POSTER SUBMISSIONS

Take the opportunity to discuss cutting edge research during the poster presentations:

Even Numbers: Day One, 16:45 - 18:00, Foyer
Odd Numbers: Day Two, 12:50 - 14:30, Foyer

★ **Poster Flash Talk**

1. ★	**Annotating plant genomes using Nanopore direct RNA sequencing**
	Matthew Parker *et al.,* University of Dundee
2.	**Cryo-electron microscopy in the University of Edinburgh as tool for structural and cell biology**
	Maarten W. Tuijtel *et al.,* University of Edinburgh
3. ★	**Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated editing of the *SNCA* gene**
	Yixi Chen *et al.,* University of Edinburgh
4. ★	**3D-organotypic models and a role for connexin signalling in Psoriasis**
	Patricia Martin *et al.,* Glasgow Caledonian University
5. ★	**Inferring cell state transition dynamics from pluripotent stem cell heterogeneity**
	Linus Schumacher *et al.,* University of Edinburgh
6. ★	**Investigating the invasive behaviour of ovarian cancer using optical coherence tomography**
	Amelia Hallas-Potts *et al.,* University of Edinburgh
7.	**Tau antibodies for the detection, management and treatment of Alzheimer's disease**
	Richard Lofthouse, University of Aberdeen
8.	**Analysis of chemical ecology in actinomycetes by mass spectrometry-based metabolomics techniques**
	Laia Castaño *et al.,* University of Strathclyde
9.	**Engineering a multicellular patterning platform to control cell signalling**
	Fokion Glykofrydis *et al.,* University of Edinburgh
10. ★	**A dual-assay platform for contraceptive screening**
	Franz S. Gruber *et al.,* University of Dundee
11.	**Machine Learning for Super Stable Transcripts**
	Jessica Birt *et al.,* University of Edinburgh
12.	**Bayesian networks for biological systems: Do I have enough data?**
	V. Anne Smith, University of St Andrews
13.	**Whole-genome sequencing approaches to elucidate the evolution of chromosome organization in *C. auris***
	Zoe Ross *et al.,* University of Aberdeen
14.	**Exploiting the natural solvent tolerance of Pseudomonads for plastic platform chemical production**
	Charles Begley, University of Strathclyde

15.	**How does a trypanosome change its spots? Decrypting immune avoidance in human trypanosomes**
	Felix Warren *et al.,* University of Glasgow
16.	**Mechanistic, structural, and functional studies of secreted heparan sulfate degrading enzymes**
	Agnieszka Bogucka *et al.,* University of St Andrews
17.	**Engineering Sulfatases for Biomedical Applications: Biochemical and Structural Characterisation of the Extracellular Sulfatases Sulf1 and Sulf2**
	Catriona Haberland *et al.,* University of St Andrews
18.	**Keap-ing Nrf2 in check for neuroprotection in Alzheimer's disease**
	Fiona Kerr *et al.,* Glasgow Caledonian University
19.	**Analysis of the Trypanosoma *congolense* cell division cycle**
	Emily J. Allan *et al.,* University of Glasgow
20.	**Cas12 derived synthetic transcription factors for easy, synergistic transactivation**
	James Bryson *et al.*, University of Edinburgh
21.	**Developing a Super-Resolution Method for the Detection of Alpha-Synuclein Aggregates**
	Alex Chappard, University of Edinburgh
22.	**Combining CRISPR genome editing with UK biobank analysis suggests a role for a novel enhancer in male alcohol abuse and chronic anxiety**
	Alasdair MacKenzie *et al.,* University of Aberdeen
23.	**Rotational 3D Microscopy for Organoid Studies**
	Ewa Guzniczak & Graeme Whyte, Heriot Watt University
24. ★	**Four-dimensional Super-Resolution imaging in living intact pancreatic islets**
	Adrian Garcia-Burgos *et al.,* Heriot-Watt University
25. ★	**A low cost electrochemical platform for detection of DNA antibiotic resistance genes and iron scavenging agents from potentially pathogenic bacteria**
	Andrew Ward *et al.,* University of Strathclyde
26.	**Immunoassay on complementary metal oxide semiconductor photodiode array**
	Ana-Maria Nastase *et al.,* University of Glasgow
27.	**Using the Scottish biomass, draff: a circular economy approach towards biomass use**
	Flora Foltanyi *et al.,* University of St Andrews
28.	**Using *Clostridium difficile* LMW SLP contributes to acute disease in the mouse**
	Filipa Baltazar Da Costa Vaz *et al.,* University of Glasgow
29.	**Visualising α-Synuclein oligomers using super resolution microscopy**
	Craig Leighton *et al.,* University of Edinburgh
30.	**Polyomics analyses reveal a role for nucleobase transporter in gentamicin-attenuated *Leishmania mexicana***
	Abdulbaset M Kabli *et al.,* University of Glasgow
31.	**Implementing CRISPR-Cas9 System for Enhanced Production of Taxol Oxygenated Precursors in *Saccharomyces cerevisiae***
	Behnaz Nowrouzi *et al.,* University of Edinburgh
32.	**Functional Characterisation of the Translocator Protein (TSPO), implications for Age-Related Macular Degeneration (AMD)**
	Lincoln Biswas *et al.,* Glasgow Caledonian University

33.	**Advanced bioprocessing strategies for Taxol biosynthetic pathway optimisation using *S. cerevisiae* microbial cell factories**
	Laura Walls *et al.,* University of Edinburgh
34.	**Multimodal, multiphoton platform for label-free nonlinear optical imaging**
	Alison McDonald *et al.,* University of Edinburgh
35.	**Ubiquitin Conjugating Enzymes in *Leishmania*: When do they work, with whom, and can they be targeted?**
	Daniel Harris *et al.,* University of Glasgow

Flash Talk - Day 2

1. Annotating plant genomes using Nanopore direct RNA sequencing

Authors: **Matthew Parker,** Kasia Knop, Anya Sherwood, Nick Schurch, Geoff Barton and Gordon Simpson

Affiliation: School of Life Sciences, University of Dundee, United Kingdom

E-mail: m.t.parker@dundee.ac.uk

Abstract:

Producing high quality transcriptome annotations of crop genomes is arguably as important as generating the genome sequences themselves, because it facilitates understanding of what those genomes encode. This is essential for rational genetic improvement and gene editing strategies. Current genome annotation pipelines often involve RNA sequencing using short read sequencing methods. Producing high quality transcriptomes from short reads involves complex reassembly methods which stitch reads back together and try to infer the patterns of RNA processing events such as splicing and poly(A) site choice. Nanopore direct RNA sequencing, however, produces full length single molecule reads, in which information on all RNA processing events, including splicing, 5' and 3' site choice, poly(A) tail length, and RNA modifications, can be captured unambiguously. The poster will present work we have done to optimise Nanopore direct RNA sequencing for this purpose in *Arabidopsis,* and the application of this work to water yam (*Dioscorea alata)* and Shea (*Vitellaria paradoxa*).

