modest but critical changes in the position of side chains. The *T. brucei* MetRS studies revealed unexpected conformational changes upon inhibitor binding. The methionine pocket is considerably enlarged by one ring system of the inhibitors, a second, auxiliary, pocket (AP) is absent before inhibitor binding and is the result of conformational selection upon binding the second ring system. Precise analysis of inhibitor contacts in the AP was followed up by synthesis of variants of initial inhibitors, where substituents were added to the ring occupying the AP. It appeared that small differences in substituent size led to small differences in the binding pocket with large consequences for affinity and EC_{50} values. MetRS inhibitors were obtained with EC_{50} values for *T. brucei* growth in the sub-nanomolar range. Building on the studies in *T. brucei* MetRS, promising inhibitors of the homologous *T. cruzi* MetRS were obtained with low EC_{50} values for *T. cruzi*. Structural information can clearly be useful in the design of new inhibitors, but the translation from Ångstroms to kilocalories is not trivial, in particular when conformational changes are occurring. Moreover, understanding the effect of substitutions on pharmacological properties remains challenging.

Biochemical characterisation of heparan sulphate and its role in *Leishmania* infection of host macrophages - A10688
Presenter: **Marissa Maciej-Hulme**, *Post-doc, Keele University*

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

Leishmaniasis 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 both human monocyte and macrophage HS show specific changes occur to the modification of HS during differentiation, resulting in the display of different sulphation patterns within the glycosaminoglycan 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, thus potentiating an alternative therapeutic approach for parasite infection.

Discovery of Novel Antimalarial Agents by Structure-Based Design and Multi-Parameter Lead Optimisation - A10808
Presenter: **Martin McPhillie**, *Postdoc, University of Leeds*

Authors: **M J McPhillie**[3]; J A Gordon[3]; P J Kocienski[3]; S Wittlin[1]; C W Roberts[4]; G McConkey[3]; A P Johnson[3]; R McLeod[2]; C W Fishwick[3]
[1] Swiss Tropical & Public Health Institute, Switzerland; [2] University of Chicago, United States; [3] University of Leeds; [4] University of Strathclyde

Our research group at Leeds has expertise in the structure-guided optimisation of small molecule inhibitors of two parasitic diseases: malaria and toxoplasmosis. A validated drug target in both causative organisms is the mitochondrial electron transport chain (mtETC) responsible for (i) maintaining an electro-potential across the inner membrane and (ii) regeneration of ubiquinone to support pyrimidine biosynthesis. Our research efforts have focussed on two elements of this chain: cytochrome bc_1 (complex III) and dihydroorotate dehydrogenase (DHODH). Here we describe the lead optimisation of two novel compound series targeting these proteins.

Dissection and rational engineering of the biosynthetic pathway to enacyloxin, a promising anti-Gram-negative antibiotic- A10739
Presenter: **Dr Joleen Masschelein**, *Postdoctoral Research Fellow, University of Warwick*

Authors: **J Masschelein**[1]; P K Sydor[1]; D Griffiths[1]; T R Valentic[2]; A Gallo[1]; C Jones[3]; L Song[1]; S C Tsai[2]; J R Lewandowski[1]; E Mahenthiralingam[3]; G L Challis[1]
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Enacyloxin IIa is a polyketide antibiotic with potent activity against Gram-positive and Gram-negative bacteria that targets ribosomal elongation factor Tu.1-5 It has been identified as a metabolite of *Burkholderia ambifaria* AMMD and shown to have clinically-relevant activity against *Acinetobacter baumannii*, a problematic multidrug-resistant Gram-negative pathogen. Despite its promising biological activity, enacyloxin IIa is unlikely to find direct clinical application, given the densely-packed array of similar and potentially labile functional groups in the antibiotic. Enacyloxin biosynthesis has recently been mapped to an 80 kb gene cluster in the sequenced genome of *B. ambifaria* AMMD and a pathway for its biosynthesis has been proposed, involving assembly of the 27-carbon acyl chain by a modular polyketide synthase (PKS). The polyketide chain undergoes various modifications by on-/post-PKS tailoring enzymes, including halogenation, hydroxylation, carbamoylation and oxidation. Detailed structural and biochemical analyses have provided insights into an unusual mechanism of chain release in enacyloxin biosynthesis, showing that the enzymatic machinery is able to accommodate (1R, 3R, 4S)-3,4-dihydroxycyclohexane carboxylic acid (DHCCA) as well as a diverse set of analogues. Using a variety of rational engineering approaches, including mutasynthesis, gene deletion and gene replacement strategies, we are currently exploiting this biosynthetic knowledge for the production of novel analogues with improved pharmacological properties.

References: [1] Watanabe T, Izaki K, Takahashi H. New polyenic antibiotics active against gram-positive and gram-negative bacteria. I. Isolation and purification of antibiotics produced by *Gluconobacter* Sp. W-315. . J Antibiot (Tokyo) 1982, 35:1141-1147. [2] Watanabe T, Sugiyama T, Takahashi M, Shima J, Yamashita K, Izaki K, Furihata K, Seto H. New polyenic antibiotics active against gram-positive and gram-negative bacteria. IV. Structural elucidation of enacyloxin IIa. J Antibiot (Tokyo) 1992, 45:470-475. [3] Watanabe T, Okubo N, Suzuki T, Izaki K. New polyenic antibiotics active against gram-positive and gram-negative bacteria. VI. Non-lactonic polyene antibiotic, enacyloxin IIa, inhibits binding of aminoacyl-tRNA to A site of ribosomes. J Antibiot (Tokyo) 1992, 45:572-574. [4] Zuurmond AM, Olsthoorn-Tieleman LN, Martien de Graaf J, Parmeggiani A, Kraal B. Mutant EF-Tu species reveal novel features of the enacyloxin IIa inhibition mechanism on the ribosome. J Mol Biol 1999, 294:627-637. [5] Parmeggiani A, Krab IM, Watanabe T, Nielsen RC, Dahlberg C, Nyborg J, Nissen P. Enacyloxin IIa pinpoints a binding pocket of elongation factor Tu for development of novel antibiotics. J Biol Chem 2006, 281:2893-2900. [6] Mahenthiralingam E, Song L, Sass A, White J, Wilmot C, Marchbank A, Boaisha O, Paine J, Knight D, Challis GL. Enacyloxins are products of an unusual hybrid modular polyketide synthase

Mediated polyvalent and cooperative interactions of drugs define mechanical force on microbial susceptibility- A10821

Presenter: **Dr. Joseph Ndieyira**, *University College London*

Authors: **J W Ndieyira**[2]; J Bailey[2]; S B Patil[2]; M Vögtli[2]; M A Cooper[4]; C Abell[3]; R A McKendry[2]; G Aeppli[1]

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Molecular recognition depends on the specificity of interactions between a ligand and receptor and regulates a wide variety of biological activities in medicine. In pharmacology for example, the goal is to interfere with pathological biochemical pathways by docking molecules on the active sites. Vancomycin, a powerful antibiotic against streptococcal and staphylococcal strains including methicillin-resistant *Staphylococcus aureus*, kills bacteria by inhibiting cell-wall biogenesis. Molecular-scale changes in the docking site on the bacterial cell wall can confer resistance to vancomycin. In contrast, oritavancin and related antibiotics show remarkable activity against vancomycin-resistant bacteria, thus violating the historic lock-and-key and induced-fit theories. Here, we show that the efficiency of substrate binding to activate bactericidal activity can be dramatically improved at a surface in contrast to solution environment. In addition, we find that while a single binding mechanism in a pre-existing ensemble of conformational states dominates the behavior of susceptible targets, it fails dramatically for drug-resistant phenotypes. A more general model, taking account of multiple binding and surface effects, provides a compact description of how chemistry and mechanics are combined to effect bactericidal activity against resistant bacteria. Subsequently, the mechanobiological effects on the extracellular matrix transduce into large-scale mechanical forces that bacteria cannot ultimately withstand, causing cell death. Our methodology is generally applicable for obtaining rapid and quantitative pre-clinical understanding of the modes of action of drugs and offers lessons for developing effective therapies.

