QMB Abstracts: Queenstown Molecular Biology Main Meeting

Q1: Twenty years of recombineering and recent developments

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Recombineering is the methodology to engineer DNA in *E. coli* using homologous recombination (HR) mediated by the phage protein pairs, Redα/Redβ from lambda or RecE/RecT from Rac, which co-operate as 5'-3' exonuclease and SSAP (single strand annealing protein) interacting pairs (1). Recombineering is the method of choice to engineer large molecules like BACs and is also applied to a variety of other applications, including retrofitting (e.g. BAC transposons, 2), stitching, prokaryotic genome engineering, synergism with site specific recombination, seamless site directed mutagenesis and high throughput engineering for genome-wide projects (3).

Investigations into the HR mechanism employed by these phage pairs revealed a new paradigm operating at the replication fork (4) and the unexpected realization that full length RecE, as opposed to short RecE or Redα, utilizes a different HR mechanism that does not depend on ongoing replication (5). Consequently we defined an unexpected division within paired exo/SSAP HR mechanisms and opened a new avenue of applications including direct cloning from genomic DNA preparations and multi-piece DNA assemblies. Recently we have extended these applications with a new method termed 'ExoCET', which permits the cloning of a specific DNA region up to at least 50kb long from mammalian genomes amongst other improvements (6).

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3. Skarnes et al, (2011) *A conditional knockout resource for genome-wide analysis of mouse gene function.* Nature 474, 337-42.
4. Maresca et al, (2010) *Single stranded heteroduplex intermediates in lambda Red homologous recombination.* BMC Molecular Biology 11:54 4.
5. Fu et al, (2012) *Full length RecE enhances linear-linear homologous recombination and facilitates direct cloning for bioprospecting.* Nature Biotechnology 30, 440-6.
6. Wang et al, (2018) *ExoCET: Exonuclease in vitro assembly combined with RecET recombination for highly efficient direct cloning from complex genomes.* Nucleic Acids Res. 46, e28.

Q2: Histone FLIM-FRET microscopy reveals spatiotemporal regulation of chromatin organization by the DNA-damage response.

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Here we describe a biophysical method to measure chromatin organisation in live cells with nucleosome level resolution. The method is based on a localised phasor image correlation analysis (ICS) of FLIM-FRET microscopy data acquired in human cells co-expressing H2B-eGFP and H2B-mCherry. This multiplexed approach produces spatiotemporal maps of nuclear wide chromatin compaction and quantifies the stability, size and spacing between detected chromatin foci. We used this method in cells where double strand breaks (DSBs) were induced by near- infrared laser micro irradiation to assay chromatin dynamics during the DNA damage response (DDR). These experiments revealed that ATM and RNF8 directed rapid local chromatin decompaction at DSBs, coupled with formation of a stable ring of compact chromatin surrounding the repair locus. Based on these data we built a longevity map of sites with high FRET indicating the time scale of large scale compaction events directed by ATM and RNF8. Then by use of a phasor-based ICS analysis we identified the locations where the DDR shapes local and global chromatin dynamics and demonstrate the utility of phasor ICS-FLIM analysis of histone FRET for the study of chromatin biology.

Q3: Bioactive (Non)-Natural Products: Diversity-oriented Synthesis Based on Biomimetic Strategies

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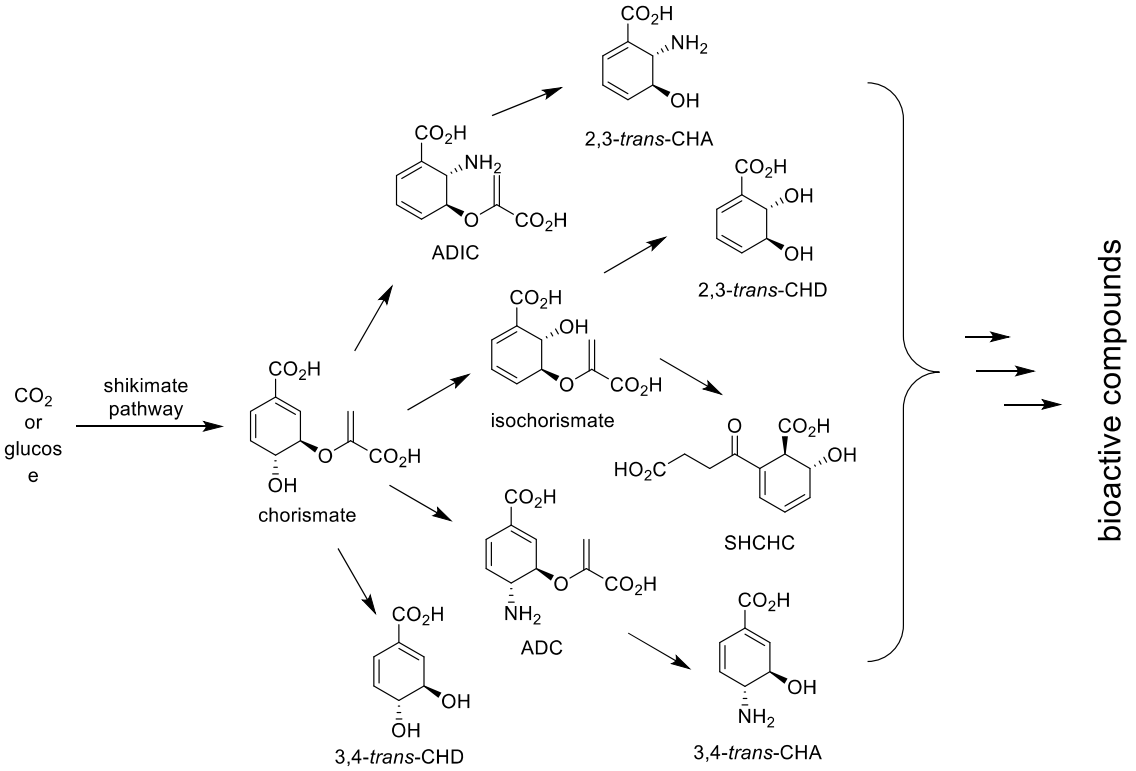
Diversity-oriented strategies are widespread in biosynthesis, or it may be more appropriate to state that biosynthesis is the archetype of diversity-oriented synthesis. We work towards the elucidation and application of diversity-oriented aspects of biosynthesis and biocatalysis, such as stereoselective phenolic coupling, chorismate-derived microbial products,[1] or the use of multi-purpose biocatalysts for C-C bond formation.

By using diversity-oriented biomimetic strategies, e.g., promiscuous enzymes, branching points such as chorismate, or keto-enol tautomerization, we introduced novel biocatalytic syntheses of small bioactive molecules. By this means, HMG-CoA reductase inhibitors, antiviral compounds, and antibiotics have been synthesized.[2] The long-term goal of this project is the identification of advantageous trajectories[3-4] for the synthesis of putative new bioactivities without the need for the synthesis of huge compound libraries.



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3. M. Beigi et al., *Regio- and Stereoselective Aliphatic–Aromatic Cross-Benzoin Reaction: Enzymatic Divergent Catalysis*, Chem. Eur. J. 2016, 22, 13999.
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Q4: New chemical probe technologies: applications to diagnostic imaging and target identification

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Hydrolases are enzymes that often play pathogenic roles in many common human diseases such as cancer, asthma, arthritis, atherosclerosis and infection by pathogens. Therefore tools that can be used to dynamically monitor their activity can be used as diagnostic agents, as imaging contrast agents for intraoperative image guidance and for the identification of novel classes of drugs. In the first part of this presentation, I will describe our efforts to design and synthesize small molecule probes that produce a fluorescent signal upon binding to a protease target. We have identified probes that show tumor-specific retention, fast activation kinetics, and rapid systemic distribution making them useful for real-time fluorescence guided tumor resection and other diagnostic imaging applications. In the second half of the presentation I will describe a chemical proteomic screen using an activity based probe that identified 10 previously uncharacterized *S. aureus* serine hydrolases that mostly lack human homologues. One hydrolase, FphB, can process short fatty acid esters, exhibits increased activity in response to host cell factors, is located predominantly on the bacterial cell surface in a subset of cells, and is concentrated in the division septum. Genetic disruption of the *fphB* gene confirms that the enzyme is dispensable for bacterial growth in culture but crucial for establishing infection in distinct sites *in vivo*. A selective small molecule inhibitor of FphB effectively reduces infectivity *in vivo*, suggesting that it may be a viable therapeutic and diagnostic imaging target for the treatment and management of *Staphylococcus* infections.

Q5: Biased signalling of synthetic cannabinoids through CB_1 receptors

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Cannabis has been used by humans for its medicinal and intoxicating effects for centuries, and these effects have been primarily attributed to its principal bioactive component Δ^9-tetrahydrocannabinol (THC). THC is an agonist at two G protein-coupled receptors (CB1 and CB2), with psychoactivity attributed to activation of central CB1. Many synthetic cannabinoid receptor agonists were developed as molecular laboratory tools for the study of the endocannabinoid system. In the past decade these compounds have entered the recreational drug market, with over 240 synthetic cannabinoids identified by government agencies in more than 100 countries, making them the most rapidly proliferating class of "designer drug"[1]. Synthetic cannabinoids are increasingly linked to serious adverse reactions, including death. During July to September 2017 at least 20 deaths were linked to the compounds AMB-FUBINACA and 5F-ADB in New Zealand.[2] Initial studies of a range of synthetic cannabinoids shows them to be high affinity and high potency agonists, but to date no clear correlation(s) have emerged between *in vitro* affinity or *in vivo* potency and toxicity.[3-6] We have compared the signalling of AMB-FUBINACA and 5F-ADB to THC in a range of in vitro assays in HEK-hCB1 cells. We find that the synthetic ligands have significantly greater efficacy and potency than THC in driving the recruitment of β-arrestin-1 and 2. Arrestin proteins are involved in internalisation of the receptors and may mediate signalling events. Further studies will examine if activation of these pathways is linked to the toxicity reported for these compounds.

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4. Banister SD, Longworth M, Kevin R, Sachdev S, Santiago M, Stuart J, Mack JB, Glass M, McGregor IS, Connor M, and Kassiou M (2016) *Pharmacology of Valinate and tert-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues.* ACS Chem Neurosci 7.
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6. Gamage TF, Farquhar CE, Lefever TW, Marusich JA, Kevin RC, McGregor IS, Wiley JL, and Thomas BF (2018) M*olecular and Behavioral Pharmacological Characterization of Abused Synthetic Cannabinoids MMB- and MDMB-FUBINACA, MN-18, NNEI, CUMYL-PICA, and 5-Fluoro-CUMYL-PICA*. J Pharmacol Exp Ther 365.

Q6: The influence of charge on the binding of lipids to membrane proteins

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Membrane proteins are an important class of proteins that reside in the membranes of organelles and cells. These membranes are composed of lipid molecules, which are crucial to the solubilisation of membrane proteins, but are also important for the function of these proteins. It is challenging to determine the molecular details of lipid-membrane protein interactions, making it difficult to unravel how particular lipids may influence membrane proteins, and the conditions under which they do this. An important interaction between lipids and membrane proteins occurs through association of charged headgroups of lipids and amino acid side chains of proteins. These interactions may be sensitive to the protonation status of both partners, and to explore this we used a combination of native mass spectrometry, computational, and electrophysiology approaches. We found that the outer membrane protein F (OmpF) has highly charge-sensitive lipid binding, while the charge-induced effects for other proteins in outer, mitochondrial or inner membranes varied substantially.

To understand these different sensitivities, we performed an extensive bioinformatics analysis of membrane protein structures and found a wide distribution of basic and acidic residue densities in the headgroup-binding regions of different membrane proteins. We correlated these densities to the observed lipid binding behaviour and by coarse-grained molecular dynamics simulations, in mixed lipid bilayers, further implicated changes in charge as being important for lipid binding. Focussing on OmpF and using electrophysiology and mass-spectrometry-based ligand-binding experiments, at low pH, we show that the lipid POPG can maintain OmpF channels in open conformations for extended time periods. Since the outer membrane is composed almost entirely of anionic lipopolysaccharide, with similar headgroup properties to POPG, such anionic lipid binding could prevent closure of OmpF channels, thereby increasing access of antibiotics that use porin-mediated pathways.

Q7: Cancer Cell Plasticity in Cancer Development and Progression to Metastatic Disease

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Not all cancer cells within a tumour are created equal. In particular, tumours contain subpopulations of cancer cells endowed with a highly aggressive nature termed tumour-initiating cells (TICs), which in turn are linked to metastasis. To date, however, the identity of those aggressive cells, and the mechanisms that determine their aggressive state, remain elusive. Importantly, we have shown that non-TICs and TICs are plastic cell populations, where bi-directional conversions between the two states can readily occur[1-3].