Notes:

2. Cryo-electron microscopy in the University of Edinburgh as tool for structural and cell biology

Authors: **Maarten W. Tuijtel**, Marcus D. Wilson

Affiliation: Wellcome Centre for Cell Biology, University of Edinburgh

E-mail: Maarten.Tuijtel@ed.ac.uk

Abstract:

Cryo-electron microscopy (Cryo-EM) can be used to study biological material in its near-native state at high resolution. The Cryo-EM Facility of the University of Edinburgh offers a wide range of techniques to address a variety of scientific questions, ranging from cell biology to structural biology of a single protein. We offer training and support for 2D-imaging, Single-Particle Analysis and 3D reconstruction, Electron Cryo-Tomography and Electron Diffraction. Depending on the project, users can be trained in sample-preparation techniques, operation of the Cryo-EM, and data processing. In the near future, we are hoping to expand our techniques to include (Cryo-) Correlative Light and Electron Microscopy (CLEM) and Focussed-Ion-Beam (FIB) milling, which allows access to thick mammalian cells.

We welcome new users and projects, please get in touch: cryoem@ed.ac.uk!

Notes:

3. Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated editing of the *SNCA* gene

Authors: **Yixi Chen[1,2],** Karamjit Singh Dolt[1], Nicola J Drummond[1], Maurice A Canham[1], Susan Rosser[2], Terry Baker[3], Marco Kriek[3], Patrick Downey[4], Tilo Kunath[1,2]

Affiliations:
[1]MRC Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, UK.

[2]UK Centre for Mammalian Synthetic Biology, The University of Edinburgh, Edinburgh, UK

[3]UCB Pharma Ltd., 208 Bath Road, Slough, SL1 3WE, UK

[4]UCB BioPharma Sprl., Chemin du Foriest 1, 1420 Braine-l'Alleud, Belgium

E-mail: s1573487@sms.ed.ac.uk

Abstract:

An emerging treatment for Parkinson's is cell replacement therapy. Authentic midbrain dopaminergic (mDA) neuronal precursors can be differentiated from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs). These laboratory produced mDA cells can mature into functional dopaminergic neurons and are currently in clinical trial of Parkinson's. However, clinical trials with human fetal mesenchephalic cells have shown that cell replacement grafts in Parkinson's are susceptible to Lewy body formation suggesting host-to-graft transfer of α-synuclein pathology.

Here we have used CRISPR/Cas9 technology to delete the endogenous α-synuclein gene (*SNCA*) in a clinical-grade hESC line to generate $SNCA^{+/-}$ and $SNCA^{-/-}$ lines. These hESC lines were first differentiated into mDA neurons, and then challenged with recombinant α-synuclein pre-formed fibrils (PPFs) to seed the formation for Lewy-like pathology as measured by phosphorylation of serine-129 of α-synuclein (pS129-αSyn).

Wild-type neurons were fully susceptible to the formation of protein aggregates positive for pS129-αSyn, while $SNCA^{+/-}$ and $SNCA^{-/-}$ neurons exhibited significant resistance to the formation of this pathological mark, conferring a measure of resistance to Lewy pathology.

Since α-synuclein may have important functions in synaptic terminals and resistance to neuro-invasive viruses, future work will assess biological functions of these genetically manipulated neurons.

Notes:

4. 3D-organotypic models and a role for connexin signalling in Psoriasis

Authors: **Erin M O'Shaughnessy[1],** Laura Garcia-Vega[1], William A Duffy[2], Mozheh Zamiri[2,3], Patricia E Martin[1]

Affiliations:
[1] Department of Life Sciences, School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, G4 0BA, UK; [2]Department of Dermatology, University Hospital Crosshouse, Kilmarnock KA2 0BE, UK, [3] Department of Dermatology, Queen Elizabeth University Hospital, Glasgow, UK

E-mail: Patricia.Martin@gcu.ac.uk

Abstract:

Psoriasis is considered as a T-cell driven disease (inside-out). Elevated levels of opportunistic skin pathogens e.g. *Staphylococcus aureus* play a role in disease progression (outside-in). Connexin26 (Cx26), a gap junction protein, is upregulated in psoriatic tissue and we hypothesise is an important driver of psoriasis.
Biopsies from normal and psoriatic patients (PP) were processed for immunohistochemistry. Keratinocytes were exposed to peptidoglycan (PGN) isolated from *S. aureus* for 6 hours in the presence or absence of Cx-channel blockers, ELISA assays monitored IL-6 levels, Immunofluorescence determined protein expression. Protein expression profiles of 3D-organtoypic models of human keratinocytes and fibroblasts derived from normal and PP were assessed.
Cx26 protein levels were upto 40x higher in psoriatic epidermis. Cx43 remained restricted to basal layers, but was post-translationally modified. In a keratinocyte model cell-line PGN induced enhanced Cx26 but reduced Cx43 and E-cadherin expression. Exposure to Cx-channel blockers attenuated the PGN induced IL-6 response. In 3D- organotypic models PP-fibroblasts had a higher pro-inflammatory status than normal keratinocytes. In keratinocytes cultured on PP-fibroblasts Ki67 and Cx26 expression were enhanced while E-cadherin levels were reduced.
In conclusion, Cx26 expression in psoriatic keratinocytes is driven by outside-in and inside-out mechanisms and inhibition of Cx26 is of therapeutic benefit.

Funded by the Psoriasis Association

Notes:

5. Inferring cell state transition dynamics from pluripotent stem cell heterogeneity

Authors: **Linus Schumacher [1],** Jochen Kursawe [2], Valerie Wilson [1], Anestis Tsakiridis [3], Alexander Fletcher [4]

Affiliations:
[1] MRC Centre for Regenerative Medicine, University of Edinburgh, UK
[2] School of Mathematics and Statistics, University of St Andrews, UK
[3] Department of Biomedical Sciences, University of Sheffield, UK
[4] School of Mathematics and Statistics, University of Sheffield, UK

E-mail: Linus.Schumacher@ed.ac.uk

Abstract:

The notion of cell states is increasingly used when classifying cellular behaviour in development, regeneration, and cancer. This is driven in part by a deluge of data comprising snapshots of cell populations at single-cell resolution. Yet quantitative predictive models of cell state transitions remain lacking. Such models would allow us to fully leverage datasets to gain a quantitative understanding of cell state transitions; and help production of specialised cell types from stem cells in vitro.

We explore to what extent cell state transition rates can be inferred quantitatively from snapshot data, for early cell fate decisions in primitive streak-like populations derived from epiblast stem cells. We adopt a Bayesian inference approach to infer cell state transition rates and their uncertainties, using in vitro data of transcription factor expression in culture conditions maintaining pluripotency. With this data-driven modelling approach we identify statistical dependencies between transcription factors indicating regulatory interactions through Bayesian model comparison. We compare models of varying complexity to the available data, and compute each model's evidence, and can thus incorporate model uncertainty into any predictions. This method is be generally applicable to binary gene expression data from cell populations, and can be extended to analyse single-cell level clonal data.

Notes:

6. Investigating the invasive behaviour of ovarian cancer using optical coherence tomography

Authors: **Amelia Hallas-Potts[1],** Jessica Lim[1], Jonathan Mason[2], Michael Churchman[1], Charlie Gourley[1], Pierre Bagnaninchi[2], C. Simon Herrington[1]

Affiliations:
[1]Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh, EH4 2XR, UK
[2]Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, EH16 4UU, UK

E-mail: s1325283@sms.ed.ac.uk

Abstract:

Ovarian tumours commonly spread along peritoneal surfaces and rarely invade deep into tissues. To accurately investigate ovarian tumour behaviour *in vitro* a biological technique that recapitulates the *in vivo* behaviour over time is required.