Intelligent fragment-based approaches to antibacterials- A10534
Presenter: **Prof Chris Abell**, *University of Cambridge*

The talk will describe the development of inhibitors of enzymes involved in key metabolic processes in *Mycobacterium tuberculosis*. It will outline the principles of the approach and the key techniques involved. Specific case studies will be described e.g. against cytochrome P450 enzymes.

Chemical Proteomic Target Discovery and Validation in Infectious Disease- A10549
Presenter: **Prof Ed Tate**, *Imperial College London*

My group develops chemical biology approaches to identify and validate potential drug targets, particularly in the field of protein post-translational modification. In this talk I will discuss our recent work in the field of protein lipidation (acylation, cholesterylation and prenylation), where we have contributed to validation of protein targets in infectious diseases caused by parasites (malaria, leishmaniasis, trypanosomiasis), bacteria and viruses. I will also illustrate how we have used chemical tagging technologies in an analytical platform for quantification and identification of protein lipidation in live cells and animals, providing the first insights into how lipidation changes in response to drug treatment at the whole proteome level. This research has enriched our understanding of these traditionally challenging classes of protein modification, and delivered novel small molecules into pre-clinical development.

Acknowledgements: I am particularly grateful to The Wellcome Trust, which has supported our work on trypanosomatids since 1988. I also thank all of my colleagues in the Division of Biological

Chemistry & Drug Discovery and the Drug Discovery Unit who's work I am drawing on in this overview.

Tres Cantos Open Lab: Present and future of this collaborative model for diseases of the developing world drug discovery- A10787
Presenter: **Dr Maria Marco-Martin**, *GlaxoSmithKline*

Since 2010, GlaxoSmithKline Tres Cantos Medicines Development Campus (TC-MDC) has adopted an open innovation strategy that fosters collaboration and transfer of knowledge with academia and biotech, with the final aim to deliver new effective medicines for DDW (malaria, tuberculosis and kinetoplastid diseases). As part of this approach, the Tres Cantos Open Lab (www.openlabfoundation.org) at TC-MDC provides visiting scientists funding and access to GSK facilities, compound collections and drug discovery expertise, in an attempt to exploit a novel model of collaboration for DDW medicine discovery and development. The Open Lab-funded partnerships focus on development of novel drug discovery tools, exploration of new molecular targets and phenotypic assays in high throughput screening programs and optimization of novel lead molecules. Part of these successful collaborations will be highlighted in this communication. With the aim to fill the portfolio for these neglected diseases and guarantee a sustainable pipeline, the Open Lab program is currently on the lookout for drug discovery opportunities with the potential to dramatically affect our capacity to discover novel promising drug candidates

Enabling Moenomycin A to target resistant Gram Negative Bacteria- A10624
Presenter: **Dr Ishwar Singh**, *Senior Lecturer, School of Pharmacy, University of Lincoln*

Authors: **I Singh**[1]
[1] *School of Pharmacy, University of Lincoln*

By 2050, antimicrobial resistance (AMR) will cause mortality to more people than cancer. Resistant Gram negative bacteria present a serious challenge due to limited or no treatment options for infections using current antibiotics. There is therefore an urgent need to develop better understanding on how to target drug-resistant Gram negative bacteria.
To address the challenges from Gram negative bacteria, we have shown for the very first time that Moenomycin A (MoeA) can be effective against the resistant Gram negative bacteria. MoeA is largely inactive and unexploited against Gram negative bacteria. MoeA is the most potent naturally occurring inhibitor of a family of glycosyltransferases (GT51), highly conserved enzymes in resistant bacteria central to producing peptidoglycan. Targeting GT51 enzyme activity by MoeA is an excellent antimicrobial strategy because (1) GT51 is essential for bacterial survival and (2) no significant natural cross resistance has been reported for MoeA, despite its use for decades in animal feed. We have pioneered a unique approach to target resistant Gram negative bacteria using MoeA and have showed a substantial reduction in MIC in resistant Gram negative bacteria: 16 times for *P. aeruginosa*, 8 times for *K. pneumoniae* and 8 times for *A. baumannii*. The latest results on targeting Gram negatives using MoeA will be presented.

Post-translational Chemical Editing Method Reveals a Dynamic Interplay Amongst Cysteine Residues in Bacterial Tyrosine Phosphatases- A10543
Presenter: **Dr Jean Bertoldo**, *Visiting Researcher, University of Cambridge*

Authors: **J B Bertoldo**[3]; T Rodrigues[4]; F A Aprile[3]; O Boutureira[3]; L Rosado[1]; F Corzana[2]; H Terenzi[1]; G Bernardes[3]
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The chemical modification of cysteine residues with small molecules has become cornerstone in the modulation of drug-relevant targets in chemical biology and drug discovery. Protein tyrosine phosphatases (PTPs) of bacterial origin often display a poorly conserved, non-catalytic cysteine (Cys) within range of the conserved active site. While mainstream target-based discovery programs aim at the inactivation of catalytically essential residues, target modulation through accessible and highly active non-catalytic Cys residues constitutes an emerging paradigm in chemical biology and molecular medicine. Herein, we disclose a post-translational chemical editing method to install dehydroalanine (Dha), without prior alteration of enzyme amino acid sequence or deleterious enzyme inactivation, in a non-catalytic Cys with exquisite regioselectivity. Unexpectedly, we unveiled a long-range dynamic interplay between the backdoor and catalytic Cys residues, as exemplified with the *Mycobacterium tuberculosis* virulence factor protein tyrosine phosphatase A (PtpA) and *Yersinia enterocolitica* tyrosine phosphatase YopH. Our findings suggest an alternative mechanism of redox regulation in bacterial PTPs and provide a solid rationale for designing effective and innovative antimicrobial PTP-targeting chemical probes.

Treatment of cutaneous leishmaniasis using sustained release delivery systems loaded with a novel chalcone - A10983
Presenter: **Prof. Bartira Rossi- Bergmann**, *Associate professor, Universidade Federal do Rio de Janeiro*

Authors: B Rossi-Bergmann[1]
[1] Federal University of Rio de Janeiro, Brazil

Current chemotherapy of cutaneous leishmaniasis (CL) uses systemic administration of toxic drugs that cause severe adverse reactions. Moreover these require multiple painful injections that lead to a high therapy evasion rate. Consequently new drugs with better delivery systems are required. We report the development of a single-dose treatment for CL based on PLGA polymeric particles loaded with a plant-derived synthetic chalcone. Encapsulated chalcone afforded enhanced intracellular efficacy and provided effective, long-lasting parasite growth control when given

subcutaneously to *Leishmania amazonensis*-infected mice, with only a transient local inflammatory reaction as seen by histopathological analysis. Full details of these advances together with studies to probe the molecular target and mode of chalcone action will be presented.

Use of Photo-Affinity Labeling to Determine the Trypanosomatid Protein Target of Analogues of the Natural Product Chamuvarinin- A10851
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 B Tulloch[1]; M K Zacharova[1]; G J Florence[1]; T K Smith[1]
[1] *University of St Andrews*

New drugs against trypanosomatid diseases are desperately needed due to the toxic nature of currently used drugs, and the increase of drug resistance. The acetogenins are a natural product family of potent Complex I inhibitors, however the analogues of chamuvarinin, an acetogenin first synthesized by our group, show high activity against bloodstream form *Trypanosoma brucei*, in which Complex I is not an essential protein. Using photo-affinity labeled (PAL) derivatives of chamuvarinin, we have begun to identify the protein target of these compounds in the parasites *T. brucei, T. cruzi* and *L. major*. Using fluorescent microscopy, we have shown that these compounds primarily target the mitochondrion of the trypanosomatids. Using mass spectrometry and specific pull-downs, we have identified that the protein target is the mitochondrial FOF1 ATP synthase, which is supported by protein modelling. The evidence produced using these PAL-labeled compounds supports our phenotypic assays, which indicate that ATP synthesis is the pathway involved in our compounds mode of action.