Using various metastasis models, we have now discovered that certain primary tumors elicit a systemic inflammatory response involving IL-1β-expressing innate immune cells that infiltrate TIC tumours colonizing a metastatic microenvironment. At the metastatic site, IL-1β maintains TICs in a ZEB1-positive differentiation state, preventing TICs from generating highly proliferative E-cadherin-positive progeny. Thus, when the inherent plasticity of TICs is impeded, overt metastases cannot be established. Ablation of the pro-inflammatory response or IL-1R inhibition relieves the differentiation block and results in metastatic colonization. Among lymph node-positive breast cancer patients, high primary tumor IL-1β expression is associated with better overall survival and distant metastasis-free survival. Our data reveal complex interactions that occur between primary tumors and disseminated TICs that could be exploited to improve patient survival.

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2. Chaffer, C.L. et al. *Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity*. Cell 154, 61-74 (2013).
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Q8: Long non-coding RNAs as drivers of tumor progression and metastasis

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Genome-wide studies revealed that most of the human genome does not encode for proteins and identified thousands of non-coding RNAs. Of these non-coding transcripts, long non-coding RNAs (lncRNAs) represent the largest and most diverse class. They are defined by length (>200 nt), transcribed by RNA polymerase II and commonly originate from intergenic regions. LncRNAs can be capped, spliced and polyadenylated but lack a significant open reading frame. Members of this class have been implicated as regulatory molecules in many cellular functions including epigenetic gene regulation, splicing and translation. Recently, lncRNAs emerge as crucial players in cancer by impacting acquired capabilities of cancer cells such as cell proliferation, angiogenesis or invasion.
We identified 30 potentially oncogenic lncRNAs in breast cancer, termed Mammary Tumor Associated RNAs (MaTARs). The expression of MaTARs correlates with breast cancer subtype and/or hormone receptor status, indicating potential clinical relevance. In addition, MaTARs are expressed in a tumor-specific manner, making them excellent candidates for drug development as systemic therapeutic intervention will only affect cancer cells. To functionally validate the role of MaTARs, we performed antisense knockdown experiments *in vitro* and *in vivo*, resulting in reduced mammary tumor growth and metastasis. Loss-of-function models generated by CRISPR/Cas9 genome editing or CRISPR interference (CRISPRi) further confirmed that MaTARs are driving tumor progression.

Ongoing studies are investigating the molecular mechanism by which MaTARs function. Our results suggest that lncRNAs are likely important drivers of tumor progression and represent promising new therapeutic targets.

Q9: Regulation of IL-6 is pivotal to outcome in colorectal cancer.

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It is well known that Interleukin 6 (IL-6) has high relevance to cancer progression and metastasis, but just how it contributes is unclear. Less well known, is that the *IL6* gene is a direct target of the most frequently altered protein in cancers, p53.

We wanted to better understand how (and why) *IL6* was regulated by p53, and whether its two functional homologs p63 and p73, also contributed to its control. Importantly, p63 is expressed as two isoforms, the shorter of which ΔNp63, is heavily implicated in cancer progression.

We therefore updated predictions of p53 family members in the *IL6* promoter and discovered that a single nucleotide polymorphism (SNP), with well-documented associations with inflammatory related pathologies, altered a predicted binding site for p63. We then undertook molecular and functional studies that supported this notion, indicating the SNP has direct effects on IL6 expression, sensitivity to p63, and also cancer cell behaviour. Next we examined public datasets and identified associations between the SNP and cancer progression for a number of different cancer types, including lung cancers, head and neck cancers and colorectal cancer (CRC). Then, to confirm this in the setting of CRC, we undertook the most comprehensive genomic analysis of CRC in New Zealand performed to date, and were able to test the SNP's relationships with mutation rates in principal cancer genes, with clinical data including survival, disease recurrence and metastasis, and also with more than 50 other known prognostic and/or functional SNPs. These data together, provide a comprehensive snapshot of CRC genetics in Southern New Zealand, and they confirm that the SNP is a powerful prognostic tool for CRC. Moreover, they illuminate the central contribution of IL-6 signalling to this disease. We expect these findings to have relevance to treatment decisions for CRC in the near future.

Q10: Putting the Brakes on Cell Death to Limit Inflammation

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Apoptosis, necroptosis and pyroptosis are regulated forms of cell death serving to eliminate obsolete, damaged or infected cells. The rapid clearance of apoptotic cells by phagocytic cells limits the response of innate immune cells to apoptosis, but excessive apoptosis that compromises the integrity of a barrier tissue such as the skin or intestinal epithelium will promote inflammation by creating points of entry for microbes. In contrast to apoptosis, necroptosis and pyroptosis are proinflammatory forms of cell death because cell lysis releases intracellular components that alert innate immune cells and trigger an inflammatory response.

In certain contexts, tumour necrosis factor (TNF) or ligands for Toll-like Receptors 3 and 4 engage either the apoptotic machinery through activation of the protease caspase-8 or the necroptotic machinery through activation of the kinase RIPK3. These signalling events are regulated by the kinase RIPK1 because cells expressing catalytically inactive RIPK1 are resistant to killing. Mice expressing catalytically inactive RIPK1 also do better than their wild-type counterparts in models of ischemia-reperfusion injury, inflammatory bowel disease, and systemic inflammation. Consequently, many groups are now investigating the potential therapeutic benefits of small molecule inhibitors of RIPK1.

Q11: Dawn of the dead: cell death by the zombie protein, MLKL

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In 2012, Mixed lineage kinase domain-like (MLKL), a catalytically-dead ("zombie") cousin of conventional protein kinases, termed a pseudokinase, was implicated as the key effector in the programmed necrosis (or necroptosis) cell death pathway. This pathway has been implicated in innate immunity, the pathogenesis of inflammatory diseases, and tissue injury arising from ischemic stroke or acute kidney injury. As a result, an improved fundamental knowledge of MLKL's activation mechanism is of enormous interest as we and others look to target the pathway therapeutically.

Our work has focused on the molecular mechanism underlying MLKL activation and how its cell killing function is regulated by its pseudokinase domain. These studies have unveiled MLKL's pseudokinase domain as a jack of many trades: a molecular switch, as a protein interaction domain for recruiting co-effectors, and as a suppressor of the killer function of the adjacent four-helix bundle domain. Recently, our structural, biochemical, biophysical and cellular biology studies of MLKL activation have revealed a number of surprises, including an unexpected divergence in the mechanisms underlying the human and mouse necroptosis pathways.

Q12: Multi-day *in vivo* imaging of bone marrow niche fate regulation and remodelling in cancer and immunity.

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It is widely hypothesised that complex interactions between immune cells, pathogenic populations and cancers with their surrounding microenvironments may contribute to homeostatic cell function and disease development. In light of this interdependency, novel interventions that target specific stromal cell lineages and their interactions with blood cell subsets in disease are being investigated. To investigate this, we are studying mouse and humanised models of autoimmunity, hematological malignancies and protective immunity. We have developed intravital microscopy methods that allow us to monitor the same cells and microenvironments in the bone marrow (BM) and peripheral immune organs long-term including repeated imaging sessions over multiple days. Using this approach, we have observed lineage specific interactions of haematopoetic cells with stroma and microenvironments in the BM. For example, we observed highly dynamic interactions and promiscuous distribution of T-ALL cells throughout the BM, without any preferential association with microenvironments that was maintained during development of chemotherapy resistance. However, this behavior was lineage specific and not a conserved feature of pathogenic, malignant or protective blood cells from other immune cell lineages.

Interestingly, while progression is independent of the stroma, accumulation of malignant lymphocytes leads to rapid, selective remodeling of the endosteal space, resulting in a complete loss of mature osteoblastic cells. This outcome shifts the balance of endogenous BM stroma towards a composition associated with less efficient hematopoiesis. However, we observe lineage specific tissue remodeling hardwired to the origin of the malignant cells. This novel, dynamic analysis highlights that future therapeutic interventions should target cell-specific mechanisms, in order to combat the invasion and survival of pathogenic blood cells. These studies provide the foundation for future studies investigating the metastasis of other tumours as well as homeostatic hematopoiesis.

Q13: Investigating bacterial virulence mechanisms in the search for future antibacterials

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Bacterial pathogens deploy an arsenal of virulence factors to establish infection and cause disease. At the forefront of the infection process are bacterial surface components, which are responsible for host colonisation and pathogen adhesion. Autotransporters (AT) proteins are the largest group of surface adhesins in Gram-negative bacteria. These proteins play a central role in controlling bacterial interactions with their environment. They allow bacteria to aggregate with other bacteria, adhere to human cells, and form biofilms - all key facilitators of bacterial persistence and pathogenesis. Despite their abundance and critical roles in pathogenesis, to date the mechanisms of action and structures of less than 0.01% of the total ATs have been investigated.

We are using X-ray crystallography, extensive biophysical techniques, immunoassays and cellular assays to investigate AT function. Our work has revealed that a head-to-tail self-association of the AT Antigen 43 (Ag43) between neighbouring cells promotes bacterial aggregation and biofilm formation.
We have produced the structures of a further AT proteins and we are now starting to elucidate the mechanisms of action for diverse ATs. So far, we have found that different AT adhesins promote bacterial aggregation using subtle variations in this self-association mechanism compared to Ag43. We are also starting to uncover in atomic detail how AT adhesins like UpaB and TibA bind epithelial surfaces. Furthermore, we are breaking new ground towards understanding how glycosylation regulates the function of multifunctional AT adhesins.

Finally, we are using this new knowledge to successfully develop methods for disrupting AT function.

Q14: The assembly of TRIM proteins and the disruption of retroviral infection

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Mammalian genomes contain evidence of prolonged and persistent invasion by retroviral pathogens in the form of endogneous retroviral elements. Consequently, cells have developed mechanisms to disrupt key stages of the retroviral lifecycle to prevent and contain infection. The TRIM protein family members Trim5α and Trim28 are two such proteins.

Members of the TRIM protein family are charactersised by a conserved N-terminal *TRI*partite *M*otif consisting of a RING domain, one or two Bbox domains and a coiled-coil region. This conserved architecture suggests a conservation of function. However while Trim5α acts during the early post-entry stages of the retroviral lifecycle, recognising the incoming retroviral capsid, preventing reverse transcription and integration, Trim28 acts after integration to silence transcription from the integrated provirus.

To investigate the mechanism of restriction we have undertaken structural and biophysical experiments to examine how assembly of the tripartite motif affects the function of both Trim5α and Trim28. In both proteins the Bbox domain mediates assembly with structures of these domains demonstrating alternate assembly interfaces. Functional assays also demonstrate differing roles with Trim5α function dependent upon assembly, while a single Trim28 dimer is sufficient for silencing.

Q15: Structure and mechanism of the SAMHD1 HIV-1 restriction factor

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SAMHD1 is a post-entry cellular restriction factor that inhibits HIV-1 replication in myeloid-lineage and resting $CD4^+$ T cells. The mechanism of SAMHD1 restriction has been disputed but the predominant theory is that SAMHD1 dNTP triphosphohydrolase activity blocks HIV-1 infection by reducing the cellular dNTP pool to a level that does not support viral reverse transcription. A large body of structural and biochemical studies have demonstrated that the active form of SAMHD1 is a protein tetramer that contains four regulatory allosteric sites each accommodating a deoxynucleotide/nucleotide pair and four active sites that hydrolyse the dNTP substrates. In addition, other studies have shown that the dNTP triphosphohydrolysis reaction is regulated by tetramer stability, controlled by SAMHD1 phosphorylation at residue T592. However, although, this wealth of information has contributed significantly to our understanding of SAMHD1 restriction, regulation and activation the molecular details of the catalytic mechanism of dNTP hydrolysis have remained unclear. Therefore, to elucidate the molecular mechanism of dNTP triphospho-hydrolysis by SAMHD1, we now have undertaken comprehensive enzymological studies employing deoxynucleotide substrate and activator analogues and determined crystal structures of catalytically active SAMHD1 with dNTP-mimicking, competitive inhibitors. These analogue studies uncovered inhibitors of SAMHD1 and also revealed the capacity for SAMHD1 to be activated by and hydrolyse existing antiviral and anticancer drugs. The SAMHD1-inhibitor co-crystal structures show in atomic detail how dNTP substrates are coordinated at the SAMHD1 active site and reveal how the protein chemically activates a water molecule to mount a nucleophilic attack on the phospho-ester bond in the dNTP substrate. In conclusion, these studies now provide the molecular details of the SAMHD1 reaction mechanism demonstrating how dNTP substrates are hydrolysed and enable more accurate prediction of whether new and existing antiviral and anticancer drugs are hydrolysed by SAMHD1.

Q16: Acute Rheumatic Fever: How Antibodies and Complement link the Chain from Streptococcus A to the Heart

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Acute rheumatic fever (ARF) is an autoimmune sequela that can develop after a Streptococcus A infection. Carditis, the most severe ARF manifestation, can lead to permanent heart valve damage and rheumatic heart disease. The rates of ARF remain unacceptably high in Maori and Pacific children in New Zealand. While immunoglobulin and complement were first observed in the myocardium and mitral valves of children who had died from cardiac failure following ARF over 50 years ago (Kaplan 1964), contemporary investigations of complement pathways in ARF have been lacking. The aim of this study was to determine how complement links with antibodies to drive inflammation in ARF in patients recruited into the nation-wide Rheumatic Fever Risk Factors study. The concentration of 17 complement factors together with 6 different immunoglobulin isotypes and subclasses were measured in participant sera using bead-based immunoassays (BD Cytometric Bead Array and Luminex xMAP). An integrative statistical approach (combining feature selection and principle component analysis) was utilised to analyze relationships among immunoglobulin and complement in ARF patients stratified by concentration of C-reactive protein (CRP). Just 4 of the 23 analytes accounted for 82% of the variance between high CRP patients and healthy controls. Notably, patients in the high CRP group exhibited linked IgG3/C4 responses. This suggests a dominant role for the classical complement pathway, initiated by antibodies of an IgG3 isotype, in ARF associated inflammation. The contribution of circulating IgG3 antibodies to pathogenesis was further explored by examining antibody specificity. Data showing marked differences in the level of IgG3 produced in response to key Streptococcus A virulence factors and efforts to profile the autoantibody repertoire in ARF patients will be presented.