Live imaging of multiple samples over 5 days was established on a commercial optical coherence tomography (OCT) system. This has been applied to ovarian cancer cell lines cultured on top of a collagen/fibroblast matrix. Live imaging was used to record cell line behaviour over several days without damage to the samples, and imaging of multiple samples over time was achieved. Explant techniques are currently being developed to investigate the behaviour of patient primary tumour and omentum samples *ex vivo* to develop a tumour explant assay; to date, images have been acquired over a one month time course.

The development of this assay provides a long-term live imaging tool to investigate therapeutics that inhibit tumour attachment and invasion into the omentum. In the future it will be important to determine whether this assay is predictive of *in vivo* tumour behaviour and whether therapeutic response can be replicated *ex vivo*.

Notes:

7. **Tau antibodies for the detection, management and treatment of Alzheimer's disease**

Authors: **Richard Lofthouse**

Affiliations:
The University of Aberdeen, The Scottish Biologics Facility and TauRx

E-mail: r03rl17@abdn.ac.uk

Abstract:

Clinical trials for the treatment of Alzheimer's disease (AD) are being hampered by a distinct inability to detect future patients at an early enough stage for new or the currently available approaches to be effective. New methods to diagnose the early stages of AD and with a greater degree of certainty, are therefore required. Tau protein appears to hold a central role in the pathology of AD and therefore there is growing interest in the diagnostic merits of measuring Tau levels in both CSF and plasma.

Using phage display we have generated a large number of single chain antibody fragments (scAbs) that recognise Tau. With the use of next generation protein interaction technologies, we have been able to extensively characterise these binders. Carrying forward those with the best properties to create a panel of antibodies capable of recognising a wide variety of Tau fragments with high specificity and affinity (pM range). We hope to use this antibody panel to study the blood of patients with Alzheimer's to see if we can detect Tau fragments that are upregulated due to disease. Whilst also investigating the therapeutic potential of these antibodies as inhibitors of tau aggregation.

Notes:

8. Analysis of chemical ecology in actinomycetes by mass spectrometry-based metabolomics techniques

Authors: **Laia Castaño**[1] Paulina Rakowska[2] Blair Johnston[1,2] Michael Chrubasik[1] and Katherine R Duncan[1*]

Affiliations:
[1]Strathclyde Institue of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom
[2] National Centre of Excellence in Mass Spectrometry Imaging, National Physical Laboratory, Teddington, United Kingdom

E-mail: laia.castano-espriu@strath.ac.uk

Abstract:

Mass spectrometry-based metabolomics has become a powerful tool for the study of chemical ecology. With the recent advances of mass spectrometry technologies and bioinformatics tools it is now possible to study the interactions with the environment and within microorganisms living in the same ecological niche. The bacterial order actinomycetales are responsible for the production of 65-70% of microbially produced specialised metabolites with diverse biological activities. Some actinomycetale strains contain over 30 biosynthetic gene clusters encoding for these metabolites. However, it is estimated that only 10% of these genes are typically transcribed in a mono-culture setting (normal laboratory conditions). Furthermore, it has been observed that microbial interactions may induce these cryptic gene clusters providing a defence mechanism. Microbial interactions were assessed in tri-cultures (three strains) and co-cultures (two strains) using 49 actinomycetale strains, two *Pseudomonas* and one *Bacillus* strain. It was found that 29 strains showed altered phenotypes as a result of bacterial competition. The analysis of these interactions with LC-MS revealed the production of metabolites that were specific to the tri-cultures and co-cultures. Our findings suggested that microbial interactions induced the production of metabolites. These interactions, will be subjected to imaging mass spectrometry in order to visualize the distribution of metabolites directly from solid media.

Notes:

9. **Engineering a multicellular patterning platform to control cell signalling**

Authors: **Fokion Glykofrydis**, Elise Cachat, Elaine Dzierzak, Jamie A. Davies

Affiliations:
Centre for Discovery Brain Sciences, The University of Edinburgh

E-mail: s1316049@sms.ed.ac.uk

Abstract:

Tissue engineering strategies based on stem cell differentiation and organoid formation suffer from variable efficiency and disorganization of anatomical domains. Whereas significant work has focused on understanding the composition of stem cell niches *in vivo*, bottom-up efforts in engineering synthetic niches *in vitro* have been limited. Here, we present our work in generating cell-based, self-organizing platforms to control cell signalling using synthetic biology. First, we program pattern-formation in mammalian cells, so that heterotypic adhesions drive the formation of hyperuniform cell distributions. CRISPR genome editing was used to generate transgenic HEK-293 lines each expressing heterophilic ICAM-1/MAC-1/LFA-1 integrins and dedicated fluorescent reporters in a drug-inducible manner. Molecular and cell-based assays show successful overexpression of adhesion molecules coupled to enhanced adhesion to heterotypic cells. We observed the arrangement and patterning of cells by fluorescence microscopy at various cell ratios. Moreover, we coupled Wnt3A production to a pre-established phase-separation patterning system, driving organized ligand emission from self-organizing multicellular islands. We co-cultured engineered Wnt3A producers with murine E11.5 metanephric mesenchyme, and evaluated the induction of nephrogenesis via immunofluorescence. Through this work, we aim to provide proof-of-concept that patterned multicellular systems can impart control in cell-fate decisions *in vitro*, contributing to the development of synthetic niches.

Notes:

10. **A dual-assay platform for contraceptive screening**

Authors: **Gruber FS[1,2],** Johnston Z[1,2], Barratt CL[2], Andrews P[1]

Affiliations:
1, National Phenotypic Screening Center, School of Life Sciences, University of Dundee
2, Division of Systems Medicine, School of Medicine, University of Dundee

E-mail: f.s.gruber@dundee.ac.uk

Abstract:

Current male methods of contraception are limited to condoms, withdrawal or vasectomy. The development of new male contraceptives has been lagging behind due to lack of an effective high-throughput phenotypic screening platform. We have developed an automated robotic screening platform, which quantifies compound activity against two key attributes of human sperm: motility and acrosome reaction. This platform is currently capable of screening up to ~2,400 cpds/day – a big leap forward for drug discovery in andrology. We have screened several libraries comprising a total of about ~30,000 cpds and we are currently screening our NPSC diversity library (~100,000 cpds). We have gathered the first starting points, which passed medicinal chemistry triaging. Those compounds are being investigated in functional assays for their potential as a safe and effective male contraceptive.

Notes:

11. **Machine Learning for Super Stable Transcripts**

Authors: **Jessica Birt**, Matthew Dale, Susan Rosser

Affiliations:
University of Edinburgh

E-mail: s1672446@sms.ed.ac.uk

Abstract:

Artificial intelligence techniques, such as machine learning, are providing promising new approaches to the rational design of biological circuits. Using sequence data alone, models predicting biological behaviour can be developed and used to screen novel sequences for potential activity. We are applying machine learning algorithms to large scale datasets of mRNA half-lives to determine the contribution of 3' UTR regions to transcript stability. Insights derived from this model can be used to generate super stable transcripts not found in nature, which we are now testing in mammalian cell lines. Such transcripts have potential applications in such areas as RNA-based therapeutics, biologics production and vaccinations.