Translating the trypanosome surface- A10550
Presenter: **Prof Mike Ferguson**, Regius Professor of Life Sciences, University of Dundee

In this talk, I will overview the commonalities and peculiarities protein N-glycosylation, glycosylphosphatidylinositol (GPI) membrane anchoring and nucleotide sugar biosynthesis in trypanosomes and the opportunities therein for therapeutic exploitation. The desire to exploit these, and several other, discoveries led groups at The University of Dundee to establish a Drug Discovery Unit that both translates parasite biology through to preclinical candidates and develops drug candidates phenotypically, performing drug target deconvolution at the end of the process. Examples of both approaches will be given. The Drug Discovery Unit capabilities established for parasitic diseases are also applied to innovative targets in several other therapeutic areas, and some of these will be briefly mentioned.

Posters in number order

Molecular Signalling and Protein/Ligand interactions in the Enterococcal Serine/Threonine Kinase IreK- Poster 1 : A10343
Presenter: **Mr. Christopher Thoroughgood**, *PhD Student, University of Warwick*

Authors: **C W Thoroughgood**[2]; A M Dixon[2]; J E Dworkin[1]; D I Roper[2]
[1] Columbia University, United States; [2] University of Warwick

The regulatory control of gene expression by external stimuli in bacteria is poorly understood but is fundamental to bacterial signaling, environmental response and antibiotic resistance. It has been known for sometime that almost all bacteria use multiple membrane bound histidine kinases and cytoplasmic response regulator proteins to form two-component systems (2CS) to respond to external stimuli. However, the nature of these protein-ligand interactions and how that interaction is transmitted through the membrane is still largely unexplored. It is becoming increasing clear that many bacterial also contain single Serine-Threonine kinase (STK) proteins, similar to those found in eukaryotes, which are responsible for more global extracellular signal responses.
Between groups at Columbia (USA) and Warwick (UK), we have identified IreK as a unique STK in pathogenic Enterococci that appears to have a highly significant role in peptidoglycan and related antibiotic resistance. The aim of this project is to dissect the molecular architecture and interaction of the Enterococcal IreK. We have already assembled a molecular tool kit of proteins, ligands and chemical probes to explore this system. We are interrogating IreK extracellular domain ligand interactions using SPR and NMR structural characterization of these domains to monitor the ligand interaction. We aim to describe IreK ligand recognition to intracellular signaling.

Developing Peptide-Mimetics for the Treatment of Cutaneous Leishmaniasis-
Poster 2 : A10791
Presenter: **Miss Hannah Bolt**, *PhD student, Chemistry Department, Durham University*

Authors: **H L Bolt**[1]; G A Eggimann[1]; P W Denny[1]; S L Cobb[1]
[1] Durham University

Leishmaniasis is a neglected tropical disease that is endemic in over 80 countries and caused by insect vector-borne protozoan parasites. Distinct *Leishmania* species can cause cutaneous leishmaniasis which leads to significant scaring and mucosal damage (mucocutaneous leishmaniasis) or visceral leishmaniasis that causes life-threatening organ damage. Most current treatments rely on drugs that have severe side-effects and emerging drug resistance could lead to ineffective theraputics in the near future. Antimicrobial peptides have been investigated as new compounds to treat CL. However, the intrinsic chemical and biological instability of peptides can present challenges. Recently we have shown for the first time that peptoids, peptide-mimetic molecules that have improved stability *in vivo*, are promising leads in the search for new anti-

Leishmanial theraputics. We have identified potent compounds against the clinically relevant *L. mexicana* axenic amastigotes and significantly against *Leishmania* infected macrophages. Differential susceptibilities of the amastigotes and insect stage promastigotes to our peptoid library will be explained and the scope and limitations of these compounds as potential topical treatments for CL will be highlighted.

Autoinducer-antibiotic conjugates - a Trojan horse approach to antibiotic resistance- Poster 3 : A10857
Presenter: **Ms Lois Overvoorde**, *PhD student, University of Cambridge*

Authors: **L M Overvoorde**[1]; Y R Baker[2]; D R Spring[1]
[1] University of Cambridge; [2] University of Oxford

Many bacteria use quorum sensing to coordinate behaviours such as swarming, virulence factor production and biofilm formation. They communicate via the excretion and uptake of small molecules known as autoinducers. With our library of autoinducer-antibiotic conjugates, we aim to hijack the autoinducer uptake apparatus to facilitate the influx of known antibiotics, in a strategy that has already successfully been employed with sideophore-antibiotic conjugates. We focus on the autoinducers produced by *Pseudomonas aeruginosa* as it is a significant human pathogen which displays high resistance to many antibiotics and uses quorum sensing to coordinate its group behaviours. Derivatives of these autoinducers are coupled with derivatives of ciprofloxacin using either a copper(I)-catalysed azide-alkyne cycloaddition or an S_N2 reaction. Ciprofloxacin was chosen as it is commonly used against P. aeruginosa but resistance to it is developing. It is hoped that the autoinducers will deliver the attached ciprofloxacin into the cell, thus potentially increasing its potency or even restoring its efficacy against resistant strains.

Analysis of differential susceptibility to miltefosine in *L. (V.) braziliensis* clinical isolates: investigation of potential mechanisms of miltefosine resistanc- Poster 4 : A11209
Presenter: **Prof. Adriano Cappellazzo Coelho**, , *Universidade Estadual de Campinas*

Authors: **A C Coelho**[1;2]; C R Espada[1]; L I Pereira[2]; F Ribeiro-Dias[2]; M L Dorta[2]; P R Machado[3]; E M Carvalho[3]; V Hornillos[4]; A U Acuña[5]; S R Uliana[1]
[1] Universidade de São Paulo, São Paulo, Brazil; [2] Universidade Federal de Goiás, Goiânia, Brazil; Universidade Federal da Bahia, Salvador, Brazil; [4] Instituto de Investigaciones Químicas, CISC, Seville, Spain; [5] Instituto de Química Orgánica, CISC, Madrid, Spain;

Leishmania (Viannia) braziliensis is the main etiological agent of cutaneous leishmaniasis in Brazil, where approximately 21,000 new cases of the disease were notified in 2014. The arsenal available for treatment is limited and inadequate due to parenteral administration, toxicity, high cost and emergence of resistance. Miltefosine is an oral drug, already in use for cutaneous leishmaniasis in Colombia and visceral leishmaniasis in India, where pentavalent antimonials are no longer effective

due to drug resistance. Miltefosine is not approved for leishmaniasis treatment in Brazil but recent clinical trials have indicated its higher efficacy when compared with pentavalent antimony. Data on the susceptibility of *L. (V.) braziliensis* Brazilian clinical isolates from different geographical regions is not available. In this study, we evaluated the susceptibility to miltefosine of 16 clinical isolates, which were not previously exposed to the drug. Half maximal effective concentrations varied by a factor of 6 to 15 for promastigotes and amastigotes, respectively.
Trying to elucidate the reason for these differences, we evaluated the miltefosine uptake in promastigotes of these isolates, using a fluorescent miltefosine analogue (Miltefosine-BODIPY) by flow cytometry. These findings showed a positive correlation between susceptibility and uptake of the drug. The miltefosine transporter and its subunit Ros3 are responsible for the transport of miltefosine into the cell in *Leishmania*. Thus, we investigated whether polymorphisms in the miltefosine transporter gene could explain the differential susceptibility phenotype. Miltefosine transporter genes (LbrM 13.1380 and LbrM 13.1400) sequences from three isolates and from *L. (V.) braziliensis* M2903 reference strain were determined. Although some polymorphisms were found in both genes, none could explain the differential susceptibility and uptake observed among the clinical isolates.