Q17: Metagenomic Exploration of New Zealand Marine Sponge Microbiomes for Bioactive Peptides and Polyketides Biosynthetic Gene Clusters

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Marine sponges are the most primitive multicellular animals on our planet, however these seemingly simple organisms harbour complex assemblages of hundreds microbial species participating in an intricate network of metabolic exchange. The microbiomes associated with sponges appear to be broadly different from other free living and symbiotic populations, and produce an astounding diversity of bioactive secondary metabolites.

Given that many of our best antibiotics and oncology drugs have been derived from microbes, the plethora of bacterial species present in marine sponges presents an exciting opportunity for the discovery of new microbial metabolites with potential medical relevance. Accessing a sustainable supply of these metabolites however, requires an innovative approach, as the majority of sponge associated species are likely to be uncultivable in a laboratory setting.

In this talk I will present recent efforts to discover the biosynthetic gene clusters for the cytotoxic polyketides mycalamide and pateamine from the microbiome of the New Zealand marine sponge *Mycale hentscheli*. I will also present results from a wider shotgun metagenomic survey aimed at discovering new peptide and polyketide compounds with potential applications as antimicrobials.

Q18: The capsule regulatory network of *Klebsiella pneumoniae* defined by density-TraDISort

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Klebsiella pneumoniae infections affect infants and the immunocompromised, and the recent emergence of hypervirulent and multi-drug resistant *K. pneumoniae* lineages is a significant healthcare concern. Capsule is an essential virulence factor in *K. pneumoniae*, and overproduction of capsule is associated with hypervirulence and the ability to cause community-acquired infections. The full details of *K. pneumoniae* capsule biosynthesis and regulation are not known. We developed a novel method to identify genes influencing capsule production, called density-TraDISort, and show that it is applicable to different species of capsulated bacteria. We have used this method to explore capsule regulation in two clinically-relevant *Klebsiella* strains; *K. pneumoniae* NTUH-K2044 (capsule type K1), and *K. pneumoniae* ATCC 43816 (capsule type K2). Multiple genes required for full capsule production in *K. pneumoniae* were identified, many of which have not previously been linked to capsule regulation. Our unique experimental setup also allowed us to identify putative suppressors of capsule in *K. pneumoniae* NTUH-K2044. We generated targeted deletion mutants to validate the results of our screen and have further explored the roles of three novel *K. pneumoniae* capsule regulators identified in our screen: MprA, SlyA and SapBCDF. We show that capsule production is at the centre of a complex network involving multiple global regulators and environmental cues. While the majority of capsule regulators are located on the core genome of *K. pneumoniae*, there is variability between strains in terms of which regulators are deployed. Our results allow us to propose an integrated model for control of capsule in *K. pneumoniae*, and provide a robust and accessible technology for studies of other capsulated bacterial species.

Q19: Medicine and Me: impacting chronic disease

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A premise of precision health is that continuous individual health monitoring will allow early disease detection, early intervention, increased quality of life and reduced health costs. Cardiovascular disease (CVD) remains a leading cause of mortality worldwide. Atherosclerosis, a primary CVD risk factor, begins early prior to clinical signs. We have focused on diagnosing early atherosclerosis by analyzing 100s of aortic and left anterior descending coronary arteries obtained from individuals (<50 years old) using high content quantitative proteomics workflows to determine network re-wiring and regulatory features associated with early atherosclerosis. Early atherosclerosis-tissue-secreted proteins using targeted mass spectrometry assays in plasma of individuals with varying degrees of CVD with estimated ROC of >95%. Implementation of these and other markers using volumetric absorptive microsampling devices for remote blood collection and our robust automated sample preparation workflows we are carrying out continuous patent-centric screening of a mid-risk CVD population. The other requirement is development of drugs able to act on subsets of individuals. In this regards we have determined the effect of disease-induced post-translational modifications on high jacketing kinase pathways. The ability to identify drug targets to the disease proteome using high throughput IPSC derived organoid coupled with high content proteomics will be discussed.

Q20: Transcriptionally induced enhancers during macrophage activation and tuberculosis infection

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Enhancers are crucial regulatory elements capable of activating transcription of target genes at distance through direct interaction via DNA looping. Enhancers themselves can be bi-directionally transcribed to generate divergent enhancer RNAs (eRNAs), often long noncoding RNAs of unknown function. The expression of eRNAs correlates with corresponding target gene expression. However, the specific roles of enhancers and eRNAs in different cells and tissues remain largely unexplored. Here, I will highlight our recent work on transcribed enhancers and gene promoters in macrophages. Through the integration of time-line transcriptome data with high-throughput conformation capture and histone modification data, we identified a robust set of transcriptionally active enhancers and studied their influence during macrophage activation and infection over time. We find that sets of enhancers gain transcriptional activity in these processes. These activities correlate with expression changes of important process-specific genes. Our results suggest that enhancers and eRNAs play important roles during macrophage activation as well as host response to tuberculosis infection. Non-coding RNAs derived from enhancer loci might constitute promising new disease markers or targets for fighting infection.

Q21: Molecular mechanisms underlying appetite regulation

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Excessive energy intake, especially on a long-term basis, underlies the development of obesity and associated comorbidities. Extremely high levels of consumption are particularly evident upon presentation of palatable tastants, specifically those rich in sugar. Avid intake of sweet diets occurs regardless of an absence of energy deficit and despite potential negative consequences arising from, for example, elevated plasma osmolality or stomach distention. Surprisingly, the neural circuit responsible for terminating consumption due to satiety or in order to protect internal milieu, comprising such powerful anorexigens as melanocortins or oxytocin (OT), appears to respond insufficiently to overeating driven by sweet reward. Here, by focusing on studies in laboratory mice and rats, we delineate evidence suggesting that chronic sugar consumption dampens meal-end activation of the brainstem-hypothalamic OT network that promotes satiety. We show that habitual exposure to sweet ingestants reduces OT expression in the hypothalamus to the levels that typically correspond to the hungry state. Finally, we suggest that palatability-driven activity of descending opioid pathways at least to some extent facilitates this diminished responsiveness of the OT system to intake of sweet foods.

Q22: Molecular pharmacology of CGRP-family receptors: migraine and beyond

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G protein-coupled receptors are important drug targets in a range of therapeutic areas. The recently approved monoclonal antibody drug for migraine, Aimovig, targets the calcitonin gene-related peptide (CGRP) receptor, which is a neuropeptide-binding receptor that is expressed in sensory neurons. This presentation will cover the rationale for targeting this receptor and highlight some of the opportunities and challenges that remain for CGRP and related receptors in migraine and beyond.

Q23: Energy dysfunction in heart disease

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Mitochondria are essential for heart function as they produce most of the energy required by cardiomyocytes through oxidative phosphorylation. Mutations in genes required for mitochondrial function compromise cardiac function and cause heart disease, however, links between the pathological mutations and how their effects on mitochondrial function contribute to the development of heart disease are missing. Recently we developed two different models of heart disease caused by allelic loss of the *Ptcd1* gene that encodes the mitochondrial protein, pentatricopeptide repeat domain protein 1 (PTCD1), causing either dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy (HCM). To understand the role of mitochondrial dysfunction in cardiomyopathy we have conditionally knocked out *Ptcd1* in mice hearts, which results in DCM and compare this model to heterozygous *Ptcd1* mice that develop HCM, providing two physiologically relevant models to study the effects of single gene defects on the development of different heart pathologies as a result of mitochondrial dysfunction.

PTCD1 haploinsufficency results in increased RNA metabolism, in response to decreased protein synthesis and impaired RNA processing that alters the biogenesis of the respiratory chain, causing mild uncoupling and changes in mitochondrial morphology. This mouse model develops adult onset obesity, liver steatosis and DCM. The *Ptcd1* knockout model revealed PTCD1 is essential in mitochondrial ribosome stability via binding to the 16S rRNA, pseudouridylation, and correct biogenesis of the mitochondrial large ribosomal subunit. In this model mitoribosome stability is compromised and therefore retrograde signaling activates the mTOR pathway, upregulating cytoplasmic protein synthesis and pro survival factors, in response to reduced mitochondrial translation. These knockout mice develop severe HCM that leads to premature death.

Q24: Illumina Award Winner

Q25: ThermoScientific Award Winner

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
Q26	Synthesis of dual adjuvanted peptide vaccines for cancer immunotherapy	Juby Mathew	Ferrier Institute, Wellington.
Q27	Lactate racemization, a story of so much more than just a nickel	Matthias Fellner	University of Otago,
Q28	The de novo DNA methyltransferase 3 (DNMT3) is transcriptionally and post-translationally regulated during a major transition in the honeybee life cycle	Carlos Cardoso-júnior	University Of São Paulo, Brazil.
Q29	Dynamin Structure and Function Hinging on Ryngos	David Almeida Cardoso	Children's Medical Research Institute, Westmead, NSW, Australia.
Q30	Tr69957, a Novel Sugar Transporter Involved in Sugarcane Bagasse Degradation in Trichoderma reesei	Karoline Nogueira	University of Sao Paulo. BRAZIL.
Q31	Peroxiredoxin 1 contribution to hydrogen peroxide signalling	Johannes Weijman	University of Otago.
Q32	Evidence for an allosteric drug binding site in the lipid kinase PI3Kalpha	Jack Flanagan	Auckland Cancer Society Research Centre, University Of Auckland
Q33	The rate of the peroxidase reaction catalysed by cytochrome c is influenced by the oxidation of methionine 80	Gabrielle Wood	University of Otago.
Q34	Avoidance of stochastic mRNA:ncRNA interactions in successful recombinant protein expression in Escherichia coli	Chun Shen Lim	University Of Otago.
Q35	Genomic hijacking - how parasitic worms manipulate their hosts	Edwina Dowle	University of Otago
Q36	Understanding the extent of TRAF RING hetero-dimerization and activity	Anubrita Das	University of Otago
Q37	PilVax – a Novel Peptide Delivery Platform for the Development of Mucosal Vaccines	Catherine Jia-yun Tsai	University of Auckland

Q38	Antiviral properties of class IV human deacetylase, HDAC11 during influenza A virus infection	Matloob Husain	University of Otago
Q39	Sequential cleavage of antiviral host factor, Histone Deacetylase 6 is caused by lysosome-associated caspases in influenza A virus infected cells	Mazhar Hussain	University of Otago
Q40	The mechanism and significance of the degradation of human cortactin during influenza virus infection	Da-yuan Chen	University of Otago
Q41	Structural investigation of TRIM proteins in Autophagy	Michael J Barnett	University of Auckland
Q42	Prediction of the host domain for partial viral genomes from metagenomes	Thomas Nicholson	University of Otago
Q43	Characterising the interaction between Fv1 and members of the mammalian ATG8 family	Jamie Taka	University of Auckland
Q44	Investigation of lysozymes from Trichomonas vaginalis	David Reeves	University of Auckland
Q45	Characterisation of the TRIM28/Krab-ZFP interaction in transcription silencing	Eugene Sun	University of Auckland
Q46	Investigating a multi-species antibiotic-resistant infection using MinION sequencing	Jenny Draper	ESR
Q47	A bidentate Polycomb Repressive-Deubiquitinase complex is required for efficient activity on nucleosomes	Martina Foglizzo	University Of Otago
Q48	The Effect of an Acute Bout of High Intensity Exercise on Levels of the Mitochondrial-Derived Peptide, MOTS-c in Healthy Young Adults	Mr. Jonathan Woodhead	University of Auckland
Q49	A role for β-catenin in skeletal muscle glucose metabolism	Stewart Masson	University of Auckland
Q50	CRISPR/Cas9 screening to identify lncRNAS as novel therapeutic targets in triple-negative breast cancer	Megan O'Malley	University Of Otago
Q51	MicroRNA and extracellular vesicle biomarkers for colorectal cancer.	Annabelle Greenwood	University Of Otago, Wellington
Q52	The long non-coding RNA MaTAR17 is a new driver of tumour progression.	Debina Sarkar	University Of Otago
Q53	Developing a light grain for translating optogenetics into clinical treatment	Mehak Janjua	University of Auckland

Q54	Soluble urokinase plasminogen activator receptor is a strong prognostic indicator of mortality in acutely breathless patients	Janice Chew-Harris	University of Otago, Christchurch
Q55	Exploring the interactions of lysozyme with metal-based compounds	Matthew Sullivan	University of Auckland
Q56	Detection, differential sorting and transfer of lung cancer-associated long noncoding RNAs (lncRNAs) via tumor-released exosomes	Aileen Geobee Uy	University of the Philippines Diliman
Q57	Locus-specific concordance of genomic alterations between tissue and plasma circulating tumor DNA (ctDNA) in metastatic melanoma	Leslie Calapre	Edith Cowan University, Australia
Q58	Exploring the genetic robustness of ncRNA and protein	Stephanie McGimpsey	University of Canterbury
Q59	High-throughput Metagenome Sequencing Pipeline using either a Reference-Based or Reference-Free Approach	Melanie Hess	AgResearch
Q60	Targeting Long Non-Coding RNAs to Inhibit Colorectal Tumor Progression and Metastasis	Brandon Wright	University of Otago
Q61	Using novel compounds to delay cyst growth in Polycystic Kidney Disease	Lorissa Mcdougall	University of Otago
Q62	Standardising Purification and Defining Receptors for Lipoprotein(a)	Catherine Dean	University of Otago
Q63	Genetic variants in the SLC2A9 locus confer risk for hyperuricemia in Māori and Pacific Island individuals	Padmini Parthasarathy	University of Otago
Q64	Streptococcus pyogenes nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity	Adrina Jethanand-Khemlani	University of Auckland

Q26: Synthesis of dual adjuvanted peptide vaccines for cancer immunotherapy

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By generating potent cytotoxic T lymphocyte (CTL) responses vaccines can target and kill cancer cells. Peptide-based vaccines are well-defined, can be highly specific and safe because they contain the most basic antigenic components (avoiding off-target effects of other vaccine vectors) and highly scalable but often lack immunogenicity. The addition of vaccine adjuvant is a common strategy often employed to overcome this limitation. A variety of compounds, each acting via distinct pathways, have been exploited, alone or in combination, to create peptide-based vaccines.