Notes:

12. **Bayesian networks for biological systems: Do I have enough data?**

Authors: **V. Anne Smith**

Affiliations:
School of Biology, University of St Andrews

E-mail: vas1@st-andrews.ac.uk

Abstract:

Bayesian networks are flexible, probabilistic statistical models capable of modelling many types of interactions (e.g., linear, U-shaped, combinatoric) simultaneously, and which excel at distinguishing direct from indirect influence. They can handle noise and stochastic process, making them well-suited to modelling biological systems. However, Bayesian networks are notoriously 'data-hungry', requiring hundreds or even thousands of data points to fully recover an underlying network. In many biological applications, a high number of variables and relatively low number of data points means that only a partial network recovery – the strongest interactions – are expected. However, some systems, such as neuronal systems, produce enough data that full network recovery may be possible. But how is one to know whether more data would recover yet more interactions? Here, I present investigations into and techniques for assessing whether a given dataset is recovering the majority of a system of interest.

Notes:

13. **Whole-genome sequencing approaches to elucidate the evolution of chromosome organization in *C. auris***

Authors: **Zoe Ross**, Gustavo Bravo Ruiz, Alexander Lorenz, Neil Gow

Affiliations:
MRC Centre for Medical Mycology, University of Aberdeen

E-mail: z.ross@abdn.ac.uk

Abstract:

The fungal pathogen *Candida auris* has emerged in the last 10 years as a multidrug resistant yeast and is causing hospital outbreaks worldwide. The infections caused by *C. auris* are invasive and have high mortality rates. This yeast can also colonize a patient's skin and persist on surfaces for weeks making it an important clinical concern. Four geographical clades of *C. auris* have been identified from South Asian, East Asian, South American and South African locations. Isolates within a clade are almost clonal, but strains from different clades are genetically distinct from each other, differing by thousands of SNPs.

To understand the intra-species genome evolution of *C. auris*, and to test whether there is more than just sequence difference, we generated whole-genome sequences of *C. auris* clinical isolates from each of the four clades using Nanopore and Illumina sequencing platforms. Complete genome sequences were assembled into chromosome-sized contigs, revealing multiple chromosome rearrangements between isolates of different clades. Evolutionary aspects of the chromosome biology of this pathogen will be discussed.

Notes:

14. **Exploiting the natural solvent tolerance of Pseudomonads for plastic platform chemical production**

Authors: **Charles Begley**

Affiliations:
The University of Strathclyde, IBioIC

E-mail: charles.begley@strath.ac.uk

Abstract:

The use of sub-optimal chassis microorganisms such as *E. coli* for whole cell biocatalysis of organic solvents leads to processes fraught with cell viability/toxicity issues, which can be attributed to the interactions of solvents with cell membranes. Hydrophobic solvents partition into cell membranes, disrupting bilayer integrity resulting in cell death
The well documented natural tolerance of *P. putida* to industrially and environmentally relevant solvents such as toluene, benzene, cyclohexane, & p-xylene, has led to interest in applying these organisms for the industrial scale production of organic solvents. Its metabolic flexibility and ability to tolerate oxidative stress, coupled with their extensive efflux repertoire makes *P. putida* an excellent organism for industrial processes as well as bioremediation
While some of the solvent-tolerance conferring mechanisms of *P. putida* have been elucidated e.g. reduced membrane permeability, headgroup alteration, vesicle formation and efflux, the identification of the genetic basis of long-term solvent tolerance mechanisms would be greatly beneficial for the engineering of industrially relevant chassis organisms. We are using a variety of functional genomic approaches to determine the molecular basis of *P. putida* tolerance towards high value solvents used by the plastics industry.

Notes:

15. **How does a trypanosome change its spots? Decrypting immune avoidance in human trypanosomes**

Authors: **Felix Warren,** Richard Burchmore, Richard McCulloch, Catarina Gadehla, Martin Llewellyn

Affiliations:
The University of Glasgow, MRC

E-mail: f.warren.1@research.gla.ac.uk

Abstract:

For a pathogen, avoiding the host immune system is crucial for its survival. Trypanosoma brucei have a well described process for host immune avoidance using variable surface glycoproteins (VSG), called antigenic variation. This process is thought to rely on transcriptional and recombinational mechanisms producing a huge diversity of VSG mRNA, which is largely dependent on the presence of VSG pseudogenes. How and where VSG pseudogenes recombine to form VSGs is unknown, and how VSG coat diversity reflects VSG mRNA diversity is unknown. More widely, if and how genomic changes infer surface protein rearrangements under immune pressures are not well defined in related species. Importantly, in Trypanosoma cruzi and Leishmania spp., strategies for immune evasion are much less clearly understood and any link between genomic, transcriptomic and surface proteome variation due to immune pressure has not been examined. Using new techniques not previously undertaken in these parasites for elaborating their spatial proteomics, we hypothesise that following immune pressures, proteomic evasion strategies may be connected to genomic rearrangements of the kinetoplastids examined here. By adding to the current knowledge of proteomic developments in these parasites we hope to further inform basic biology and potential translational therapies.

Notes:

16. **Mechanistic, structural, and functional studies of secreted heparan sulfate degrading enzymes**

Authors: **Agnieszka Bogucka,** Laura S. Griffin, Tracey M. Gloster

Affiliations:
University of St Andrews

E-mail: ab420@st-andrews.ac.uk

Abstract:

There is limited structural and functional insights into sulfatases despite these enzymes being implicated in a number of lysosomal storage diseases and even cancer metastasis. This information is needed to aid in design of treatments such as enzyme replacement therapy, gene therapy, substrate reduction therapy, or molecular chaperone therapy for lysosomal storage diseases. Design of specific enzyme inhibitors, or modulators, which could act as molecular chaperones and allow for correct folding of the proteins and/or improvement of activity of these enzymes is of interest, which would overcome the main disadvantage encountered by other treatments of crossing the blood brain barrier. We are studying the human N-acetyl glucosamine-6-sulfatase, N-sulfoglucosamine-3-sulfatase, and glucuronate 2-sulfatase that are involved in heparan sulfate degradation in the lysosome. The project is focused on over-expressing the enzymes, with a view to mechanistic kinetic studies to understand the activity and substrate specificity. In addition attempts will be made to crystallize the enzymes and solve their structures by X-ray crystallography. With this information we will identify or design small molecule inhibitors or activators that could be beneficial in stabilizing the sulfatases, which in the future may be beneficial in the design of therapies for lysosomal storage disorders.

Notes:

17. **Engineering Sulfatases for Biomedical Applications: Biochemical and Structural Characterisation of the Extracellular Sulfatases Sulf1 and Sulf2**

Authors: **Catriona Haberland**, Dr Tracey Gloster and Dr Gordon Florence

Affiliations:
CRITICAT CDT, School of Chemistry, North Haugh, University of St Andrews, KY16 9ST, Scotland.
School of Biology, Biomedical Science Research Complex, North Haugh, University of St Andrews, KY16 9ST, Scotland.

E-mail: cgh6@st-andrews.ac.uk

Abstract:

Sulf1 and Sulf2, the only sulfatases known to be secreted into the extracellular matrix, are singular in that they can post-synthetically alter the sulfation pattern of glycosaminoglycans, specifically heparan sulfate (HS) and heparin (Hp). By hydrolysing 6-O-sulfate groups on glucosamine units, the Sulfs are able to modulate the interactions of HS and Hp with different binding partners, including chemokines, growth factors, morphogens, and also viral proteins. The variety of binding partners reflects the diversity of cell-cell and cell-matrix interactions the Sulfs are able to modulate, such as developmental processes, tumour growth, anticoagulation and pathogen invasion.