A large demography movement role in cutaneous leishmaniasis incidence from Southwest of Iran- Poster 5 : A10784
Presenter: **Dr. Mohammad Hossein Feiz Haddad**, *Member of Staff , Ahvaz Jundishapur University of Medical Sciences*

Authors: **M H Feiz Haddad**[3]; K Safaei[1]; R Feiz Haddad[2]; B Jahani[1]
[1] Ahvaz Jundishapur University of Medical Sciences , Iran; [2] Department of Nursing, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran; [3] Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background and Objectives: In recent years, a religious festival movement as one of the largest human gathering drew an estimated 20 million pilgrims at the holy confluence of the Iraq. This phenomenal movement and its impacts have gone for the most part undocumented. To address this new phenomenon in terms of leishmaniasis prevalence, authors decided to study and evaluate collected data to monitor and preparedness as well as response to public health emergencies at the event. This study also describes the trends of the cutaneous leishmaniasis around the event with particular emphasis on preventive and mitigation strategies; the capacity for surveillance and response to disease outbreaks and implications of lessons learned for future religious mass gatherings. Methodology: A cross-sectional demography and epidemiological study was carried out on examined 2637 patients with cutaneous leishmaniasis during righteous festival monitoring trends fluctuations on the disease for the period of 2011 to 2016. Furthermore, Biometric parameters included age, gender, season, habitation situation, and lesion numbers', sites', and sizes' were recorded in cutaneous leishmaniasis epidemiologic data summary forms. Findings & discussion: Of 2637 patients, 1174 (44.5%) were females and 1463 (55.5%) males. Maximum and

minimum prevalence were observed in winter (52.33%) and summer (7.62%) which trend fluctuation was matched perfectly with the timing of this religious community (Table 1). Furthermore, maximum infection rate was recorded for patients above 20 year-old (45.3%) and minimum rate was documented for patients over 60 year old (0.9%). Habitation situation showed 1557 (59%) were settled in temporary accommodations and 1080 (41%) were housed in tents which probably set up on hosts liars during of the festival. From lesion location point of view most lesions were on hands (37.5%), face (30%), feet (26.3%), and other organs (6.2%) and the number of lesions ranged from 1 to 5 and sized varied from 0.5 to 5.5 cm. The trend of the disease showed a constant growth from which 2011 begun to rise and reached to a peak at 2015. Conclusion: Conclusively, cutaneous leishmaniasis became a serious dermatological health problem due to a recent great demographic movement to neighboring country Iraq with a high incidence to an already endemic area. In addition, unfavourable factors on the prevalence of cutaneous leishmaniasis in studied regions could increase the risk of exposure to leishmaniasis because study of biometric parameters in endemic regions confirmed significant effects on prevalence of cutaneous leishmaniasis during holding of this religious event. These findings can be effective for assessing disease prevention programs for the next mass gathering.

Intracellular trafficking of GFP expressing *Leishmania aethiopica* in terminally differentiated THP-1 cells.- Poster 6 : A11162
Presenter: **Medhavi Ranatunga**, *PhD Research Student, University of Greenwich*

Authors: **R M Ranatunga**[1]; G Getti[1]
[1] *University of Greenwich*

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. 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 hosts within the PV of previously infected cells.

Targeting male and female gametocytes: preventing malaria transmission- Poster 7 : A10806
Presenter: **Dr. Holly Matthews**, *Research associate, Imperial College London*

Authors: **H Matthews**[2]; M Delves[2]; C Miguel-Blanco[2]; I Molina[1]; E Herreros-Aviles[1]; J Baum[2]
[1] *GlaxoSmithKline;* [2] *Imperial College London*

Growing evidence of resistance to artemisinin threatens the shelf-life of the current frontline antimalarial combination therapies and underscores the urgent need for the discovery of novel drugs and drug-targets. Historically, antimalarial chemotherapy has been an arms race between drug-development and resistance acquisition of asexual parasites. Multi-targeted approaches are a necessity if future gains are to be made. Chemically targeting mature gametocytes to prevent parasite transmission is becoming a favoured component of eradication campaigns. In recent years the development of high-throughput antimalarial assays has permitted access to previously unexplored chemical space. To facilitate the discovery of transmission blocking antimalarials and novel druggable targets, the Dual Gamete Formation Assay (DGFA) has been developed as a high-throughput alternative to the Standard Membrane Feeding Assay (SMFA). The DGFA determines the viability of male and female gametocytes independently, in the same well, following drug exposure. From a screen of 13,533 compounds (Tres Cantos Antimalarial Set, TCAMS) pre-selected for activity against *P. falciparum* asexual blood-stages, the DGFA has identified 262 hits ($\geq$ 50% inhibition at 2 μM). Of these, 199 were male-specific, 7 were female-specific and 56 were dual-active. Despite representing only ~20 % of the sexually dimorphic population, males were more drug-sensitive than females. Future work will focus on dissecting the mode-of-action of hit compounds.

Towards new drugs for trypanosomatid diseases based on specific high-affinity inhibitors for RNA editing ligase 1- Poster 8 : A11211
Presenter: **Dr. Marios Frantzeskos Sardis**, *Research Associate, University of Edinburgh*

Authors: **M F Sardis**[2]; S Zimmermann[2]; V Feher[1]; J Sørensen[1]; C Smith[3; 4]; L Hall[2]; M Greaney[3; 4]; R E Amaro[1]; A Schnaufer[2];
[1] Department of Chemistry and Biochemistry, University of California San Diego, United States; [2] Institute of Immunology and Infection Research, University of Edinburgh; [3] School of Chemistry, University of Manchester, UK; [4] School of Chemistry, University of Manchester, UK, United States

Messenger RNA editing by uridylyl insertion/deletion is a unique process in kinetoplastid mitochondria, probably essential for their survival (Schnaufer et al., 2001) and therefore a potential drug target. The crystal structure of the catalytic domain of TbREL1 (Deng, Schnaufer et al., 2004) shows a unique active centre with a well-defined ATP binding site. We have developed a new REL1

activity assay suitable for high-throughput screening (HTS). A proof-of-concept screen against the LOPAC library and a HTS campaign of diversity and kinase inhibitor-focused compound libraries at the Dundee Drug Discovery Unit resulted in a hit rate of 1.7% and 0.77%, respectively. Hit optimisation led to the identification of several promising compound series with potency up to an IC50 of 20 nM.
Furthermore, we have expressed REL1 orthologs from four kinetoplastid parasites*, T. cruzi, T. congolense, T. vivax and L. donovani* that are functional in the HTS activity assay and some TbREL1 inhibitors show similar potency against these orthologs.
Aided by the orthologous proteins we are continuing the lead development of the initial hits and will present findings on structure-activity relationships, biophysical characterization, and activity against parasites.

Target elucidation of novel natural product inspired inhibitors of *Trypanosoma brucei* - Poster 9 : A10926
Presenter: **Miss Elizabeth King**, *PhD Student, University of St Andrews*

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

The natural world is host to a wide variety of biologically active compounds that successfully treat a range of diseases. Chamuvarinin, of the acetogenin family, is a natural product isolated from the roots of the bush banana plant *Uvaria chamae* and was subsequently synthesised in-house by the Florence lab. The acetogenins are cytotoxic to cancer cell-lines and chamuvarinin itself has low micromolar activity against *Trypanosoma brucei*, the causative agent of human African trypanosmiasis. A series of simplified analogues that retain the activity of chamuvarinin have been synthesised to determine the structure-activity relationships of various positions of the molecule. Motifs that increase selectivity towards the parasite have been developed and key trypanocidal elements of the core structure have been identified, aiding in the future development of new series of compounds. A key aspect of this project is the synthesis and use of photo-affinity probes in pursuit of the drug targets. Biochemical approaches with these probes have led to the identification of two candidate protein targets in bloodstream form *T. brucei*. Future efforts will involve examination of the protein-drug relationships, using genetic and biochemical approaches, which should direct synthesis of more potent and selective analogues.

Discovery of New Therapies for Leishmaniasis through the Development of Chemical Probes- Poster 10 : A11015
Presenter: **Miss Rebecca Charlton**, *PhD chemist, Department of Chemistry, Durham University*

Authors: **R Charlton**[1]; C Brown[1]; J G Mina[1]; P G Steel[1]; P W Denny[1]
[1] Durham University

Leishmaniasis is a vector-borne Neglected Tropical Disease, caused by protozoan parasites of the genus *Leishmania*. There are approximately 1.3 million new cases of leishmaniasis world-wide each year claiming a significant number of lives. This infectious disease primarily affects the world's poorest countries and therefore antileishmanial treatments need to be affordable and accessible. Furthermore, given the threat of drug resistance there is an urgent need to develop a drug with a novel mode of action.