A common approach utilizes activation of Toll-like receptor (TLR), which are predominantly expressed in immune cells. TLR agonists such as unmethylated CpG (5' – C-phosphate-G – 3') oligonucleotide and Resiquimod can activate antigen presenting cells by triggering TLR9 and TLR-7/TLR8 respectively, allowing them to drive the expansion of CTLs. Another approach, which is different from most, utilizes glycolipid molecules, such as α-galactosylceramide (α-GalCer), to activate invariant natural killer T-cells. Once activated, these cellular adjuvants are able to license dendritic cells through the provision of CD40L and cytokines, leading to the production of CTLs specific to the antigen being co-presented. Synergistic CTL priming has been reported when the TLR and cellular pathways are triggered simultaneously [1].

In this research, we aim to design self-adjuvanting peptide vaccines that contain multiple adjuvants triggering distinct pathways with the overall goal of developing more powerful, but safe, cancer vaccines. Organic synthesis, chemoselective conjugation protocols and automated solid phase peptide and oligonucleotide synthesis methodologies will be used. Chemical challenges includes conjugation site, chemical linkage strategy, choice of protecting groups for selectivity, large molecular weights and differences in polarity and solubility. All of which needs to be considered with respect to the likely biological activity.

Reference

1. Thaiss, C.A., et al., *Chemokines: a new dendritic cell signal for T cell activation.* Front Immunol, 2011. 2: p. 31.

Q27: Lactate racemization, a story of so much more than just a nickel

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Lactate racemase LarA, the ninth discovered nickel-dependent enzyme, was shown to contain a newly identified vitamin B3 derived nickel cofactor. Synthesis of the cofactor involves three proteins LarB, LarC, and LarE.

Cofactor biosynthesis begins with LarB, a carboxylase/hydrolase of NaAD. LarE, a new member of the PP-loop ATP Pyrophosphatase family, then inserts two sulfur atoms. Structural analysis, combined with structure-guided mutagenesis establishes LarE as a paradigm for sulfur transfer through sacrificing its catalytic cysteine residue, only the second sacrificial sulfur transferase to be described [1].

Finally, LarC inserts a nickel atom to form a five-membered nickellacycle structure in which a stable nickel-carbon bond, as well a nickel-sulfur bond is created (a metallacycle). LarC is therefore the first cylometallase identified in nature. Structure-function characterization discovered that LarC requires cytidine triphosphate hydrolysis [2].

LarA then utilizes the cofactor for lactate racemization. Again using functional and structural methods we provided compelling evidence, that this is accomplished via a proton-coupled hydride transfer mechanism [3].

Lactate racemization is involved in lactate metabolism and cell wall assembly but the cofactor may also be used for a wide range of other yet to be discovered reactions.

1. Fellner, M., Desguin, B., Hausinger, R. P., and Hu, J. (2017) *Structural insights into the catalytic mechanism of a sacrificial sulfur insertase of the N-type ATP pyrophosphatase family, LarE,* Proc Natl Acad Sci U S A 114, 9074-9079.

2. Desguin, B., Fellner, M., Riant, O., Hu, J., Hausinger, R., Hols, P., and Soumillion, P. (2018) *Biosynthesis of the nickel-pincer nucleotide cofactor of lactate racemase requires a CTP-dependent cyclometallase,* J Biol Chem., in press

3. Rankin, J. A., Mauban, R. C., Fellner, M., Desguin, B., McCracken, J., Hu, J., Varganov, S. A., and Hausinger, R. P. (2018) *Lactate Racemase Nickel-Pincer Cofactor Operates by a Proton-Coupled Hydride Transfer Mechanism*, Biochemistry 57, 3244-3251.

Q28: The *de novo* DNA methyltransferase 3 (DNMT3) is transcriptionally and post-translationally regulated during a major transition in the honeybee life cycle.

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In the eusocial honeybee, *Apis mellifera*, workers have a highly plastic lifespan. In this caste, aging is regulated by intrinsic physiologic factors and contingent on the tasks performed by colony members: e.g. nurses are young workers that perform tasks inside the colony, such as cleaning the nest and taking care of the brood, whereas older bees are usually foragers, taking more risky tasks outside the colony, such as collecting food and water. The transition from nurse to forager is a major transition in the life cycle of a bee, but it doe not occur at a fixed time, but can be accelerated, delayed or even reversed depending on social cues and season. DNA methylation, has been shown to be a major epigenetic hallmark related to phenotypic plasticity in social insects. Recently, DNA methylation was found associated with chronological aging and behavioural maturation in honeybee workers. To gain insight into the epigenetic regulation associated with the nurse-to-forager transition, we analysed DNMT3 gene expression and the protein subcellular localization in fat body cells of workers. The results showed that DNMT3 mRNA and protein levels are higher in foragers compared to nurses. Confocal images revealed a strong DNMT3 labelling in the cytoplasm but not in the nucleus, as one would have predicted from its enzymatic activity. This localization was further investigated by immunogold labelling in TEM sections, showing that DNMT3 is associated with lipid vesicles in different cell types of the fat bodies. In line with these results, *in silico* analysis revealed a highly hydrophobic alpha-helix in the three-dimensional structure of honeybee DNMT3, which is likely the region that may interact with lipids. Taken together, our results indicate that DNMT3 is regulated at different levels, from gene expression to protein localization, during a major transition of the honeybee life cycle.

Q29: Dynamin structure and function hinging on ryngos

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Dynamins are multi-domain GTPase enzymes capable of performing the final scission of invaginated plasma membrane prior to the completion of endocytosis. Pharmacological targeting of dynamin in relevant mouse models has been shown to provide therapeutic relief for ailments as diverse as chronic kidney disease and epilepsy. We have generated a series of small molecule modulators (Ryngos) which 'lock' dynamin into a 'ring' oligomeric state that structurally differs from the 'helical' state required for endocytosis. However, these compounds exhibit different activities on enzyme activity *in vitro* (Ryngo-1: mixed-mode / Ryngo-3: stimulation). Due to their chemical similarity, it can be surmised that these pharmacological agents share a common binding pocket. To establish the binding site of Ryngos and allow for targeted drug design and dissection of dynamin residues responsible for inhibition or stimulation of activity, advanced computer modelling was initially employed. Lead compounds, Ryngo-1-23 and Ryngo-3-32, were predicted to independently localize to, and differentially interact with Hinge 1, located between middle domain and bundle-signalling element of dynamin. A partial overlap of implicated residues between Ryngo-1-23 and Ryngo-3-32 suggests drug binding to different sub-regions of Hinge 1 may be capable of imparting different actions (stimulation/inhibition) on dynamin activity *in vitro*. To validate this model, mutagenesis of implicated Hinge 1 residues was carried out and resultant mutants characterised. Biochemical assays largely support these predictions (i.e. single mutations specifically lost drug action) as well as highlight a broader role for Hinge 1 in dynamin characteristics (e.g. activity, oligomerisation, and endocytosis). To account for allosteric effects of mutation, a chemically dissimilar dynamin-targeting compound (Dynole-34-2) was employed and revealed loss of Ryngo action to be specific to Hinge 1. The data supports the proposed model of these compounds differentially interacting with a flexible hinge within dynamin, an exceptionally rare binding site for pharmacological agents.

Q30: *Tr69957*, a Novel Sugar Transporter Involved in Sugarcane Bagasse Degradation in *Trichoderma reesei*

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Trichoderma reesei (*Hypocrea jecorina*) is a saprophyte fungus involved in the degradation of cell wall polysaccharides from plants. This fungus has an astonishing ability to produce and secrete cellulases, and is the most important industrial fungus in the production of these enzymes that are used, among other purposes, in the biofuel industry, such as bioethanol. Despite a broad progress in the characterization of cellulosic enzymes in *T. reesei*, the involvement of sugar transporters in the process of deconstruction of biomass and induction of xylanases and cellulases genes expression are yet to be known. The aim of this study was characterize a novel sugar transporter, which was previously identified by our group through in silico analysis of RNA-seq data. For this, we constructed a *T. reesei* 69957-sugar transport system in *Saccharomyces cerevisiae* and a *T. reesei* mutant strain carrying a deletion of *Tr69957.* The data obtained showed that *T. reesei*69957-sugar transport system (*Tr69957*) is capable of transporting xylose, mannose, and cellobiose in *Saccharomyces cerevisiae*. The deletion of *Tr69957* in *T. reesei* affected the fungal growth and biomass accumulation, and the sugar uptake in the presence of mannose, cellobiose, and xylose. Molecular docking studies revealed that *Tr69957* shows reduced protein–ligand binding energy for interactions towards disaccharides in comparison with monosaccharides. In addition, the deletion of *Tr69957* affected the gene expression of cellobiohydrolases (*cel7a* and *cel6a*), β-glucosidases (*cel3a* and *cel1a*), and xylanases (*xyn1* and *xyn2*) in the cultures of parental and mutant strains in the presence of cellobiose and sugarcane bagasse. This work contributes to a more comprehensive understanding of the molecular mechanisms involved in the process of lignocellulosic biomass deconstruction in *T. reesei* and show for the first time that a filamentous fungus (*T. reesei*) contains a potential mannose transporter that may be involved in the degradation of cellulose.

Reference

Nogueira KMV, de Paula RG, Antoniêto ACC, Dos Reis TF, Carraro CB, Silva AC, Almeida F, Rechia CGV, Goldman GH, Silva RN (2018). *Characterization of a novel sugar transporter involved in sugarcane bagasse degradation in Trichoderma reesei*. Biotechnology for Biofuels. 2;11:84.

Q31: Peroxiredoxin 1 contribution to hydrogen peroxide signalling

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Hydrogen peroxide (H_2O_2) has transcended from being considered merely an artefact of aerobic respiration to a *bona fide* cellular signalling molecule. H_2O_2 is able to oxidise thiol groups of cysteine residues, thereby modulating protein activity. However, how H_2O_2 can specifically oxidise thiols remains perplexing because of the existence of a group of proteins named peroxiredoxins (Prdx). Prdxs are ubiquitous and, highly abundant peroxidase proteins with exceptionally high reaction rate constants with H_2O_2 (in the order of 10^7 M^{-1} s^{-1}). Within bacteria and yeast, H_2O_2-signalling is known to operate via intermolecular disulfide-bond relays, mediated by Prdxs. In mammals, disulfide-relays have been observed with Prdxs (namely Prdx1 and 2) but there are limited examples. Characterising how mammalian cells respond to H_2O_2 treatment on a system-wide scale is essential for discerning the roles Prdx1 and 2 have in mammalian H_2O_2-signalling.

We have used redox proteomics (isotope-coded affinity tag (ICAT) labelling and mass spectrometry) to investigate the role of Prdx1 in H_2O_2-signalling within a human HAP1 cell line. HAP1 cells that have Prdx1 knocked-out (Prdx1-KO) have a reduced response to H_2O_2 via the p38-MAPK stress-signalling pathway. Initial data suggests that WT and Prdx1-KO HAP1 cells exhibit similar basal levels of protein oxidation. Moreover, subtle differences in the response of WT and Prdx1-KO cells to treatment with H_2O_2 point towards Prdx1 acting as both a scavenger and signalling-molecule. These preliminary studies indicate that redox proteomics using ICAT will enable a greater appreciation of the role that Prdx1 in H_2O_2-signalling.