This project will attempt to expand our understanding of the biochemical and structural characteristics of the Sulfs and their interaction with glycosaminoglycans, and to subsequently assess their potential in biomedical applications. To address this full-length proteins and individual domains will be expressed, purified and crystallised for structure determination by X-ray crystallography. Furthermore, kinetic and substrate specificity assays will aim to provide an insight into the structure-activity relationship of the Sulfs. Based on these results potential inhibitors, protein stabilisers or activity enhancers will be designed, synthesised and tested. Additionally, protein engineering trials will be carried out to develop potential biocatalysts or protein therapeutics.

Notes:

18. Keap-ing Nrf2 in check for neuroprotection in Alzheimer's disease

Authors: **Aikaterina Miari[1*], Guillermo Ortiz-Pasamontes[2*],** Mohamed Elsharkasi[1], Selina Wray[3], Geoffrey Wells[4] and Fiona Kerr[1,2]
* These authors contributed equally to this work

Affiliations:
1. Department of Biological & Biomedical Sciences, School of Health & Life Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow, G4 0BA.
2. Department of Biomedical Sciences, School of Applied Sciences, Edinburgh Napier University, 9 Sighthill Court, Edinburgh, EH11 4BN.
3. Institute of Neurology, University College London, Gower Street, London, WC1E 6BT.
4. UCL School of Pharmacy, University College London, 29/39 Brunswick Square, London, WC1E 6BT.

E-mail: Fiona.Kerr2@gcu.ac.uk

Abstract:

Nrf2 is a master regulator of cytoprotective genes, and a promising target for prevention of Alzheimer's Disease (AD). Clinically, however, classical Nrf2 activators exert off-target toxicity, and new pharmacological strategies for safer neurological activation of Nrf2 are required. We have shown that direct disruption of the protein-protein interaction (PPI) domain between Nrf2 and its negative regulator Keap1 can protect neurons and restore Nrf2 activity, without inducing toxicity, in *Drosophila* and mouse neuron models of AD. Our work aims to translate these findings to human systems and to identify the molecular effectors of this neuro-protective strategy.

Preliminary results suggest that the chemical Keap1-Nrf2 PPI disruptor 18e reduces synaptic loss in human induced pluripotent stem cell (hiPSC) neurons exposed to natural, patient-relevant, Aβ oligomers, in correlation with Nrf2 activation. Assessment of 84 antioxidant genes, using an RT^2 Oxidative Stress PCR Profiler Array, uncovered 2 upregulated and 42 down-regulated genes in Aβ vs control cells. Further qPCR analysis of a subset of these genes revealed that specific molecules associated with neuronal protection downstream of Keap1-Nrf2 disruption. Further work is required to determine their causal role in neuro-protection and potential as new pharmacological targets for preservation of Nrf2 function and neuronal protection in AD.

Notes:

19. **Analysis of the Trypanosoma *congolense* cell division cycle**

Authors: **Emily J. Allan,** Michael P. Barrett, Jonathan Wilkes, Tansy C. Hammarton

Affiliations:
University of Glasgow

E-mail: e.allan.1@research.gla.ac.uk

Abstract:

Animal African trypanosomiasis is a wasting disease endemic to sub-Saharan Africa, where it has a prominent impact on cattle production and is responsible for losses worth up to US$ 5 billion per annum. Trypanosoma congolense, one of the causative agents of this disease, has recently been the subject of increased research interest, due to both its economic significance and amenability to laboratory culture. However, the cell division cycle of T. congolense has yet to be described. This project seeks to map the order of replication and segregation of different organelles and to define the signalling pathways and checkpoints that regulate the T. congolense cell cycle. Our initial investigations have shown that kinetoplast segregation occurs prior to mitosis, as in T. brucei. Whilst analysis of the T. congolense kinome is still ongoing, we have identified 12 cyclin-dependent kinases, including one T. congolense-specific CRK and at least 8 cyclins, although orthologues of T. brucei CYC2, CYC9 and CYC11 appear to be missing. Further, our preliminary analysis of cell cycle checkpoints using the kinase inhibitor flavopiridol to arrest the cell cycle suggests there may be some differences in cell cycle regulation between the T. congolense and T. brucei.

Notes:

20. **Cas12 derived synthetic transcription factors for easy, synergistic transactivation**

Authors: **James Bryson**[1]**,** Prof Steven Pollard[2] and Prof Susan Rosser[1]

Affiliations:
[1]UK Centre for Mammalian Synthetic Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3BF, United Kingdom

[2]MRC Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom EH16 4UU

E-mail: james.bryson@ed.ac.uk

Abstract:

Increasing numbers of researchers are adopting CRISPR technologies, with Cas9 forming a fundamental tool for many molecular biology laboratories. To date, much work has gone into expanding the Cas9 toolkit, with the generation of catalytically dead 'dCas9' variants fused to different transactivator domains to make synthetic transcription factors for the up-regulation of targeted genes. The more recently discovered CRISPR/Cas12a possesses interesting properties when compared to Cas9; it can be targeted by a single crRNA (compared to a fused crRNA-tracrRNA for Cas9) and possesses the ability to process transcripts containing multiple of these crRNAs into single crRNAs without requiring secondary proteins. This makes Cas12a a very powerful platform, when looking to target multiple loci within the genome simultaneously. The following work seeks to leverage these strengths by generating synthetic transcription factors, in particular validating and characterising a dFnCas12a-VPR variant which possesses the shortest characterised PAM sequence within the Cas12a family. This enables a tool that not only has the inherent advantages of the Cas12a family, but further possesses a PAM that enables comparable targeting density to the SpCas9 system, potentially offering a new gold standard for the binding modules of synthetic transcription factors, repressors and chromatin modifiers.

Notes:

21. **Developing a Super-Resolution Method for the Detection of Alpha-Synuclein Aggregates**

Authors: **Alex Chappard**

Affiliations:

UK DRI, University of Edinburgh

E-mail: A.J.A.Chappard@sms.ed.ac.uk

Abstract:

Aberrant protein aggregation is a predominant feature of many neurodegenerative disorders. The pathological hallmark of Parkinson's disease is the presence of Lewy bodies in the substantia nigra. Aggregated alpha-synuclein forms the primary filamentous component of these Lewy bodies, and as such is a key area of research. Evidence suggests that small alpha-synuclein oligomeric species (as opposed to fibrils) are the key cytotoxic species in Parkinson's disease. Notably, these smaller aggregate species are difficult to characterise due to their small size, high level of heterogeneity, and low abundance. Critically, there is currently a lack of ways to detect, identify, and characterise these smaller aggregates, while no methods have been developed which specifically target alpha-synuclein aggregates.

The aim of this work is to fully develop a new super-resolution method to detect and characterise these smaller aggregates. This PAINT-based method, which we term 'Peptide-PAINT', is designed to specifically target alpha-synuclein aggregates at the nanoscale. This method requires the use of small, alpha-synuclein-specific designer fluorescent peptides in order to detect and bind alpha-synuclein, which will be used to characterise aggregate species. This method will be characterised fully in vitro and in cells before moving into dopaminergic neurons, human post-mortem tissue, and cerebrospinal fluid.