We have identified the *Leishmania* enzyme inositol phosphorylceramide synthase (IPCS) as a potential drug target. This membrane-bound enzyme is involved in the biosynthesis of the sphingolipids and has a role that differs from its mammalian orthologue which provides the potential to access selective drugs. An attractive strategy for drug development for NTDs is repurposing existing drug structures. Following this approach, a small set of known bioactive compounds (NINDS set) was screened against the *Leishmania* major IPCS. Following secondary screening against promastigote forms of the parasite, four compounds with activity greater than pentamidine were identified for further study. This presentation will discuss these results with a focus on work adapting the leading compound as a probe to further validate IPCS as the drug target.

Analysis of Protein Stability and Protein-Ligand Interactions for Drug Discovery by Thermal Shift Assay- Poster 11 : A10984
Presenter: **Miss Emily Cardew**, *PhD Student, Durham University*

Authors: **E M Cardew**[1]
[1] *School of Biological and Biomedical Sciences, Durham University*

A thermal shift assay (TSA) is a high-throughput fluorescence-based biophysical technique for assessing protein stability based on protein melting temperature (Tm). A non-covalent dye, namely SPYRO Orange, is used; upon binding the hydrophobic core of thermally denatured protein, a fluorescence signal is emitted at a time-point that corresponds to the Tm. Two screens, namely the Durham Salt and Durham pH screens, have been developed and are currently being commercialised by Molecular Dimensions Ltd. The screens can be used in a TSA to assess the thermal stability of a protein in a broad range of solutions. Utilising the Durham Screens offers the chance to improve your protein purification and discover potential crystallisation conditions. Protein-ligand interactions can also be explored using compound libraries in a TSA, where ligand binding is indicated by a positive shift in Tm. NAMI is a GUI-based Python programme for automatic TSA data analysis and interpretation (Groftehauge et al., 2015). NAMI facilitates the rapid determination of Tm values and generates a colour-coded table of all results, allowing simple visual analysis. Overall, combining the use of the Durham Screens and compound libraries in TSAs with data analysis in NAMI generates a powerful tool for the assessment of protein stability for structural studies and structure-based drug design.

Microorganisms and activation of the immune response- Poster 12 : A10863

Presenter: **Kingsley O. Chikere**, *skin care professional, computer science*

Authors: **K O Chikere**[1]
[1] *University of Lagos, Nigeria*

The mammalian immune system has innate and adaptive components, which cooperate to protect the host against microbial infections. The innate immune system consists of functionally distinct 'modules' that evolved to provide different forms of protection against pathogens. It senses pathogens through pattern-recognition receptors, which trigger the activation of antimicrobial defences and stimulate the adaptive immune response. The adaptive immune system, in turn, activates innate effector mechanisms in an antigen-specific manner. The connections between the various immune components are not fully understood, but recent progress brings us closer to an integrated view of the immune system and its function in host defence.

Catch me if you can: Using ATP affinity chromatography for *Leishmania* drug target deconvolution- Poster 13 : A11024
Presenter: **Mr Olivier Leclercq**, *Technician, Institut Pasteur*

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

Existing anti-leishmanial drugs are compromised by severe side effects and emergence of parasite resistances, calling for the discovery of new drug candidates to drive anti-parasitic chemotherapy. We recently identified hit compounds with anti-leishmanial activity by screening ATP-like compound libraries using target-based and phenotypic assays. Our current efforts are directed toward drug target deconvolution to reveal off-target effects of hit compounds identified by screening against recombinant *Leishmania* casein kinase CK1.2, and identify the unknown targets of hit compounds identified by screening against *L. amazonensis* infected primary macrophages. We use a proteomic strategy combining competition assays, successive ATP affinity chromatography, 2D-DiGE and MS analyses to evaluate the specificity of CK1.2 hit compounds, and discover novel targets based on the hits identified by phenotypic screening. As a first step towards the validation of this technology, we identified *Leishmania* ATP-binding proteins (ATPome). MS analysis of eluted fractions revealed 1405 putative ATP-binding proteins, including 241 proteins with a Gene Ontology annotation ATP-binding (GO: 0005524), including 96 protein kinases, thus establishing the first ATPome in any Trypanosomatid. We recovered 1164 proteins from our assay, including 456 hypothetical proteins that bear no overt homology to any ATP-binding protein. These proteins are either indirectly enriched through interaction with ATP-binding proteins, or directly enriched through yet uncharacterized, parasite-specific domains able to bind ATP. Our current efforts will be presented to (i) distinguish between these two possibilities, including enrichment analyses under high salt concentrations known to eliminate protein-protein interactions, (ii) identify novel domains by bio-informatics analyses, and (iii) apply our ATPome analysis for drug target discovery.

Molecular Signalling and Protein/Ligand interactions in the Enterococcal Serine/Threonine Kinase IreK- Poster 14 : A10608
Presenter: **Mr. Christopher Thoroughgood**, *PhD Student, University of Warwick*

Authors: **C W Thoroughgood**[1]; A M Dixon[1]; D I Roper[1];
[1] *University of Warwick*

The regulatory control of gene expression by external stimuli in bacteria is poorly understood but is fundamental to bacterial signaling, environmental response and antibiotic resistance. It has been known for sometime that almost all bacteria use multiple membrane bound histidine kinases and cytoplasmic response regulator proteins to form two-component systems (2CS) to respond to external stimuli. However, the nature of these protein-ligand interactions and how that interaction is transmitted through the membrane is still largely unexplored. It is becoming increasing clear that many bacterial also contain single Serine-Threonine kinase (STK) proteins, similar to those found in eukaryotes, which are responsible for more global extracellular signal responses.
Between groups at Columbia (USA) and Warwick (UK), we have identified IreK as a unique STK in pathogenic Enterococci that appears to have a highly significant role in peptidoglycan and related antibiotic resistance. The aim of this project is to dissect the molecular architecture and interaction of the Enterococcal IreK. We have already assembled a molecular tool kit of proteins, ligands and chemical probes to explore this system. We are interrogating IreK extracellular domain ligand interactions using SPR and NMR structural characterization of these domains to monitor the ligand interaction. We aim to describe IreK ligand recognition to intracellular signaling.

Chemical Validation of Methionyl tRNA Synthetase (MetRS) as a Druggable Target in *Leishmania* donovani- Poster 15 : A11172
Presenter: **Leah Torrie**, *Biochemistry Team Leader, University of Dundee*

Authors: **L S Torrie**[1]; D A Robinson[1]; L Stojanovski[1]; F Simeons[1]; S Manthri[1]; J Thomas[1]; A Neuhs[1]; K D Read[1]; I H Gilbert[1]; A H Fairlamb[1]; M De Rycker[1]
[1] *University of Dundee*

Methionyl tRNA synthetase (MetRS) has been chemically validated as a drug target in the kinetoplastid parasite *Trypanosoma brucei*. In the present study we investigate the validity of this target in the related trypanosomatid *Leishmania donovani.* Following development of a robust high-throughput compatible biochemical assay, a compound screen identified DDD806905 as a highly potent inhibitor of *Ld*MetRS (K_i 18 nM). Crystallography revealed this compound binds to the methionine pocket of MetRS with enzymatic studies confirming DDD806905 displays competitive inhibition with respect to methionine and mixed inhibition with respect to ATP binding. DDD806905 showed activity, albeit with different levels of potency, in various *Leishmania* cell-based viability assays, with on-target activity observed in both *Leishmania* promastigote cell assays and a *Leishmania tarentolae in vitro* translation assay. Unfortunately this compound failed to show efficacy in an animal model of leishmaniasis, with high levels of plasma protein binding and

sequestration of this dibasic compound into acidic cellular compartments providing possible explanations for this failure to translate. Although DDD806905 is not a developable anti-leishmanial compound, MetRS remains an attractive anti-leishmanial drug target.