Q32: Evidence for an allosteric drug binding site in the lipid kinase PI3Kα

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The class IA phosphatidylinositol-3 kinase PI3Kα, phosphorylates the membrane embedded phospholipid phosphatidylinositol-4,5-phosphate ($PI(4,5)P_2$) making $PI(3,4,5)P_3$, which is a secondary messenger that promotes the membrane localisation of proteins with specialist domains that recognize the phosphorylated inositol unit. To perform this reaction, PI3Kα needs to bind its ATP substrate and interface with the cells plasma membrane to find its $PI(4,5)P_2$ substrate. The gene encoding PI3Kα is one of the most mutated in cancer, and many of the mutations create hyper-activated enzymes. Based on this, there is a huge effort to find inhibitors that are selective for the PI3Kα enzyme, with the current molecules all blocking the ATP binding site. Despite this, the effect of these inhibitors on membrane binding has yet to be investigated, even though they bind to the region of the protein most involved in membrane binding and substrate recognition.

Using a Forester resonance energy transfer (FRET) reporter system and biolayer interferometrry, we have investigated the effect of ATP blockers on the interaction between PI3Kα and a synthetic membrane using mixed lipid liposomes.

We identified one molecule that had a dramatic effect on wild type PI3Kα membrane binding compared to a common oncogenic form that has better membrane binding capability. We also observed a similar effect using biolayer interferometry to follow protein-liposome interactions with immobilised liposomes. In dissecting the function of some of the drug-ATP binding site interactions, we developed a reactive probe molecule that led to the unexpected identification of a new binding site outside of the active site.

We hypothesise that this new site is responsible for the membrane binding effect via an allosteric mechanism. These new data provide evidence that some PI3K inhibitors have two modes of action and further exploration of this could be used to develop new types of PI3Kα inhibitor.

Q33: The rate of the peroxidase reaction catalysed by cytochrome *c* is influenced by the oxidation of methionine 80

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Cytochrome *c* is proposed to catalyse the peroxidation of the phospholipid cardiolipin in mitochondria. There are reports that this peroxidase reaction is a necessary step in the intrinsic apoptosis pathway. The reaction has a lag phase followed by a steady state before the reaction rate levels off. Cytochrome *c* differs from other peroxidase enzymes as the heme group is hexa-coordinated. It is believed that during the lag phase of the reaction the methionine residue bound to the heme iron is oxidised. This disrupts the bond between the methionine and the heme, making cytochrome *c* penta-coordinate and able to catalyse the peroxidation of substrate.

Both naturally and non-naturally occurring mutations in cytochrome *c* are known to change the rate of the peroxidase reaction. The purpose of this research was to determine whether this difference in activity is due to the protein dynamics changing when cytochrome *c* is mutated. To address this, the temperature dependence was determined for the steady state of the reaction and used to estimate the change in heat capacity. The results showed that the change in heat capacity is the same for wildtype and mutant cytochrome *c*. Therefore, protein dynamics do not play a role in the observed activity changes as they do not differ with cytochrome *c* mutation.

The effect of substrate concentration on the peroxidase reaction was also considered. As substrate concentration is increased, the steady state rate of the reaction decreases, and the lag phase lasts longer. This suggests that high concentrations of substrate inhibit the oxidation of the methionine residue associated with the heme group, inhibiting the formation of an active penta-coordinate peroxidase enzyme. We propose that differences in peroxidase activity are due to changes in the oxidation of the methionine, with higher concentrations of substrate having an inhibitory effect.

Q34: Avoidance of stochastic mRNA:ncRNA interactions in successful recombinant protein expression in *Escherichia coli*

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The most common solution to overcome low expression of heterologous proteins in *Escherichia coli* is optimization of codon usage. However, this does not always solve the expression problem. We recently discovered that the 'avoidance' of stochastic mRNA:ncRNA interactions is a common feature of highly expressed endogenous proteins and has been verified with green fluorescence reporters. To investigate whether this finding is useful for designing synthetic gene, here we study TargetTrack, a structural genomics database developed by the Protein Structure Initiative. Other features affecting expression such as biosynthetic cost and tRNA copy number were also examined.

Q35: Genomic hijacking - how parasitic worms manipulate their hosts

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Parasites routinely manipulate the behaviour of their hosts to enhance their survival and transmission. One of the most extraordinary of these host manipulations is the water-seeking behaviour that some nematodes and hairworms induce in their hosts so that the worms might exit the host in a suitable environment and reproduce. The worm hijacks the host's central nervous system forcing the normally terrestrial host to seek water. Once water is found the adult worm, often equal to or bigger than the host, erupts in an explosive frenzy, sacrificing the host, so that the parasite might complete its lifecycle. This amazing alteration in behaviour is induced by worms spanning two phyla (Nematoda and Nematomorpha) and is observed in a variety of arthropod hosts, notably crickets, weta and earwigs. Host manipulations are the consequence of genes in the parasite genome modifying the hosts' phenotypic traits. But the development and genetic control of these behavioural modifications are not well understood as experimentally tractable systems are rare. Here we use total RNA sequencing to access transcriptomic changes in both the parasite and host brain with the goal of identifying how host manipulations develop and the genetic mechanisms behind them. Using two phyla (Nematoda and Nematomorpha) of parasites that induce the same behavioural manipulation in two different insects (earwigs and weta) also enabled us to determine the similarity of the genetic mechanism(s) across divergent lineages. Despite the similarity of the behavioural manipulation in the groups in one system we found extremely high rates of a co-occurring virus. We hypothesis that in this system the behavioural manipulation may not be induced by the worm but by the co-occurring virus.

Q36: Understanding the extent of TRAF RING hetero-dimerization and activity

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TNF Receptor Associated Factors (TRAFs) are a family of seven adaptor proteins that regulate both cell survival and apoptosis in many immune response pathways. Dysregulation or mutation of TRAFs has been linked to development of B cell lymphomas and several inflammatory disorders1. TRAFs act as scaffolds to recruit downstream substrates and are also thought to modulate signal transduction via ubiquitylation. TRAFs are defined by the presence of a C-terminal coiled coil domain as well as a TRAF-C domain. These domains form a stable trimer and the TRAF-C domain interacts with the cytoplasmic domains of various receptor peptides. At the N-terminus most TRAFs have a RING domain followed by four-five Zn finger domains and these are important for ubiquitylation. Structural and biochemical studies have shown that dimerization of the RING domain is crucial for the E3 ligase activity of TRAF62. Although both the trimeric receptor binding domain, and dimeric E3 ligase activity have been well studied, it is uncertain how these TRAF-C/CC trimers and RING dimers assemble/associate during signal transduction.

Surface conservation mapping of the RING domains from TRAFs that co-localize during signalling shows a highly conserved RING dimer interface indicating the possibility of TRAF RING heterodimer formation. We have investigated the importance of RING dimerization for ubiquitylation and whether RING heterodimers can form between co-localized TRAFs. Our studies suggest a novel molecular mechanism mediating ubiquitin transfer by dimeric TRAF RING domain. In addition, our *in vitro* biochemical assays show that TRAF RING heterodimers can form. This is an exciting observation that suggests a higher level of complexity in TRAF-mediated signalling is possible.

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Q37: PilVax – a Novel Peptide Delivery Platform for the Development of Mucosal Vaccines

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Vaccines remain the most cost-effective and feasible means of infectious disease control in the community. Well-defined synthetic vaccines based on individual peptides are specific and safe. However, peptide antigens are usually poorly immunogenic and sensitive to proteolytic degradation, thus require costly conjugation to carrier proteins and administration with potentially toxic adjuvants. Lactic acid bacteria have become promising vehicle for delivering active molecules to mucosal sites. We have developed PilVax, a novel peptide delivery platform that allows the presentation of a stabilised and highly amplified peptide as part of the group A streptococcus (GAS) serotype M1 pilus structure (PilM1) on the surface of the food-grade bacterium *Lactococcus lactis*. Pili (*sing.* Pilus) are hair-like protrusions from the bacterial cell surface. GAS pili are mainly formed by multiple copies of the highly immunogenic, covalently-linked backbone pilin (Spy0128). We identified several surface-accessible, structurally-flexible loop regions within Spy0128 that can be replaced with the model peptide OVA324-339. The modified pilus structure was expressed on the surface of *L. lactis* by a plasmid harbouring a strong, constitutive promotor. Pili assembly and peptide display were analysed by western blot and flow cytometry using specific antibodies. Intranasal immunisation of PilVax generated strong mucosal and systemic antibody responses in mice. We have further show that it is possible to insert more than one peptide into the same integration site, and peptide epitopes can be incorporated into structurally similar but antigenically different pilus structure. PilVax also provides benefits such as higher safety and lower production and transportation costs. Furthermore, the needle-free mucosal administration route is an additional advantage for the use in developing countries, where efficacious vaccines are most needed.

Q38: Antiviral properties of class IV human deacetylase, HDAC11 during influenza A virus infection

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Influenza virus is an ever-evolving human pathogen and presents a constant threat to global public and animal health. Therefore, there is an undeniable need to identify missing links – both host and viral – that are critical for influenza virus pathogenesis to develop alternative, effective and long-lasting anti-influenza virus strategies. Histone deacetylase 11 (HDAC11) is the most recently discovered deacetylase and is classified as sole member of class IV. We and others have recently demonstrated that at least one member of each of the other three human HDAC classes – I, II and III, exhibit antiviral properties against influenza A virus (IAV). Consistent with this, we demonstrate herein that human HDAC11 exhibits anti-IAV properties too. We found that RNA interference-mediated knockdown of HDAC11 augments the IAV growth kinetics in human lung epithelial cells, A549 by up to 1 log. One of the ways HDAC11 exerts its anti-IAV function is by being a part of the IAV-induced host antiviral response. We found that the kinetics of both IAV- and interferon-induced innate antiviral response is significantly delayed in HDAC11-depleted cells. Further, in the absence of HDAC11 expression, there was a significant decrease in the expression of interferon-stimulated genes – IFITM3, ISG15, and viperin, previously implicated in anti-IAV function. One of the ways IAV antagonises HDAC11 is by downregulating its expression in host cells. We found that there was up to 93% reduction in HDAC11 transcript levels in A549 cells in response to IAV infection. HDAC11 is the smallest HDAC with majority of its polypeptide assigned to catalytic domain. Further, evolutionarily, it seems to be the least evolved and most closely related to common ancestral HDAC gene(s). Furthermore, HDAC11 has also been described as a *deacylase*. Therefore, our findings present exciting prospects for further investigations into significance of HDAC11 in virus infections.

Q39: Sequential cleavage of antiviral host factor, Histone Deacetylase 6 is caused by lysosome-associated caspases in influenza A virus infected cells

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Influenza A virus (IAV) is one of the most successful human pathogens. It causes an acute febrile respiratory disease in humans, commonly known as flu. IAV causes regular epidemics, unpredictable pandemics, and fatal zoonotic outbreaks. Lack of universal IAV vaccine and effective antiviral drug warrant a thorough understanding of IAV-host interactions. Our lab and others have discovered that host histone deacetylase 6 (HDAC6) plays a pivotal role in IAV infection by restricting the assembly of IAV and inducing the innate immune response against IAV. Viruses are known to antagonize antiviral host factors by various mechanisms to replicate efficiently in host cells. Therefore, the aim of my PhD is to determine the mechanisms IAV employs to circumvent the antiviral function of HDAC6. So far, I have found that IAV downregulates HDAC6 expression in human lung epithelial cells both at mRNA (>95% after 24 hours) and polypeptide (~75% after 24 hours) level, and in a dose-dependent manner. A further time-course kinetic analysis revealed that HDAC6 mRNA and polypeptide levels decrease gradually over the course of the infection. The downregulation of HDAC6 polypeptide level is potentially occurring through its proteolytic cleavage as two smaller fragments (~98 and ~125 kDa) appear in infected cells. The perturbation of lysosomal, proteasomal and apoptotic pathways, three main protein degradation pathways in mammalian cells, revealed that lysosome-associated caspases sequentially cleave HDAC6 polypeptide in infected cells. Further, the knockdown of the expression of executor caspases, caspase-3, -6, and -7 indicated that caspase-6-activated caspase-3 cleaves the HDAC6 polypeptide in infected cells. A further investigation of caspase-3 cleavage sites in HDAC6 polypeptide will add to the knowledge related to the antagonism of HDAC6 by IAV.

Q40: The mechanism and significance of the degradation of human cortactin during influenza virus infection

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Influenza virus remains one of the major threats for global public health and economy. The lack of a universal vaccine, variable efficacy, and difficulty in strain prediction for annually-formulated vaccines and growing resistance to existing antiviral drugs press the need to find targets with potential universal treatment and prevention against influenza virus. To contribute to this overarching goal, we study the molecular basis of influenza virus-host cell interactions.