Overall, the work presented here introduces this new Peptide-PAINT method, including results gained thus far and plans for future development.

Notes:

22. **Combining CRISPR genome editing with UK biobank analysis suggests a role for a novel enhancer in male alcohol abuse and chronic anxiety**

Authors: **Alasdair MacKenzie,** Connor Davidson, Toni-Kim Clarke and Andrew McEwan

Affiliations:

University of Aberdeen

E-mail: alasdair.mackenzie@abdn.ac.uk

Abstract:

Chronic anxiety is a major co-morbidity of male alcohol abuse which accounts for 7.9% of male deaths annually. In order to explore a possible regulatory process linking these disorders we used comparative genomics to identify a highly conserved polymorphic enhancer region (GAL5.1) 42kb 5' of the GAL gene that is known to co-regulate alcohol intake and mood. GAL5.1 drove reporter gene expression in the same hypothalamic cells that expressed GAL mRNA and UK biobank analysis revealed a significant association of the stronger GG allele of GAL5.1 to alcohol abuse and anxiety in men (n=115,865; p=0.0008). In order to determine the physiological role of GAL5.1 we used CRISPR/CAS9 genome editing to disrupt of GAL5.1 in mice. Analysis of GAL mRNA expression in these mice showed a major reduction in amygdala and hypothalamus. In addition, alcohol intake in both sexes almost ceases whilst anxiety was significantly reduced in male mice mirroring the sexual dimorphism seen in humans. Our subsequent identification of an EGR1-PKC-epsilon driven pathway involved in modulating the GG allele of GAL5.1, but not the CA allele, opens the possibility of the development of personalised therapeutic approaches to treat male anxiety and alcohol abuse.

Notes:

23. **Rotational 3D Microscopy for Organoid Studies**

Authors: **Ewa Guzniczak** & Graeme Whyte

Affiliations:
Heriot-Watt University, School of Engineering and Physical Science, Department of Biological Chemistry, Biophysics and Bioengineering Edinburgh Campus, Edinburgh, EH14 4AS, Scotland

E-mail: eg100@hw.ac.uk

Abstract:

The ability to produce organoids consisting of thousands of cells clustered together offers many opportunities in studying the complex cell and tissue interaction in an in vitro model system, however when moving from flat 2D cultures into 3D organoids, additional complications arise. One difficulty is the ability to image the cells within the organoid with high resolution. 2D culture is ideal for generating detailed microscopy images capable of the nanometre-scale resolution, as the cells are spread out on in a single, flat layer on top of optically transparent glass. If the cells instead are arranged into a 3D spheroid, the capability to obtain detailed microscopy images is reduced in part due to the number of cells light has to pass through, each of which scatters the light and reduces the intensity and resolution obtainable.

By controllably rotating the organoid, it is possible to image it from different directions and minimise the depth of cells through which the light has to travel to produce a fluorescent image, allowing for its 3D structure to be reconstructed with more detail. Here we present non-contact methods for holding and controllably orienting individual cells, clusters and organoids which can be used to rotate and image them from many different directions providing a valuable analysis tool for detailed imaging studies.

Notes:

24. **Four-dimensional Super-Resolution imaging in living intact pancreatic islets**

Authors: **Adrian Garcia-Burgos,** Prof Rory Duncan, Prof Shareen Forbes, Dr Alison Dun

Affiliations:
Heriot-Watt University

E-mail: ag101@hw.ac.uk

Abstract:

Diabetes is a world-wide problem caused by either altered insulin secretion and/or response to insulin in the organism. Insulin is a hormone, specifically produced and secreted by beta-pancreatic cells and regulates the metabolism in the organism. A relatively new treatment for Type-1 Diabetes is pancreatic islet transplantation. Pancreatic islet transplantation stabilises glycaemic control in Type-1 diabetes; notably, Scotland has world-leading expertise in this therapy and a highly successful transplantation programme. Therefore, much fundamental and translational biology remains to be determined.

My PhD aims to study the release of insulin at the level of intracellular granule pools in so-called β-Cells in situ within pancreatic islets, before and after transplant, using mouse models and human donor islets, combined with high-resolution 4-D microscopy and molecular biology. Additionally, I am working on different projects to understand more about pancreatic islets response to the isolation process pre and post-transplant. Mitochondrial density and shape could be used as a proxy for prediction of the clinical performance. International collaboration to try different fluoroprobes, labelling alpha and beta cells. Using different microscopy techniques to achieve super-resolution. In my poster, I will discuss the background to the project, along with some early data I have acquired.

Notes:

25. A low cost electrochemical platform for detection of DNA antibiotic resistance genes and iron scavenging agents from potentially pathogenic bacteria

Authors: **Butterworth, A.[1],** Peveler W.J.[2], Corrigan, D.K.[1], Ward, A.C.[3]

Affiliations:
1. Department of Biomedical Engineering, University of Strathclyde
2. School of Chemistry, University of Glasgow
3. Depart of Civil and Environmental Engineering, University of Strathclyde

E-mail: andrew.c.ward@strath.ac.uk

Abstract:

Electrochemical techniques hold huge potential within the life sciences sector in terms of the ability to detect biological substances at low cost in a manner than can be integrated into an existing laboratory based experiments. Furthermore, there is a pressing need for diagnostic sensors to detect pathogenic organisms in a wide range of scenarios such as in healthcare and agriculture in order to support antimicrobial stewardship and early detection of disease. Through the use of an extremely simplified instrument, costing less than £5 per unit, we have shown that it's possible to detect the presence of DNA from the oxacillin resistance gene, OXA-1. In addition to this, we are now exploring the use of the platform to detect the presence of siderophores, through the use of a sensor array containing synthetic metal binding groups that can transduce the presence of siderophores produced by infection-causing bacteria and ultimately lead to their identification. We will use these tools to "fingerprint" bacterial siderophores, exploiting the heterogeneity of iron chelating agents produced by different species of microorganism to identify not only the siderophores present but potentially the microorganism present in a sample.

Notes:

26. **Immunoassay on complementary metal oxide semiconductor photodiode array**

Authors: **Ana-Maria Nastase,** Chunxiao Hu, Valerio F. Annese, Dharmendra S. Dheeman, David Cumming, Simon Rogers, Michael P. Barrett

Affiliations:
University of Glasgow

E-mail: a.nastase.1@research.gla.ac.uk

Abstract:

Immunoassays remain one of the most sensitive method of detection for infectious diseases. Concomitantly, the world of electronics is dominated by the low cost, mass-manufactured complementary metal oxide semiconductor (CMOS) technology, which has made a huge impact on sensing technology. By combining immunoassay techniques and CMOS technology a powerful tool of detection for infectious diseases could be developed. The aim of this project was to develop an immunoassay which can be used on the CMOS platform for the detection of Human African Trypanosomiasis (HAT).