Design and synthesis of cytochrome bc1 inhibitors towards the treatment of toxoplasmosis- Poster 16 : A11241
Presenter: **James Gordon**, *PhD student, University of Leeds*

Authors: **J A Gordon**[1]; C W Fishwick[1];
[1] *University of Leeds*

Toxoplasmosis is a highly prevalent apicomplexan parasite, estimated to infect around a third of the world's population, with an exceptionally broad range of outcomes ranging from apparently asymptomatic to causing potentially fatal toxoplasmic encephalitis. Current treatments are unsuitable for a number of reasons; including side effects leading to intolerance as well as crucially being unable to entirely eliminate the parasite from the host. This can then lead to the potential of recrudescence only preventable by long term prophylactic treatment. The cytochrome bc_1 complex as a vital component of the electron transport chain has been validated as promising target for protozoan infections, including evidence of upregulation in the dormant bradyzoite life stage. A number of series are being developed against this target, in particular a number of quinolone/pyridine based series. These series however have suffered from a number of flaws, most notably solubility. In this work we attempt to address a number of these intrinsic flaws while building upon the promising activity these series have obtained. By exploring simple rationally directed scaffold jumps we have reached a series of tetrahydroquinolones (THQ'S) with improved solubility while demonstrating good cellular activity and impressive *in vivo* efficacy.

Pannexin-1 mediate *Trypanosoma cruzi* Invasion into Cardiac Myocytes- Poster 17 : A11238
Presenter: **Mr. Ivan Barria**, *Universidad de Antofagasta*

Authors: **I Barria**[2]; J Guiza[2]; J Gonzalez[2]; J C Saez[1]; J L Vega[2];
[1] *Pontificia Universidad Católica de Chile, Chile;* [2] *Universidad de Antofagasta, Chile*

Introduction: *Trypanosoma cruzi*, the causative agent of Chagas disease induces repetitive cytosolic Ca^{2+} promoting *T.cruzi* invasion in cardiac cells. The aim of our study was to evaluate the role of pannexin-1 hemichannels (Panx1-HC) in repetitive cytosolic Ca^{2+} transients induced by *T.cruzi* in cardiomyocytes. Methods: Intracellular Ca^{2+} signal was evaluated by Ca^{2+} sensitive dye Fluo-3. The functional state of hemichannels was evaluated by dye uptake measurements in neonatal rat cardiomyocytes or HeLa cells stably transfected with Panx1 exposed to T.cruzi. The invasion was determinated by fluorescence imaging. Results: In cardiac cells, exposure to trypomastigotes evoked repetitive Ca^{2+} transients with a frequency of 0.026 +/- 0.004Hz similar to ATP-induced Ca^{2+} transient frequency of 0.038 +/- 0.003Hz. These effects were blocked by

[10]Panx1(100 uM) or probenecid (500 uM), which are Panx1-HC blockers. Exposure to trypomastigotes increased dye uptake 4 fold in cardiac myocytes, and 2 fold in HeLa-Panx1 cells compared to uninfected cells. This phenomenon was completely prevented by [10]Panx1(100uM). The invasion was significantly blocked by [10]Panx1 to 1.3% infected cells vs 16% infected cells under control conditions. Discussion: These results provide evidence that *T. cruzi* induced opening of Panx1-HC promotes signaling events that favour invasion in cardiac tissues.

Magnetic Hyperthermia as a Novel Approach for Treatment of Cutaneous Leishmaniasis- Poster 18 : A11201
Presenter: **Dr. Helen Price**, *Lecturer in Bioscience, Keele University*

Authors: S Oates[2]; C Hoskins[1]; N Telling[1]; **H P Price**[2];
[1] Institute for Science and Technology in Medicine, Keele University; [2] School of Life Sciences, Keele University

The use of magnetic nanoparticles to produce heat (magnetic hyperthermia) has gained considerable interest in development of novel cancer therapies due to the increased sensitivity of cancerous cells to heat shock. In the current study, we are analysing the potential of magnetic hyperthermia to be applied to the treatment of cutaneous leishmaniasis as an inducible, controlled and localised form of thermotherapy. We have confirmed there are significant 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 25°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.

Protein Tyrosine Phosphatases in malaria parasite - a potential drug targets!-
Poster 19 : A11198
Presenter: **Rajan Pandey**, *PhD student, ICGEB*

Authors: **R Pandey**[1]; R Kumar[1]; D Brady[2]; P Malhotra[1]; R Tewari[2]; D Gupta[1];
[1] International Centre for Genetic Engineering and Biotechnology, India; [2] University of Nottingham

Plasmodium phosphatases and kinases are involved in all essential processes in the parasite life cycle. Using *in silico* techniques we were able to identify 67 putative phosphatases in *P. falciparum* based on conserved domain and its substrate preference, of these 32 are identified as protein phosphatases. Among the 67 identified *Plasmodium* phosphatases, 5 were identified as potential tyrosine phosphatases (PTP1, PTP2, PRL, YVH1 and PTPLA). The tyrosine phosphatases share a common catalytic mechanism mediated by CYS, ARG and ASP residues. The protein family has a distinctive signature motif, (I/V)HCXXGXXRS, harbouring the crucial catalytic cysteine residue necessary for the PTP activity. Tyrosine phosphatases in higher eukaryotes are involved in

around 2% protein dephosphorylation reaction and play essential role in signalling, cell growth, differentiation and cell cycle progression control. Two of the *P. falciparum* PTPs (PRL and YVH1) have been biochemically analysed and are likely to be essential for parasite survival whereas PTP1, PTP2 are non-essential and PTPLA is essential during sporozoite development in *P. berghei*. PRL phosphatase is prenylated, secreted and has been shown to be involved in the host invasion process making it as potential drug target. PTPLA, is a PTP like protein where proline residue is present instead of an active arginine in the distinctive signature motif. It is present in both host and parasite. In this, we will present our results with respect to two *Plasmodium* PTPs; PRL (structure based *in-silico* drug screening) and PTPLA (cellular localization and phenotypic analysis using GFP-tag and gene knockout).

Sensitive Amperometric Immunosensor for Malaria Detection - Poster 20 : A11188
Presenter: **Ms. Aver Hemben**, *Student, Cranfiled University*

Authors: **A Hemben**[1]; I E Tothill[1];
[1] *Cranfield University*

Plasmodium falciparum histidine – rich protein 2 (*Pf*HRP 2) was selected in this work as the biomarker for the detection and diagnoses of malaria. An enzyme linked immunosorbent assay (ELISA) was first developed to evaluate the immunoreagents suitability for the sensor construction. A disposable sensor was used with gold working electrode and an integrated counter and reference electrode to develop the immunosensor for *Pf*HRP 2, and enable the low cost, easy to use and sensitive detection of malaria. The gold sensor chip was first characterised and then applied to immobilise the anti- *Pf*HRP 2 monoclonal antibody as the capture antibody. A sandwich ELISA assay format was then constructed using horseradish peroxidase (HRP) as the enzyme label and the electrochemical signal generated using 3,3,´5,5'tetramethyl-benzidine dihydrochloride (TMB) /H_2O_2 system. The performance of the assay and the sensor were optimised and characterised achieving a limit of detection of 1.05 ng mL^{-1} in buffer. The assay signal was then amplified using gold nanoparticles conjugated detection antibody-HRP and a detection limit of 0.5 ng mL^{-1} was achieved enhancing the sensor sensitivity. The assay conducted in 100% serum yielded 1.18 ng mL^{-1} and 0.25 ng mL^{-1} in buffer andAuNP assays. This sensor format is ideal for malaria detection and on-site analysis in resource-limited settings where implementation of malaria diagnostics is essential in control and elimination efforts. **Keywords:**Malaria detection, *Pf*HRP 2, parasites, immunosensor, biosensor, rapid diagnostic test.