Recently, we demonstrated that influenza A virus (IAV) exploits host cytoskeleton scaffolding protein, cortactin for infection. We found that RNA interference (RNAi)-mediated knockdown of cortactin expression in human cells inhibits IAV infection, whereas the overexpression of cortactin promotes IAV infection. However, during the late stage of IAV infection, cortactin polypeptide undergoes degradation by lysosome-associated apoptotic pathway. In this follow-up study, our aim is to further understand the mechanism of cortactin degradation and elucidate the significance of it during influenza virus infection. So far, we have found that lysosomal protease, cathepsin C is involved in cortactin degradation as the knockdown of cathepsin C mRNA rescued cortactin polypeptide by 90% in IAV-infected cells. Furthermore, caspase 3 – a key executor protease of apoptotic pathway is directly involved in cortactin degradation as the knockdown of caspase 3 rescued cortactin polypeptide in IAV-infected cells by 87%; whereas, the knockdown of caspase 6 and 7 – the other executor proteases did not. Finally, the CaspDB database – a caspase cleavage site prediction tool predicts the presence of at least 34 caspase cleavage motifs throughput the human cortactin polypeptide. A sequential site-directed mutagenesis of those motifs indicate that cortactin polypeptide is cleaved at multiple sites during IAV infection though a final outcome of this exercise is still awaited. Our goal is to generate a degradation-resistant cortactin polypeptide variant to elucidate the role of human cortactin during influenza virus infection.

Q41: Structural investigation of TRIM proteins in Autophagy

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Members of the TRIM protein family are characterised by an N-terminal TRI-partite Motif, containing a RING domain with E3 ligase activity, up to two B-box binding domains, and a coiled-coil domain. With over 80 members in the human genome, TRIM proteins are involved in a diverse range of cellular processes including innate immune surveillance and transcriptional silencing.

Recent studies[1] have suggested that a majority of TRIM proteins may also have roles in the regulation of Autophagy, an important cellular process for the degradation and recycling of cytosolic components and maintaining homeostasis.

In this hypothesis, the TRIM protein acts as a scaffold and interacts with key autophagy machinery including Beclin-1, ULK1, and mammalian ATG8 homologues (mATG8). The principal mechanism for localising to the autophagophore membrane is via binding to a mATG8, such as LC3B, which are embedded within the membrane by C-terminal lipid modification. It is proposed that TRIM proteins also act as a selective autophagy receptor, targeting substrates to the membrane via an LC3-interacting region (LIR) that binds LC3B. Consequently, TRIM proteins may act as receptors and regulators of precision autophagy for specific targets[2].

We have recently determined the co-crystal structure of TRIM5α and LC3B, that reveals a cryptic binding motif within the coiled-coil domain of TRIM5α, making this the first example of an LIR with α-helical secondary structure.

To characterise this atypical LIR and probe the mode of binding to the other mATG8s, we have developed fusion constructs of mATG8 proteins with a C-terminal peptide derived from the helical LIR region of TRIM5α. We have so far determined two crystallographic structures for GABARAPL1 fusion constructs, however care must be taken in their biological interpretation due to the loss of secondary, tertiary, and quaternary structure elements of the α-helical LIR.

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Q42: Prediction of the host domain for partial viral genomes from metagenomes

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Diverse viruses are integral parts of biological systems. New technologies including metagenomic analysis has allowed many more viral sequences to be found. Its is expected that virome metagenomes contain a mix of sequences that may be from viruses infecting eukaryotic, bacterial or archaeal hosts. However, one challenging part of viral metagenomics has been identifying the likely host from a partial viral sequence. Archaeal viruses are apparently the least common, and their sequences are underrepresented in databases. They are not easily detected in viromes, we address this problem here. A set of Hidden Markov Models was made for all viral proteins from DNA viruses with known hosts in each of the three domains of cellular life (Archaea, Bacteria, Eukarya). These models were analysed to identify those found specifically in viruses from one domain. In a test set of viral proteomes from viruses with known hosts, multiple models matched proteins and predicted the correct host. By using these 24 199 models we were able to predict the domain for polycistronic sequences from metagenomes. Most predictions were for Bacteria or Eukarya hosts, but the program also enabled prediction of Archaeal viruses from metagenomic sequences.

Q43: Characterising the interaction between Fv1 and members of the mammalian ATG8 family

Taka, J.R.H, Black, M., Keown, J.R., Goldstone, D.C.

The co-evolution of retroviral pathogens and mammals has led to development of intracellular defence systems to combat retroviruses. Retroviral replication can be restricted at multiple stages of the retroviral life-cycle by anti-viral proteins termed restriction factors. Fv1 is the prototype restriction factor that was first discovered to determine susceptibility of mouse cells to MLV. Fv1 has phenotypic similarities to the unrelated restriction factor TRIM5α, both recognise the viral capsid lattice and block replication prior to integration of the provirus into the host genome. The similarity extends to the functional domain architecture of an N-terminal dimerization domain and a C-terminal recognition domain.
TRIM5α has been proposed to act as an autophagic receptor for recruitment of HIV-1 capsid to the autophagosome by interacting with the mammalian ATG8 protein family. The ATG8 family interact with proteins containing a well characterised LIR binding motif principally consisting of two hydrophobic residues that bind conserved hydrophobic pockets in the ATG8 proteins. Recently, the Fv1 N-terminal domain (20-200) was demonstrated to bind to the ATG8 protein LC3B.

We investigated binding of Fv1 to the ATG8 family by sedimentation analytical centrifugation (SV-AUC) experiments, demonstrating Fv1 (20-200) bound all 6 members. A saturation binding experiment was undertaken on 5 ATG8 proteins showing the binding affinity for Fv1 is very weak. To identify the Fv1 binding site we made a series of Fv1 (20-200) constructs containing single aromatic-to-alanine mutations and investigated binding to LC3B by SV-AUC.

Q44: Investigation of lysozymes from *Trichomonas vaginalis*

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Trichomonas vaginalis is the most common non-viral STI in the world, with more than 143 million new infections each year. This obligate parasite of the human urogenital tract infects 5% of women and 0.6% of men worldwide causing trichomoniasis[1]. Symptoms include urethritis, vaginal pruritus, and erythema. Infection requires adherence of the protozoa to the epithelial tissue of the urogenital tract, which is inhibited by the native microbiota. Most infections are asymptomatic but confer complications such as increased cervical and prostate cancer risk, male infertility, and a 2-3-fold increased risk of HIV acquisition. Treatment of *T. vaginalis*, limited to metronidazole and tinidazole, is becoming less frequently successful as drug resistant strains are on the rise.

The *T. vaginalis* genome was published in 2007, which identified a highly expanded degradome[2]. Previous work from our group characterised nine NlpC proteins, which demonstrate activity against bacterial cell wall peptidoglycan by cleaving the peptide cross links. Overexpression of NlpC proteins may confer a competitive advantage to *T. vaginalis* in the urogenital tract.

To further explore the repertoire of peptidoglycan degrading enzymes in *T. vaginalis,* we searched the *T. vaginalis* genome for lysozyme-like proteins. Lysozymes are a family of peptidoglycan degrading enzymes which hydrolyse the glycosidic bond between the N-acetylmuramic acid and N-acetyl-D-glucosamine subunits of peptidoglycan. We identified eight candidate lysozyme like genes. Sequence analysis revealed five contained arginine rich signal sequences very similar to those previously seen in NlpC proteins. Cloning and expression of these genes revealed that removal of signal sequences was required for soluble expression. Turbidity assays may determine the ability of *T. vaginalis* lysozymes to degrade peptidoglycan extracted from *Eschericia coli* and *Bacillus subtilis*. X-ray crystallography trials for structural characterisation are underway.

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Q45: Characterisation of the TRIM28/Krab-ZFP interaction in transcription silencing

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TRIM28 (KAP1/TIF1β) acts as the universal co-repressor for the largest family of transcription factors in mammals, the Krüpple-associated box containing-Zinc Finger Proteins (Krab-ZFPs). During early embryonic development Krab-ZFP/TRIM28-mediated silencing is responsible for maintaining genomic imprints and silencing endogenous retroviruses. In addition to its transcriptional role, TRIM28 takes part in a multitude of cellular pathways and modulates processes including cell growth, differentiation, pluripotency, apoptosis, DNA repair, and cancer.

TRIM28 belongs to the TRIM protein family with over 70 members in humans. They share a conserved N-terminal tripartite motif (TRIM), also known as the RBCC as it consists of a RING domain, one or two B-box domains and an antiparallel coiled-coil (1). Using biophysical techniques we have identified the domain responsible for higher order assembly in TRIM28, and determined the crystal structure of the assembly interface. We have also performed structure-based mutagenesis to investigate the functional importance of TRIM28 self-assembly. The assembly-null mutants we have generated provide a useful tool to characterise TRIM28's interaction with Krab-ZFPs. We have determined the binding affinity and stoichiometry of TRIM28/Krab-ZFP interaction. We have undertaken Small Angle X-ray Scattering to determine the molecular envelope of the TRIM28 RBCC/MBP-Krab complex. This model suggests the Krab domain binds to the central part of the TRIM28 coiled-coil, consistent with our results from domain mapping using a series of recombinant TRIM28 truncation constructs.

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Q46: Investigating a multi-species antibiotic-resistant infection using MinION sequencing

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Carbapenemase-producing Enterobacterales (CPE) are enteric bacteria resistant to carbapenems, the antibiotic class considered the last line of defence against serious gram-negative bacterial infections. In New Zealand, infections with CPE are often associated with overseas travel. Their detection is of significant concern, as carbapenemase genes are carried on mobile elements which can easily spread under antibiotic pressure, and plasmid transmission has been implicated in hospital CPE outbreaks. Here we describe using MinION sequencing to investigate the case of a hospital patient infected with multiple species of CPE.

Four distinct carbapenemase-producing bacterial species were isolated from clinical and screening specimens from a single patient during a three-week period of hospitalisation. Standard Illumina sequencing revealed a common ~11 kb contig containing the *bla*$_{\text{NDM-1}}$ carbapenemase gene and a ~46-52 kb contig carrying the signature of IncA/C2, a highly efficient conjugative plasmid. This suggested that extensive transmission of a *bla*$_{\text{NDM-1}}$ carrying IncA/C2 plasmid between species had occurred within the patient. However, this could not be determined reliably from the incomplete Illumina short-read assemblies.

We therefore turned to Oxford Nanopore long-read sequencing, using a rapid barcoding kit on the same DNA preparations used for the Illumina libraries, and multiplexing up to 11 isolates on a single MinION flowcell. Even with the multiplexing and non-optimised DNA extraction, a simple hybrid assembly with UniCycler revealed that a novel 167 kb IncA/C2 plasmid had been transmitted between bacterial species within the patient. This plasmid carries multiple antibiotic resistance genes, including the *bla*$_{\text{NDM-1}}$ carbapenemase, thus explaining the multi-species CPE infection.

Q47: A bidentate Polycomb Repressive-Deubiquitinase complex is required for efficient activity on nucleosomes.

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Ubiquitination and deubiquitination of histone proteins are post-translational modifications that play a key role in chromatin structure and regulation of gene transcription. Of particular relevance is attachment of ubiquitin to Histone 2A on lysine residue 119 (H2AK119Ub) in humans, an epigenetic mark that represses gene expression and is removed by the Polycomb Repressive-Deubiquitinase (PR-DUB) complex.

The *Drosophila melanogaster* PR-DUB consists of the deubiquitinase protein Calypso and a binding partner Additional Sex Combs (ASX). The corresponding complex in humans comprises the proteins BRCA1-associated protein-1 (BAP1) and ASX-Like 1 (ASXL1). BAP1 has recently emerged as an important tumor suppressor, which is frequently mutated in metastatic uveal melanomas (85%), mesotheliomas (30–60%), renal cell carcinomas (15%), as well as in myelodysplastic syndromes and other neoplasms.

We have solved the crystal structure of the Calypso–ASX complex to a resolution of 3.5 Å. Using biochemical and biophysical approaches, we demonstrated that Calypso–ASX forms a bidentate complex consisting of two Calypso and two ASX molecules. Mutagenesis studies showed that the Calypso coiled-coil hairpin plays a key role in mediating bidentate complex formation. Disruption of the bidentate Calypso interface does not affect inherent catalytic activity, but impairs recruitment of the PR-DUB to nucleosomes and removal of H2AK119Ub. Mutating the equivalent surface on the human counterpart, BAP1, also compromises recruitment and activity on nucleosomes.

This work suggests that high local concentrations drive assembly of bidentate PR-DUB complexes on chromatin, favouring PR-DUB recruitment to nucleosomes and removal of H2AK119Ub. Such a model provides insights into the mechanism of distinct classes of PR-DUB mutation in tumorigenesis.

Q48: The Effect of an Acute Bout of High Intensity Exercise on Levels of the Mitochondrial-Derived Peptide, MOTS-c in Healthy Young Adults

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Regular exercise improves metabolic health and attenuates many of the major hallmarks of aging. However, we do not yet fully understand how exercise improves health of multiple organs within the body. Evidence suggests that metabolic stress caused by exercise promotes skeletal muscle to release peptides and hormones that facilitate an adaptive response to improve system-wide function. MOTS-c is a recently discovered peptide translated from the mitochondrial genome and has been shown to elicit exercise-like improvements in metabolism when administrated exogenously in mice (1). MOTS-c and exercise share a similar downstream molecular signalling target (AMPK) and therefore may be related. We hypothesized that MOTS-c expression may be increased by high intensity aerobic exercise in young adult humans.