Notes:

27. **Using the Scottish biomass, draff: a circular economy approach towards biomass use**

Authors: **Flora Foltanyi,** Christopher Lancefield, Tracey Gloster and Nicholas Westwood

Affiliations:
Schools of Biology and Chemistry, University of St Andrews, St Andrews, KY16 9ST

E-mail: ff45@st-andrews.ac.uk

Abstract:

Draff, a highly abundant and locally available lignocellulosic biomass in Scotland, is the focus of both academic research and industrial applications. One of the current significant biorefining approaches for its valorisation involves a thermal and a subsequent enzymatic pretreatment, followed by bacterial fermentation into biofuels.[1] This biorefining approach, however, might be further optimised via the application of a different pretreatment method which could provide additional value-added products. The butanosolv pretreatment developed by Lancefield *et al.*[2] is an excellent alternative as it extracts the three main components of the lignocellulosic biomass (cellulose, hemicellulose and lignin) in an usable form. It uses a biorenewable solvent and hence potentialy drives a circular economy approach. The development, optimisation and potential scale up of the butanosolv pretreatment of draff was attempted and three product streams: cellulose pulp (CP), hemicellulose-derived fraction (HDF) and *pseudo* lignin (PL), were obtained. The composition of the individual products was examined mainly via 2D HSQC NMR analysis in order to determine their industrial applicability from a biorefining point of view. As a result, the CP and HDF generated were proposed as useful substrates for bacterial fermentation following an enzymatic pretreatment step, with the potential for recycling the *n*-butanol to take a closer step towards a circular economy approach.

1 *Biorefining Potential for Scotland. Mapping bioresource arisings across Scotland*, 2017.
2 C. S. Lancefield, I. Panovic, P. J. Deuss, K. Barta and N. J. Westwood, *Green Chem.*, 2017, **19**, 202–214.

Notes:

28. ***Clostridium difficile*** **LMW SLP contributes to acute disease in the mouse**

Authors: **F. Vaz[1],** BugSlayer Consortium[1,2,3] and G. Douce[1]

Affiliations:
[1]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK
[2]Krebs Institute and Dept. of Molecular Biology and Biotechnology, University of Sheffield, UK
[3]Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, University of Newcastle, Newcastle upon Tyne, UK

E-mail: Filipa.BaltazarDaCostaVaz@glasgow.ac.uk

Abstract:

The intestinal immune system is constantly exposed to many different organisms, from commensals to pathogens, and presents an array of strategies to maintain homeostasis. For pathogens to thrive in such a hostile environment, protection against innate immune effectors is crucial, and it can be provided by surface components that maintain cell wall integrity. Accordingly, mechanisms of protection and evasion have been described for outer membranes, wall teichoic acids and capsules. However, the role of S-layer in pathogenesis remains to be clarified.

Clostridium difficile gut colonisation is associated with a wide spectrum of gastrointestinal diseases, ranging from mild to severe, acute to persistent. Pathogenicity and fulminant disease are mainly attributed to the production of Toxins A and B.

The S-layer is ubiquitous in *C. difficile* strains and, upon cleavage of the precursor protein SlpA, is composed of a conserved (HMW SlpA) and a variable (LMW SlpA) region. The LMW SlpA confers strain specificity, is highly immunogenic and faces the external environment. The S-layer has been reported as an adhesion factor to the gut epithelia. In addition, we have shown that FM2.5 strain, which does not present S-layer, is avirulent in the Golden Syrian hamster model of C. difficile infection [1]. More recently, in vivo and in vitro evaluation of this strain has suggested that delayed toxin expression could have contributed to the absence of lethal disease. Our recent studies have focused on a derivative strain, RΔD2, that whilst comprising a deletion in the LMW SlpA, still presents an assembled S-layer at the cell surface. Mice infected with RΔD2 showed limited weight loss and disease in comparison to mice infected with the wild type strain, whilst still presenting high levels of colonization and gut toxemia. Our results suggest that the S-layer contributes to pathogenicity by a yet unknown mechanism. We hypothesise that the LMW SlpA may trigger strong inflammatory responses which contribute to the severity of the disease.

Notes:

29. **Visualising α-Synuclein oligomers using super resolution microscopy**

Authors: **Craig Leighton,** Supervisors: Dr Tilo Kunath and Dr Mathew Horrocks

Affiliations:
University of Edinburgh

E-mail: s1681074@sms.ed.ac.uk

Abstract:

Parkinson's disease (PD) is clinically diagnosed, with confirmation made post-mortem. The typical motor symptoms only manifest after approximately 50-60% of midbrain dopaminergic nerve terminals have been lost, which can occur over the course of 10-15 years prior to diagnosis. This highlights the need for an early diagnostic method.

α-Synuclein is central to the aetiology of PD and evidence suggests that oligomers of the protein found in CSF are indicative of the disorder (Horrocks et al., 2016). These oligomers, however, are lowly abundant and highly heterogenous, making them challenging to study. Current methods of measuring oligomer load in CSF are indirect and yield highly variable results (Tokuda et al., 2010). A more direct oligomer detection method is therefore required.

By visualising molecules individually, single-molecule and super-resolution microscopy methods enable even the rarest of species to be characterised. We have focused on developing and using such tools to observe oligomers in which αSyn is phosphorylated at serine 129, a modification that is a highly specific biomarker in PD progression (Fujiwara et al., 2002). We will seek to correlate this post-translational modification on CSF-derived α-synuclein oligomers with disease state and explore the potential of using it as a biomarker.

Notes:

30. **Polyomics analyses reveal a role for nucleobase transporter in gentamicin-attenuated Leishmania mexicana**

Authors: **Abdulbaset M Kabli,**[1,3] Aruna Prakash,[1] Snezhana Akpunarlieva,[1] Kathryn Crouch,[1] Michael P Barrett,[1,2] and Richard J Burchmore[1,2]

Affiliations:
[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow G12 8TA, UK,
[2]Glasgow Polyomics, Wolfson Wohl Cancer Research Centre, University of Glasgow, Garscube Campus, Glasgow G61 1QH,
[3]College of Applied Medical Sciences, Al-Baha University, Al-Baha 61008, Saudi Arabia

E-mail: a.kabli.1@research.gla.ac.uk

Abstract:

Introduction: Leishmania have the ability to subvert the host immune system and adopt sophisticated strategies to develop and survive within macrophages in mammalian host. The molecular communication between host and parasite decides the outcome of infection, but is incompletely understood. We have compared genotype and phenotype of an attenuated Leishmania mexicana line with a virulent, isogenic wild type precursor. We aim to use comparative polyomics approaches to identify key virulence factors, and to explore the potential of the attenuated line as a vaccine candidate.
Methods: Log phase promastigotes of wild-type and gentamicin-attenuated line (H-line) were grown in parallel in media containing 10% FBS, and they were harvested for polyomics analyses. For proteomic analysis, protein extracts were labelled using 6-plex TMT and analyzed with LC-MS/MS. For metabolomics, metabolites were extracted with Chloroform/ Methanol/ Water (1:3:1) and analyzed with LC-MS. For transcriptomics, RNA was isolated and converted into cDNA libraries for cluster generation and sequencing.
Results: We found 18 proteins, 26 identified metabolites, and 481 transcripts were differentially expressed in H-line. Thresholds of FC ≥1.5 and FDR ≤0.05 were set up for polyomics data analyses. Correlation of polyomics datasets reveals that nucleotide metabolism is significantly altered in H-line. Furthermore, nucleobase transporter was significantly down regulated in proteomics analysis.
Conclusions: Modulation of gene expression, observed through polyomics analyses, may relate to gentamicin selection. ΔNT3 cells become more sensitive to allopurinol (purine analogue) at lower concentrations comparing to the wild-type cells, suggesting that this may contributes to Leishmania avirulence.