Identification and analysis of putative homologues of innexin in *Trypanosoma cruzi*. - Poster 21 : A11200
Presenter: **Dr. José Luis Vega**, *Assistant Professor, Universidad de Antofagasta*

Authors: I Barria[1]; J Güiza[1]; J Gonzalez[1]; J L Vega[1];
[1] *Universidad de Antofagasta, Chile*

Rationale: Innexin membrane channels (innexons) play important roles in the physiology of invertebrates organisms, and they are not expressed in vertebrates. This suggests that they might be therapeutic targets in pathogenic protozoan organisms. **Methods:** We analyzed the genomes of pathogenic protozoan giardia, plasmodium, *Leishmania* and trypanosoma. The hemichannels functional state was evaluated by dye uptake measurements into *T. cruzi.* **Results:** The search showed the existence of genes encoding putative homologues of innexin channels in protozoan. Multiple sequence alignments of insect innexin with putative protozoan innexin differ substantially. In *T. cruzi* a functional assay showed that uptake of the fluorescent dye (ethidium bromide 1+, 394.8 Da) increased in divalent cation solution free, a condition known as an inducer of opening of innexons. This effect was blocked by lanthanum (200 μM) and carbenoxolone (100 μM) two drugs described as innexon blockers. **Conclusions**: Identify innexin hemichannels in *T. cruzi* could open new pharmacological target to the design effective new therapies to Chagas disease.
Acknowledgements: This work was supported by a FONDECYT 11130013 (to JL Vega), 1131007 (to J González). I Barría holds a CONICYT-PhD fellowship.

Probe development for the identification of chalcone molecular targets in *Leishmania* - Poster 22 : A11262
Presenter: **Mr Douglas Oliveira**, *PhD Student, Durham University*

Authors: **D Oliveira**[1,2]; P G Steel[1]; P Denny[1]; B Rossi-Bergmann[2]
[1] Durham University; [2] UFRJ, Rio de Janeiro, RJ, Brazil;

Chalcones are a promising new class of antileishmanials whose target is still unknown. In previous work, we have reported the selective activity of the chalcone CH8 against several *Leishmania* spp., suggesting a conserved drug target. In this work we synthesized CH8 analogues with alkyne groups for cycloaddition coupling (Huisgen-Sharpless reaction) without affecting antileishmanial activity. To discover the protein target of CH8 we then synthesized probes containing biotin (DEO53) or a fluorophore (PGS14.4), and an azide group allowing chemical linkage to the CH8 analogues. Two CH8 analogues, NAT22 and DEO37, and two probes, DEO53 (biotin-tagged) and PGS14.4 (rhodamine-tagged) were screened against *L. amazonensis* and L. major insect stage promastigotes. NAT22 and DE037 showed excellent activity against both species, IC50 0.3-2.2μM. However, the PGS14.4 and DEO53 probes had significantly decreased activity against *L. amazonensis* and L. major, probably due to their large molecular size. To allow these molecules work to reach the chalcone target(s), L .amazonensis promastigotes were permeated (digitonin), incubated with fluorescent PGS14.4 and imaged using confocal microscopy. The probe primarily localized to the mitochondria. L .amazonensis promastigotes pre-incubated with DEO37 were then lysed by sonication and a subsequent click reaction allowed the purification of bound proteins. In summary, these probes appear to be a useful tool for the identification of the chalcone target(s) in *Leishmania* spp. Supported by: CAPES, CNPq

Peroxisomes in *Toxoplasma gondii* ? - Poster 23
Presenter: **Alison J Mbekeani**, *PhD Student, Durham University*

Authors: **A J Mbekeani** [1]; W Stanley[1]; M Meissner[2]; E Pohl[1]; P W Denny[1]
[1] School of Biological and Biomedical Sciences and Biophysical Sciences Institute, Durham University;
[2] Wellcome Trust Centre for Molecular Parasitology, University of Glasgow

The metabolism of fatty acids and cholesterol is essential to all eukaryotic organisms and occurs in various organelles, including peroxisomes. Other than lipid metabolism, peroxisomes contain many enzymes involved in several different metabolic processes. One key enzyme found in most peroxisomes is catalase. Catalases take part in the neutralization of hydrogen peroxide, thereby preventing toxic build up within cells. This enzyme has overtime become a key identifier of peroxisomes in many organisms. However, this is controversial when it comes to Toxoplasma gondii. The use of catalase as a marker for peroxisomes in this apicomplexan protozoan parasite has been disputed, and in some cases lead to the belief that the *T. gondii* does not maintain these organelles. In this research project we are taking a different approach to answer this question of T. gondii peroxisomes. Through evolution *T. gondii* has maintained, within its genome, many of the genes encoding peroxisomal proteins, named peroxins (PEX). Here we investigate the presence of peroxisomes within *T. gondii* using PEX proteins. The experimental approach taken involves the characterization of TgPEX5 and TgPEX7 proteins and their associated ligands TgSCP2 and TgThiolase respectively. TgSCP2 with a C-terminal Peroxisomal Targeting Signal 1 (PTS1), binds TgPEX5, whilst TgThiolase with an N-terminal PTS2, binds TgPEX7. Using molecular biology, reverse genetics and proteomics within the tachyzoite and bradyzoite stages of this parasite, we aim to prove or refute the presence of peroxisomes within *T. gondii*.

The role of host and parasite sphingolipid biosynthesis enzymes in *Toxoplasma gondii* proliferation and invasion- Poster 24
Presenter: **Amjed Q. Ibrahim Al-Qaisi**, *PhD Student, Durham University*

Authors: **A Q Ibrahim Al-Qaisi**[1,2] H Shams-Eldin[3], R T Schwarz[3] and P W Denny [1]
[1] School of Biological and Biomedical Sciences, Durham University, UK, [2] Biology Department, College of Science, Baghdad University, Iraq, and [3]Institute for Virology, Philipps-University Marburg, German

The phylum Apicomplexa includes many protozoan parasites that cause serious human and animal disease, for example *Plasmodium* (malaria), *Eimeria* (coccidiosis) and *Toxoplasma* (toxoplasmosis). Treatments against these parasites are limited and novel solutions are urgently required. Recently, research has focused on parasite specific features of lipid biosynthesis as drug targets. In particular the biosynthesis of sphingolipids, which have essential roles in many processes, has been highlighted as a potential target. Using the model apicomplexan *Toxoplasma gondii* we are studying the role of parasite and host sphingolipid biosynthesis in invasion and proliferation. To do this we are functionally characterizing the *Toxoplasma* sphingolipid biosynthetic pathway by focusing on the enzyme that catalyzes the first step in sphingolipid biosynthesis, serine

palmitoyltransferase (SPT). This activity is encoded by 2 differentially expressed conserved genes in *Toxoplasma* - *Tg*SPT1 and *Tg*SPT2. In parallel, the response of the host sphingolipid biosynthetic pathway to parasite infection is being investigated. Results so far demonstrate that the expression of Chinese Hamster Ovary cell host SPT and primary sphingomyelin synthase (SMS2) are unaffected by *Toxoplasma* infection, indicating that parasite manipulation of host sphingolipid biosynthesis does not occur. This supports the hypothesis that *Toxoplasma* is dependant on *de novo* biosynthesis. Metabolic labelling has shown that several distinct complex sphingolipids are synthesized independently by the parasite. Interestingly, the fungal inositol phosphorylceramide (IPC) synthase inhibitor aureobasidin A (AbA) has been reported to target *Toxoplasma* IPC synthesis. However, our results demonstrate that whilst AbA, and an orthologue, are active against the parasite, their effect on *Toxoplasma de novo* sphingolipid biosynthesis is negligible. In addition, by using *Leishmania major* as a model we are studying the role of two compounds recognised as inhibitors of IPC synthase in this kinetoplastid protozoan parasite, clemastine and a benzazepane. This work involves studying the effects of these two compounds on metabolites related sphingolipid biosynthesis. The results showed that ceramide levels increased in treated parasites, perhaps leading to parasite death via apoptosis.
Together these approaches will lead to further understanding of the roles of sphingolipid biosynthesis in parasitism and also demonstrate the possibilities of targeting the parasite pathway for therapeutic intervention. Future work will establish the localization of *Tg*SPT1 and 2, and attempt to knockout the genes encoding these proteins to study the role of *Toxoplasma* sphingolipid biosynthesis in parasite invasion and proliferation, and also its validity as a drug target.