10 healthy young males completed 10 x 60 second intervals of maximal intensity exercise interspersed with 75 seconds of rest. Muscle and plasma MOTS-c expression was significantly increased immediately post-exercise but returned to baseline following 4-hours recovery, indicating an acute response to exercise. The ribosomal 12S region encoding MOTS-c also had an increased RNA expression immediately post exercise. To investigate whether MOTS-c production primarily occurs within the muscle, C57/BL6 mouse extensor digitorum longus (EDL) muscles were isolated and contracted *ex vivo* for 10 minutes. Muscle MOTS-c levels were substantially increased following contraction, suggesting MOTS-c is produced rapidly within muscle in response to metabolic stress. Taken together, we have provided the first evidence that the mitochondiral derived peptide MOTS-c is an exercise-sensitive myokine, and it is now important to determine the fate and actions of secreted MOTS-c to understand its role in mediating exercise induced improvements in health.

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Q49: A role for β-catenin in skeletal muscle glucose metabolism

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Type 2 diabetes is a major health issue in New Zealand, to combat this a thorough understanding of insulin action is required. While considerable effort has been focused on understanding the complex signalling networks underpinning the movement of GLUT4 containing vesicles to cell surface, much is still unclear. Recent work has identified β-catenin as a component of glucose uptake into adipocytes (1), here we show a similarly important role in the major glucose clearing organ, skeletal muscle. Disruption of β-catenin in L6-myc myocytes through both siRNA-knockdown, and chemical inhibition by pyrvinium, negatively impacts insulin-stimulated skeletal muscle glucose uptake. These findings are supported by *ex vivo* experiments in which insulin-stimulated isolated mouse muscle glucose uptake is also impaired following pyrvinium treatment. Furthermore, pyrvinium also impairs AICAR stimulated glucose uptake in L6myc myotubes indicating β-catenin is involved in multiple forms of GLUT4 mediated glucose transport.

Immunoblotting in both tissue and cultured myocytes suggest insulin triggers a signalling cascade resulting in phosphorylation of β-catenin^{S552} which can be blocked by the protein kinase B inhibitor Akti-1/2. Given that inhibition of β-catenin blocks multiple GLUT4-trafficking mechanisms, it is likely that β-catenin's role in glucose uptake is in a shared component of both insulin dependent and independent uptake, potentially involving cytoskeleton rearrangement as is seen in other tissue types (2). However, further work is needed to identify the specific mechanism by which β-catenin regulates GLUT4-mediated glucose uptake.

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Q50: CRISPR/Cas9 screening to identify lncRNAS as novel therapeutic targets in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is a subtype of breast cancer classified by a lack of clinically significant levels of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in the patient's tumours. Absence of these typical markers of breast cancer mean that most TNBC patients cannot be treated by targeted therapies.

In recent years, long non-coding RNAs (lncRNAs) have emerged as a potential therapeutic target in cancer treatment. LncRNAs are a subtype of non-coding RNAs classified by a length of > 200 nt. By definition, they lack a significant open reading frame which means they are not translated into proteins. Although they do not code for proteins, lncRNAs are biochemically analogous to mRNA. Hundreds of lncRNAs have recently been discovered as key players in at least one of the hallmarks of cancer.

This project aims to assess the biological importance of lncRNAs in TNBC progression and metastasis. The lncRNAs of interest were chosen based on their overexpression in TNBC patients using data found in The Cancer Genome Atlas (TCGA). CRISPR interference (CRISPRi), a CRISPR/Cas9 system which uses a catalytically inactive Cas9 protein, is being employed to create lncRNA knockdown mutants of an epithelial human breast cancer cell line representing metastatic TNBC. We confirmed successful integration of the CRISPRi system into the TNBC cells and validated that the system was effective at silencing both protein-coding and lncRNA genes *in vitro.* We performed a loss-of-function screen using sequence specific single guide RNAs (sgRNAs) to assess the impact of lncRNAs on TNBC cell growth. This study aims to identify lncRNAs that are promising new therapeutic targets for TNBC.

Q51: MicroRNA and extracellular vesicle biomarkers for colorectal cancer.

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Earlier diagnosis and optimisation of treatment strategies for colorectal cancer (CRC) are two major areas that could improve CRC-related morbidity and mortality in New Zealand. Previously, miR-21 and miR-29a have been found to be aberrantly expressed in tumour tissue and plasma of patients with CRC. These miRNA are released from tumour cells packaged into extracellular vesicles (EVs) which are capable of travelling to the circulatory system and distant sites in the body. As such, they have emerged as potential diagnostic and prognostic markers for CRC.

To assess the ability of circulating miR-21 and miR-29a to distinguish between healthy controls (n=17) and patients with early (stage I/II, n=32) vs. late disease (stage III/IV, n=29), we examined plasma expression levels by RT-qPCR. Additionally, expression levels of miR-21 and miR-29a (RT-qPCR) and CD147 (CRC-EV marker; immunohistochemistry) were examined in the lymph nodes of 13 stage II CRC cancer patients to assess whether evidence of CRC-derived EVs were detectable in non-metastatic patients.

Plasma miR-29a expression was significantly different across patient groups ($p=0.0035$, one-way ANOVA) with statistically significant differences observed between control vs late stage CRC and early vs late stage CRC ($p<0.5$, Tukey's multiple comparison). Additionally, plasma miR-21 expression trended towards statistical significance ($p=0.07$, one-way ANOVA).
This work suggests that plasma levels of miR-29a, and potentially miR-21, could be useful markers for distinguishing between early and late stage CRC and for discrimination of CRC patients vs controls. Future work will need to extend this analysis to a larger group of patients to validate these findings and assess the prognostic value of these microRNA markers.

Q52: The long non-coding RNA MaTAR17 is a new driver of tumour progression.

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Genome-wide studies revealed that majority of the human transcriptome consists of non-coding RNAs, but their function and mode of action remains elusive. Diermeier et al., identified and characterised 30 Mammary Tumour Associated RNAs (*MaTAR*s) that are overexpressed in breast cancer compared to normal tissue. *MaTARs* have the potential to be used as therapeutic tools owing to their tumour-specific expression. Unlike other lncRNAs, upregulated expression of *MaTAR17* is not limited to breast cancer and is overexpressed in colorectal, lung and head and neck cancer. Overexpression of *MaTAR17* in several cancer types indicates a more general role of this lncRNA in tumour progression. Knockout of *MaTAR17* using the CRISPR/Cas9 system resulted in reduced cell proliferation *in vitro* and *in vivo*. Analysis using RISE (a database of RNA interactome from sequencing experiments), revealed possible RNA-RNA interaction between Microtubule Actin Filament 1 (*MACF1*) mRNA and *MaTAR17*. Furthermore, Adenomatous Polyposis Coli (*APC*) was identified as a protein interaction partner of *MaTAR17*.

Aberrant *MACF1* expression is linked to initiation of tumour cell proliferation, migration and metastasis in breast, colon and lung cancer. Additionally, *APC*, a well-characterized tumour suppressor gene contributes to development of colon and breast cancer. Investigation of probable interactions between *MaTAR17*, *MACF1* mRNA and APC will therefore provide an insight into the function of *MaTAR17* in tumour progression.

Preliminary gene expression studies using quantitative RT-PCR revealed reduced expression of *MACF1* and *APC* in *MaTAR17* knockout cells. *In vitro* localisation studies using RNA-FISH will validate RNA-RNA interaction between *MaTAR17* and *MACF1* mRNA. Ongoing studies using Chromatin Isolation by RNA Purification (ChIRP)assays will help identify possible binding sites of APC and *MACF1* to *MaTAR17*. ChIRP will also enable identification of other additional binding partners (RNA/DNA/protein) of *MaTAR17*. Investigating the molecular mechanism via which *MaTAR17* acts in the cell will aid us to explore the therapeutic potential of the lncRNA in the future.

Q53: Developing a light grain for translating optogenetics into clinical treatment

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Current optogenetic implants are created for acute animal testing which use micro LEDs at the stimulation site or fibers to transport light to the site from a remote-control unit. Clinical translation of optogenetics requires creation of a long-term implantable light source. There are currently no available devices that could be used for chronic studies in human or other large animals due to inability to demonstrate level of safety and illumination required. We propose a very small (3 x 3 x 10 mm maximum) fully implantable light source with array of LEDs encapsulated in biocompatible borosilicate glass for use in chronic large animal studies, which we have called a light grain. This hermitically sealed implant can be placed directly on the stimulation site for neural activation or inhibition.

The interaction of light with the glass encapsulation and light propagation through tissue needs to be studied to investigate volume of illumination and achievable stimulation depth. Modelling light propagation using open-source Monte Carlo simulation will provide insight into the stimulation depth achievable from our light source. This is validated with experiments on tissue-mimicking optical phantoms and in vitro experiments on mammalian brain slices of varying thickness. To validate the functionality of implant in vivo, rat pupillary response will be measured with light stimulation of the superior cervical ganglion (SCG). Stimulation occurs through opsins which are light-sensitive protein channels that allow passage of ions to depolarize the cells in response to blue light (~ 470 nm wavelength) resulting in neural activation of the SCG. Viral vectors are injected into the SCG via a 'floating pipette' and express the opsins known as Channelrhodopsin-2 (ChR2).1 The efficacy of the light grain is being determined by comparing the electrical versus ontogenetically evoked pupillary responses from the SCG.2

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Q54: Soluble urokinase plasminogen activator receptor is a strong prognostic indicator of mortality in acutely breathless patients

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Soluble urokinase plasminogen activator receptor (suPAR) is a circulating protein believed to be responsible for immune dysfunction leading to plague vulnerability. Its use in heart failure (HF) populations is unclear. We hypothesise that suPAR could prognosticate adverse outcomes for patients presenting with acute breathlessness suspicious of acute HF.

Plasma suPAR were determined in 333 patients presenting to hospital with breathlessness and in 155 separate healthy volunteers. Acute HF was adjudicated by two cardiologists blinded to all results, bar NTproBNP. Plasma samples taken at hospital presentation were interrogated for suPAR (CE marked ELISA, Virogates), hsTnT and NT-proBNP (Roche Cobas), complete with 1yr follow up data. In 155 healthy volunteers (median age 47yr, IQR: 31-57, 50% female), median suPAR was 2.0 ng/mL, IQR; 1.7-2.3, and correlated with age (Spearman's r=0.310, p<0.0001), eGFR (r=-0.285, p<0.0001), and NTproBNP (r=0.407, p<0.0001), but not BMI. In the breathless cohort (median age 73yr, IQR: 62-81, 56% female), 33% had ADHF, 14.7% with pneumonia, and 26.7% with COPD. Plasma suPAR were higher in those who died at 1yr, new HF at 1 yr, in those with ADHF, and separately, pneumonia (p=0.03). suPAR had strong 1yr prognostic ability to predict death (ROC=0.719, n=70, p<0.0001), new HF (ROC=0.707, n=59, p<0.0001) and the composite death/HF at 1yr (ROC=0.733, n=104, p<0.0001). suPAR could improve the mortality prediction at 1yr for ROC hsTnT (0.729 to 0.741) and NTproBNP (0.695 to 0.729), but did not assist the prediction of HF. After adjustment for conventional risk factors, suPAR outperformed hsTnT and NTproBNP (p=0.04) at predicting death in those with ADHF (n= 22).

These novel results show suPAR as a strong biomarker that will provide beneficial prognostic information in patients who are at risk of death, especially when combined with hsTnT and NTproBNP, and could alter future clinical approach in the management of these patients.

Q55: Exploring the interactions of lysozyme with metal-based compounds

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New Zealand suffers from one of the highest rates of cancer incidents per year, bearing a huge strain on the health system. One of best methods of treating cancer is through the use of chemotherapeutics, with platinum based anticancer agents currently used in 50% of cancer treatments.[1] However, due to the interaction with multiple binding partners beyond the major target DNA, patients treated with these platinum based therapies suffer a large number of side effects. To improve the pharmacological properties there has been a fervent search for the next 'blockbuster' metal-based anticancer drug. This search led to the advent of organometallic piano-stool complexes. This scaffold holds a number of favourable properties and through relatively small changes to the ligand structures, significant changes in the biological activity are observed.[2]

To better understand the structure reactivity relationships that these compounds have with proteins we have investigated the interactions between different organometallic compounds and the model protein hen egg white lysozyme (HEWL). In particular we have focussed on the impact of metalation on protein.[3] Using protein crystallography, the interaction of HEWL and [Ru(cymene)(NHC)Cl_2] ligand exchange was observed with the Ru centre coordinating to His15 and Arg14. In addition, the *p*-cymene ligand was cleaved which was concomitant with an oxidation of the ruthenium metal centre from Ru^{II} to Ru^{III}, as confirmed by electron paramagnetic resonance spectroscopy (EPR).[4] Changes in the metal centre alter binding characteristics and the site of interaction. These studies demonstrate that small changes in organometallic compounds can have significant effects on both their ability to bind and the mode of binding to a protein substrate.