Key words:
Leishmania mexicana, attenuation, H-line, Gentamicin, Polyomics, Nucleobase transporter

Notes:

31. **Implementing CRISPR-Cas9 System for Enhanced Production of Taxol Oxygenated Precursors in *Saccharomyces cerevisiae***

Authors: **Behnaz Nowrouzi**[a], Leonardo Rios Solis[a,b]

Affiliations:
[a] Institure for Bioengineering, School of Engineering, University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom
[b] Centre for Synthetic and Systems Biology (SynthSys), University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom

E-mail: Behnaz.Nowrouzi@ed.ac.uk

Abstract:

Taxanoids comprise a structurally and functionally diverse groups of diterpenoids naturally found in yew trees (*Taxus spp.*). Of them, particularly Taxol has high potential in cancer treatment. Owing to its low abundance in nature, its large-scale production is greatly hindered. Using synthetic biology tools, it is possible to produce this value-added plant metabolite at lower cost and greater titre in microbial hosts. However, Taxol production is still far behind the industrial production due to low yield of Taxadiene-5α-ol, an oxygenated taxane and the second intermediate in its biosynthesis pathway. The high promiscuity of the catalysing enzyme, which is a P450 enzyme, generates an spectra of oxygenated taxanes which are not involved in Taxol synthesis. We report on utilizing CRISPR/Cas9 to integrate relevant *Taxus cuspidata* genes in *Saccharomyces cerevisiae*. We illustrate the correlated production of Taxadiene-5α-ol and other oxygenated byproducts with regards to yeast growth stage and metabolism in time-series analyses. The current study has the aim to reduce the production of oxygenated taxane by varying the ratio of P450 and reductase gene copy numbers and promoters. Additionally, cofactor engineering and flux balance analysis will be performed to promote NADPH and heme availability, while reducing intracellular stress and increasing cellular biomass.

Notes:

32. **Functional Characterisation of the Translocator Protein (TSPO), implications for Age-Related Macular Degeneration (AMD)**

Authors: **Lincoln Biswas,** Annette Graham, Xinhua Shu

Affiliations:
Department of Life Sciences, Glasgow Caledonian University, Glasgow, UK.

E-mail: Lincoln.Biswas@gcu.ac.uk

Abstract:

Age-related macular degeneration (AMD) is the most frequent cause of progressive blindness disease among the elderly, representing 54% of legal blindness. A cardinal feature of AMD is an accumulation of cholesterol in sub-retinal deposits called drusen, suggesting abnormal lipid homeostasis in the progression of this disease. The present study aims to investigate a mitochondrial translocator protein, TSPO that mediates cholesterol trafficking in RPE cells and which may be involved in the pathogenesis of AMD. We found that TSPO specific ligands significantly promote cholesterol efflux in retinal pigment epithelium (RPE) cells, while deletion of TSPO impaired cholesterol efflux. The oxidised LDL uptake and accumulation markedly increased in $TSPO^{-/-}$ RPE cells due to LDLR dysregulation. Consequently, the reactive oxygen species (ROS) level and the expression of pro- inflammatory cytokines were also increased significantly in $TSPO^{-/-}$ RPE cells. We further demonstrated in mice fed with high-fat diet (HFD) the TSPO ligand, Etifoxine, has a potential effect on the reduction of retinal lipid levels. The drug also decreased the ROS generation and secretion of pro-inflammatory cytokines. Additionally, in aged mouse RPE cells, TSPO expression was reduced, and cholesterol efflux impaired. This study sheds light on molecular and cellular aspects of AMD pathogenesis and suggests that TSPO may have therapeutic potential for treating AMD patients.

Notes:

33. Advanced bioprocessing strategies for Taxol biosynthetic pathway optimisation using S. cerevisiae microbial cell factories

Authors: **Laura E Walls**[a], Behnaz Nowrouzi[a], Jonny Dennis[b], Stephen Wallace[b,c], Leonardo Rios-Solis[a,c]

Affiliations:
[a] Institure for Bioengineering, School of Engineering, University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom
[b]Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom
[c] Centre for Synthetic and Systems Biology (SynthSys), University of Edinburgh, Kings Buildings, Edinburgh, United Kingdom

E-mail: L.E.Walls@sms.ed.ac.uk

Abstract:

Taxadien-5α-hydroxylase catalyses the first oxidation step in the biosynthesis of the critical anti-cancer drug, Taxol. Despite decades of study, the promiscuous and multispecific enzyme remains a major bottleneck in biosynthetic pathway development. In this study, a strategic CRISPR-Cas9 toolkit was coupled with state of the art, industrially relevant micro (BioLector pro) and mini (Applikon MiniBio 500) bioreactor systems in order to alleviate this bottleneck and accelerate bioprocess development. High throughput in vivo microscale screening studies were performed to investigate the effects of wide-ranging processing conditions on the biosynthetic pathway simultaneously. The high throughput data facilitated rapid elucidation of the optimal conditions. As a result, key oxygenated taxane yields were enhanced to a measurable level of 39.0$\pm$5.7 mg/L in S. cerevisiae for the first time. Challenges such as biofilm formation and excess acidification were eradicated through careful optimisation during subsequent scale up experiments, further improving oxygenated taxane production to 53.2 mg/L. Preliminary high throughput micro-scale fed-batch studies have demonstrated superior productivity whilst effectively mimicking industrial scale fermentation conditions. The proposed interdisciplinary approach successfully enhanced oxygenated taxane yields in S. cerevisae, representing a major step towards the sustainable biosynthesis of the lifesaving chemotherapy drug.

Notes:

34. **Multimodal, multiphoton platform for label-free nonlinear optical imaging**

Authors: **A. McDonald;** A. Downes and A. Elfick

Affiliations:
University of Edinburgh (Scotland)

E-mail: dr.alison.mcdonald@ed.ac.uk

Abstract:

The identification of different molecular species on the microscopic scale is still a considerable challenge in many areas of biology. Typical optical analysis of biological systems is invasive and often relies on synthetic or genetic fluorescent labels to provide contrast which can be far from ideal for imaging cells in their native state. To address the need for minimally-invasive, high-speed, label-free chemical imaging, we have developed a multimodal nonlinear optical platform that combines coherent anti-Stokes Raman scattering (CARS), stimulated Raman spectroscopy (SRS), two photon excitation fluorescence (TPEF) and second harmonic generation (SHG). This hybrid microscope offers an extremely wide range of complementary information on biological systems with deeper imaging and reduced photo damage compared to conventional fluorescence microscopes. In addition, we can also combine this with atomic force microscopy (AFM) to measure the surface topography and to map the local mechanical and chemical properties of live cells, tissue and biomolecules with nm resolution.

This microscope is available for use at the BioImaging Small Research Facility in the Institute for BioEngineering at the University of Edinburgh. If you wish to learn more about these techniques, you can contact our unit at bioimaging@ed.ac.uk.

Notes:

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

Authors: **Daniel Harris,** Dr Mads Gabrielsen, Dr Boris Rodenko, Dr Farid El Oualid, Prof Jeremy Mottram, Dr Danny Huang, and Dr Richard Burchmore.

Affiliations:
University of Glasgow, Beatson Institute for Cancer Research, University of York, UbiQ Bio BV

E-mail: d.harris.1@research.gla.ac.uk

Abstract:

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

Notes:

Disruptive Technologies Conference: 25-26th Sept 2019