Characterisation of *Mycobacterium tuberculosis* genes involved in *de novo* pimelate biosynthesis – Poster 25
Presenter: **Musa Gugu**, *PhD student, Durham University*

Authors: **F M Gugu[1]; J J Harburn[1]; J D Sellars[1]; A K Brown[1]**
[1] School of Medicine, Pharmacy and Health, Durham University, Queens Campus, Stockton-on-Tees, UK

Mycobacterium tuberculosis (TB) is estimated to cause 9.6 million cases and 1.5 million deaths annually. In 2014, an estimated 190,000 people died of drug resistant strains of TB, with the highest number of positive tests for drug resistance being observed than ever before. The intrinsic resistance of the organism to a variety of antibiotics can be attributed to the complex nature of its cell wall. Biotin is an important micronutrient essential for the biosynthesis of this complex *M. tuberculosis* cell wall and is required by several enzymatic pathways as a critical cofactor. Central to the biosynthetic pathway of biotin is the priming substrate methyl-pimelate which is synthesized via a similar pathway to fatty acid biosynthesis. Methyl pimelate is utilised in the synthesis of biotin through an omega-methyl-hydrolase enzyme, BioH. The enzymes involved in the pimelate biosynthesis pathway are yet to be identified and characterised in *M. tuberculosis*. In this study, we have identified candidate enzymes which are potentially involved in pimelate biosynthesis in *M. tuberculosis* through a preliminary bioinformatics analysis using *Escherichia coli* sequences. BlastP

analysis against the *M. tuberculosis* H37Rv genome identified homologous protein sequences. *M. tuberculosis* H37Rv genes have been cloned into a variety of mycobacterial and *E. coli* expression system to enable Immobilized Metal Affinity Chromatography purification of recombinant protein candidates. We have demonstrated that proteins involved in pimelate biosynthesis in other bacteria can be identified in *M. tuberculosis* and purification of these proteins can be achieved. Future studies will involve biochemical and inhibition studies of the candidate proteins in order to generate novel anti-tubercular agents.

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Information for participants staying at Collingwood College

If you have booked University accommodation for the Conference, you will be accommodated at **Collingwood College**.

Collingwood College is one of the most modern of all Durham University's colleges and offers all the essential amenities for any event, from international conferences for hundreds of delegates to group trips exploring the riches of the area.

Bedroom Facilities

The College has 200 en-suite bedrooms and 307 standard bedrooms.

All bedrooms have the following facilities:

- Tea and coffee making facilities refreshed daily
- Free internet connection Wi-Fi available in the bedrooms
- Hollow fibre filled pillows
- Bathrooms with shaver socket outlets
- Toiletry pack containing Shower gel/Shampoo, Soap, Body Lotion and a vanity

Check In

Rooms are guaranteed to be ready by 2pm. The reception will be open from 9am-5pm**.** Outside these times, a porter will be available to show you to your room. Please use the internal telephone near to reception to call the porter if he is away from his post.

Check Out

Check out is before 10am.

Breakfast

For participants resident in Collingwood College, breakfast will be served in the college's dining hall from 8am–9am. Early breakfasts can be arranged on request.

Guest information

Smoking

Smoking is prohibited in all enclosed public places in the UK. A designated outdoor smoking area is provided at each College.

Business and Internet Facilities

Free wireless internet access is available in college and all University buildings. Login details will be provided at registration. Fax and photocopying facilities are available at the College, subject to a small charge.

Electrical Appliances

Plugs and sockets in the UK have voltage between 220-240V. Please bring appropriate adaptors for your mobile phone recharger and other appliances.

Emergency

In case of an emergency, to report any suspicious behaviour, accidents, if you require any medical attention or any other assistance please contact Reception.

Parking

Car parking is available at the college, but limited and strictly on a first come first serve basis. No spaces can be reserved. Car details will need to be registered on arrival at College Reception.

Directions to college

Set in tranquil woodland, Collingwood is close to the city centre, Durham Botanic Garden and the Oriental Museum.

Public Transport Directions

Durham Railway station is 2 miles away. From the city bus station - a short walk from the railway station – take the Arriva No 6 Bishop Auckland service which runs every 15 minutes past the Colleges on South Road.

Road Directions

Take the A690 from the A1 (M), turn left across Elvet Bridge over the traffic lights. Follow the road to New Inn crossroads, go straight over. Collingwood is the third turning on the left hand side.

For further information on how to get to Durham, please refer to the Travel section at the end of this brochure.

Travel information: How to get to Durham

By Air

NEWCASTLE AIRPORT

Newcastle airport is approximately 20 miles north of Durham. There are connecting flights from a number of European hubs including London Heathrow & Gatwick, Amsterdam, Paris and Brussels, and the airport is also served by a number of budget airlines. See www.newcastleairport.com for full details.

The Metro is available from the airport to Newcastle Central Station. The journey takes approximately 20 minutes, and Metro trains run every 7 minutes.

If you are arriving in the evening we recommend taking a taxi from the airport to avoid the risk of missing the last trains south (see taxi section).

DURHAM TEES VALLEY

Durham Tees Valley is approximately 25 miles south of Durham. Although it has fewer flights than Newcastle, there are connecting flights with Amsterdam and other cities, and it is served by budget airlines. See www.durhamteesvalleyairport.com for further details.

To reach Durham, the Tees Valley Sky Express (737) bus service runs between the airport and Darlington train station and is free for air travellers. The timetable can be viewed at www.skyexpress.co.uk.

By Train

60 InterCity trains from most major centres in the country call at Durham daily including 14 trains from London. The National Express high speed service takes under 3 hours from London King's Cross on the main East Coast line. First Transpennine Express offers frequent links to Manchester, Sheffield and Leeds,
while Cross Country links Durham with Scotland, the Midlands and the South West.
For train times and fare information call National Rail Enquiries on 08457 48 49 50 or +44(0)20 7278 5240 from overseas (note international rates apply), or visit www.nationalrail.co.uk. The following website is also useful for checking train times www.thetrainline.com

By Public Transport

There are several express coach services daily from most major cities. Durham is well served by both regional express services and the local bus network.
From the city bus station - a short walk from the railway station – take the Arriva No 6 Bishop Auckland service, which runs every 15 minutes past the Colleges on South Road. For more information visit:
http://www.traveline.info/
http://www.durham.gov.uk/busmap

By Car

Durham city centre is only two miles from the A1 (M). Leave the motorway at Junction 62 on the A690 Durham - Sunderland road and follow signs to Durham City Centre.

Durham City is served by three Park and Ride sites on the key routes into the city. These are situated at Belmont, Sniperley and Howlands. A direct bus service runs every 10 minutes to the city centre from each site. The sites offer free parking for users of the Park and Ride bus service and unlimited daily travel into the city centre and back for just £2.00 per person. Park and Ride users can also use the Cathedral Bus for free.

By Taxi

Taxis are available from Newcastle and Durham Tees valley airports.

Airport Taxis is the official taxi service for Newcastle International Airport. To make a booking, or for further information, contact:

Tel: +44 (0) 191 214 6969
Web: www.airport-taxis.co.uk

In Durham, taxis are available from the train station, on the opposite northbound side, accessible by a subway. The taxi journey to the Colleges takes 5–10 minutes.

Useful Durham Taxi Numbers:

Paddy's Taxis	0191 3866662
Pratts Taxis	0191 3864040
Durham Taxis	07961 818464
Pollys Taxis	07774634765
Dunelm Taxis	0191 3831122
Macs Taxis	0191 3841329

Durham station is in B1 on the attached map. Newcastle airport is less than 1 hour away by taxi.

The accommodation and breakfast is at Collingwood College. No. 6 on the map.

The symposium and lunches are a short walk away at the Calman Learning Centre – No. 43 on the map.