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Q56: Detection, differential sorting and transfer of lung cancer-associated long noncoding RNAs (lncRNAs) via tumor-released exosomes

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Lung cancer is the leading cause of cancer-related deaths among men and women worldwide, mainly because of the late stage at diagnosis, fast onset of chemoresistance and metastasis. Current screening tests that have been shown to reduce cancer mortality can also yield high false positive rates, underlining the need for more reliable procedures. Exosomes, nanovesicles released by both normal and cancer cells into the peripheral circulation, are emerging as promising biomarkers as they harbor genetic material reflective of their cell of origin. Moreover, exosomes shuttle functional cargo capable of modifying recipient cells upon internalization. Identification of cargo that can facilitate tumor development and progression may help elucidate lung cancer pathogenesis and ultimately, serve as targets of therapy. This study focuses on the potential of exosomal long noncoding RNAs (lncRNAs) as promoters of lung carcinogenesis and as minimally invasive disease biomarkers. Exosomes from the A549 lung adenocarcinoma cell line were isolated, and characterized by transmission electron microscopy and western blot analysis. Long noncoding RNAs CARLo5, CCAT2, and SCAL1 which are upregulated, and BANCR, MEG3 and TUG1 which are downregulated in lung cancer tissues, were selected as candidate biomarkers. Semi-quantitative PCR analysis show that the selected lung cancer-associated lncRNAs are differentially expressed in exosomes compared to their parental cells. We further hypothesized that cellular mutational status influence such cargo loading, given that an oncogene driver mutation is indicative of tumor behavior and patient prognosis. Quantitative PCR data suggest that KRAS mutations, the most common molecular aberration in non-small cell lung cancer, play a role in either cellular retention or exosome packaging of lung cancer-associated lncRNAs, which may hint at a cell's strategy for maintaining its oncogenic state. We are currently performing co-culture experiments to demonstrate the uptake of A549-derived exosomes by normal lung fibroblasts, a frequent site of lung cancer metastasis.

Q57: Locus-specific concordance of genomic alterations between tissue and plasma circulating tumor DNA (ctDNA) in metastatic melanoma.

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Circulating tumor DNA (ctDNA) may serve as a surrogate to tissue biopsy for non-invasive identification of mutations across multiple genetic loci and for disease monitoring in melanoma. In this study, we compared the mutation profiles of tumor biopsies and plasma ctDNA from metastatic melanoma patients using custom sequencing panels targeting 30 melanoma-associated genes. Somatic mutations were identified in 20 of 24 melanoma biopsies, and 16 of 20 (70%) matched-patient plasmas had detectable ctDNA. In a subgroup of seven patients for whom matching tumor tissue and plasma were sequenced, 80% of the mutations found in tumor tissue were also detected in ctDNA. However, *TERT* promoter mutations were only detected by ddPCR, and promoter mutations were consistently found at lower concentrations than other driver mutations in longitudinal samples. *In vitro* experiments revealed that mutations in promoter regions of *TERT* and *DPH3* are underrepresented in ctDNA. While the results underscore the utility of using ctDNA as an alternative to tissue biopsy for genetic profiling and surveillance of the disease, our study highlights the under-representation of promoter mutations in ctDNA and its potential impact on quantitative liquid biopsy applications.

Q58: Exploring the genetic robustness of ncRNA and protein

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Protein families can be detected across large phylogenetic distances computationally, but many non-coding (nc)RNA families cannot. We hypothesize that RNA families may undetectable due to rapidly changing nucleotide sequence while maintaining function-implying that RNA is more robust to mutation than proteins, allowing them to exhibited greater coding diversity. The relative mutational robustness of RNA and protein was tested using three methods. The nucleotide diversity of a large group of ncRNAs was compared to the nucleotide diversity of matched proteins. Compared across different levels of conservation, there was no difference in nucleotide variation between the biomolecules. We then directly tested the robustness of RNA and proteins pairs with *in vitro* and *in silico* mutagenesis of their respective genes. Individual RNA and proteins reacted to mutagenesis differently, but there was no clear pattern of increased robustness in RNA. Interesting, during the *in silico* mutagenesis, the protein tested was more robust to both point mutations and indels, despite the potential for indels to cause disruptive frameshift mutations. In this first experimental comparison of protein and RNA, we found no quantitative difference in mutational robustness. Additional work is necessary to explore potential qualitative differences as well as other forms of robustness to gain further insight into the evolution and functionality of biomolecules.

Q59: High-throughput Metagenome Sequencing Pipeline using either a Reference-Based or Reference-Free Approach

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The rumen microbiome plays an important role in ruminant food digestion, and is associated with traits such as methane production and feed efficiency. Rumen microbial profiles (RMP) are currently generated using metagenome sequencing, which is expensive and time-consuming for a large number of samples, or small subunit rRNA sequencing (e.g., 16S), which captures only a single gene. We developed a low-cost, high-throughput metagenome sequencing pipeline based on Restriction Enzyme Reduced Representation Sequencing (RE-RRS)[1] using either ApeKI or PstI restriction enzymes, with RMP generated either with or without a reference dataset. The reference-based approach involved comparing sequence reads to genome assemblies from the Hungate 1000 Collection[2] using BLAST[3], then assigning reads at the genus level with the algorithm used in MEGAN[4]. The reference-free approach involved obtaining the proportion of reads that matched a set of common (present in >25% samples) trimmed 65bp reads using TagDigger[5].

We used RE-RRS to sequence DNA extracted from rumen samples[6] from 118 high- or low-methane sheep at two time points, and generated RMP. The reference-based approach assigned taxonomies for 2-26% of sequences, while 4-16% of sequences matched to common tags for ApeKI and 33-61% for PstI. RMP were previously obtained from these samples using 16S rRNA gene sequencing[6]. The first component of a correspondence analysis of the RMP was used to compare performance between the 16S approach and RE-RRS approaches. Repeatabilities were 0.62±0.06 for all RE-RRS approaches except for the reference-free approach using the ApeKI restriction enzyme (0.44±0.07), which was similar to that from 16S (0.45±0.08). The genetic correlation with methane was greatest for the reference-free approach with PstI (0.83±0.31), followed by 16S (0.65±0.47), then the reference-based approach with ApeKI (0.59±0.32). RE-RRS is a desirable approach for high-throughput metagenome profiling. Our approach will be used to sequence thousands of rumen samples over the next year.

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Q60: Targeting Long Non-Coding RNAs to Inhibit Colorectal Tumor Progression and Metastasis

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Long non-coding RNAs (lncRNAs) are ribonucleic acid transcripts longer than 200 nucleotides in length that do not code for a protein. It is currently thought that around 60,000 lncRNA genes exist in the human genome [1] however the function of many of these genes are yet unknown. It has been found that lncRNAs play a role in disease development and cancer, thus illustrating their potential as drug targets.
Here, we investigate the role of one lncRNA in cancer using CRISPR/Cas9 genome editing and CRISPR interference (CRISPRi). CRISPRi is a method of repressing specific genes in cells by using catalytically inactive, "dead" Cas9 fused to a transcriptional repressor that sterically blocks RNA polymerase from transcribing the gene[4].

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Q61: Using novel compounds to delay cyst growth in Polycystic Kidney Disease

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Polycystic kidney disease (PKD) is caused by genetic mutations in the polycystin genes, *PKD1* and *PKD2*, producing a dysfunctional polycystin calcium channel receptor complex. This leads to changes in downstream signaling and subsequent gene expression, which releases cells from controlled proliferation and initiates cyst formation in the kidney tubules[1]. This process starts *in utero* but can take several decades before kidney function is measurably compromised, with limited treatment options available by this stage, namely dialysis and kidney transplant[1]. We are investigating novel compounds, and also drugs[2] used to treat some types of cancer which could be re-purposed for the treatment of PKD. Using *in vitro* models of cyst formation we aimed to identify if these compounds could slow cyst formation, to test their potential for delaying the onset of end stage kidney disease in PKD.

A spheroid cyst assay has been developed from the growth of kidney derived Madin-Darby canine kidney and LLC-PK1 (porcine kidney proximal tubule) cell lines in Matrigel. This assay has been used to measure the effect of various compounds on cyst growth *in vitro*. Preliminary data suggests that treating the cultures with the novel compounds and drugs can slow cyst growth over time with changes in the cells regulatory pathways.

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Q62: Standardising Purification and Defining Receptors for Lipoprotein(a)

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Elevated plasma lipoprotein(a) [Lp(a)] is a known risk factor for cardiovascular disease with no current therapies. Lp(a) shares similar structural and chemical properties with LDL, but it is bound to apolipoprotein(a) [apo(a)] which shares homology with plasminogen. Approximately 20% of the population has elevated Lp(a) levels, which are predominantly determined by an individual's genetics. Lp(a) is hypothesised to contribute to inflammation, tissue repair, and thrombosis, but it's catabolism and physiological role is yet to be clearly defined. Vast gaps in the knowledge paired with a lack of standardisation in Lp(a) research has hindered the speed at which advancements can be made.

Lp(a) uptake from plasma is primarily *via* the liver and kidney but the receptors responsible have remained largely undiscovered. Recently it was shown that the Plg-RKT receptor from the plasminogen receptor family is significant for Lp(a) uptake and metabolism in hepatocytes. It is hypothesised that Lp(a) uptake is also regulated by wider receptors in the plasminogen family (specifically those involved in Ca^{2+} pathways) and that they interact *via* its apo(a) component.

This research aims to further define cellular receptors for Lp(a) uptake. To achieve this, cells with knockouts of plasminogen receptors (S100A10 & Plg-RKT) and receptors with conflicting evidence for their effect on uptake (SCARB1 & LDLR) were treated with purified Lp(a). As large amounts of Lp(a) were needed for such treatments, a protocol for Lp(a) purification that gave good purity and yield was required. Two variations were introduced to a current method of purification to improve yield. Visualisation and quantification of Lp(a) throughout the purification steps indicated that the adjustment to the method had been successful in increasing yield whilst maintaining purity and integrity of the Lp(a) particle. Initial treatment of knockout cells with purified Lp(a) showed that Lp(a) uptake is dependent on various cellular receptors.

Q63: Genetic variants in the *SLC2A9* locus confer risk for hyperuricemia in Māori and Pacific Island individuals.

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Hyperuricemia, elevated levels of serum urate, is a prerequisite for gouty arthritis. The solute carrier family 2 member 9 (*SLC2A9)* gene that encodes a urate transporter tops the list of hyperuricemic genes. It is a key genetic determinant of serum urate levels and explains about 3% of urate variance. Gout is highly prevalent in the New Zealand Māori and other Polynesian populations. As an attempt to understand the reason for this increased prevalence, this study focused on the identification and characterisation of Polynesian-specific genetic variants within the *SLC2A9* locus conferring susceptibility to hyperuricemia, using the rare variant analysis approach.

The *SLC2A9* locus was resequenced in 809 individuals comprising hyperuricemic cases and normouricemic controls**.** Based on self-reported ancestry, the cohort was split into two subsets (Polynesian, n = 440 and European, n = 369). All Polynesians were from New Zealand while Europeans were from New Zealand and the United States. Association analysis was carried out to identify risk variants within the *SLC2A9* locus that confer risk for hyperuricemia. Multiple adjusted logistic regression analysis was carried out using R version 3.4.1.

A total of 3964 variants were identified within the locus, with 100 variants found to be significant in the Polynesian population (OR [95% CI] = 0.10 [0.01;0.88] to 5.43 [1.93;15.33], P_{OR} = 0.00028 to 0.049, $MAF_{controls}$ = 0.014 to 0.535, MAF_{cases} = 0.002 to 0.546). Twenty-five of these variants were found to be Polynesian-specific, amongst which 14 were found to be novel. These variants will be further analysed, replicated and functionally annotated in a larger cohort as a continuation of this study.

This research would provide a greater insight into the genetic causes of gout. More importantly, the identification of penetrant variants could be applied in precision medicine and public health genomics to improve health outcomes for the target population.

Q64: *Streptococcus pyogenes* nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is a human pathogen that causes a wide range of diseases, including pharyngitis, necrotizing fasciitis and toxic shock syndrome. This bacterium produces a large arsenal of virulence factors, including the cell wall-anchored *Streptococcus pyogenes* nuclease A (SpnA), which facilitates immune evasion by degrading the DNA backbone of neutrophil extracellular traps. SpnA consists of a C-terminal endo/exonuclease domain and a N-terminal domain of unknown function. Site-directed mutagenesis of SpnA has been carried out to further define the mechanism of the nuclease. The ability to degrade DNA by these recombinant SpnA mutants were either abolished or reduced when predicted metal-binding and catalytic site residues were mutated. To investigate the role of SpnA in virulence *in vivo*, *Galleria mellonella* (wax moth) larvae were used as an infection model. A GAS *spnA* deletion mutant showed reduced virulence in this model, with the *spnA wt* complementation completely restoring virulence. Interestingly, complementation with the spnA catalytic site mutant SpnA H716A only partially restored virulence. Our results outline the critical role of several predicted residues in enzymatic activity and demonstrate that nuclease activity is not exclusively responsible for SpnA-mediated GAS virulence in a *Galleria mellonella* infection model.