QMB Abstracts: Biomolecular Interactions

BI1: Chew on this: trogocytosis by *Entamoeba histolytica* enables human cell killing and immune evasion

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Entamoeba histolytica is a pathogenic amoeba and the causative agent of the diarrheal disease amoebiasis in humans. The species name (*histo-:* tissue; *lytic-:* dissolving) derives from the capacity to destroy host tissues, which is likely caused by killing of human cells and immune evasion. *E. histolytica* trophozoites ("amoebae") can invade the large intestine, causing ulceration and can enter the bloodstream. Although it is rare, amoebae that have entered the bloodstream can disseminate to other tissues (e.g., the liver, lungs, or brain), causing fatal abscesses. Little is known of the mechanisms that allow *E. histolytica* to evade immune detection and to disseminate upon entering the bloodstream. Notably, virulent strains are complement-resistant, but this is poorly understood. Amoebae also possess contact-dependent cell-killing activity that is likely to drive tissue damage, but the mechanism was previously unclear. We established a new paradigm by discovering that amoebae kill human cells by biting off and ingesting fragments, which we named "amoebic trogocytosis" (*trogo-:* nibble). We subsequently found that amoebae display human cell membrane proteins after performing trogocytosis and become protected from lysis by human complement. We are now delineating this unexpected and novel strategy for immune evasion. We are applying imaging flow cytometry, host and amoeba mutants, and a variety of host cell types to dissect the contribution of trogocytosis to immune avoidance *in vitro,* and we are using the mouse model of amoebiasis to extend these findings to pathogenesis *in vivo.* Beyond *E. histolytica,* trogocytosis is a burgeoning theme that has far-reaching applications to eukaryotic biology. Several microbial eukaryotes use trogocytosis to kill other cells. In multicellular eukaryotes, trogocytosis is used for cell-killing, cell-cell communication and cell-cell remodeling. Trogocytosis plays roles in the immune system, in the central nervous system, and during development. Therefore, an improved understanding of the mechanism and biology of *E. histolytica* trogocytosis will apply both directly to the pathogenesis of amoebiasis and broadly to eukaryotic trogocytosis in general.

BI2: MicroCT examination of the existence of an osmotic barrier between fibres and vessels in sugar maple (*Acer saccharum*)

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Sugar maples (Acer saccharum) develop elevated stem pressures when subjected to daily cycles of freezing and thawing. These elevated stem pressures develop due to the compression of gas found within fibre cells composing most of the stem. These fibre embolisms should dissolve over time due to the effects of surface tension. That they persist is hypothesised to be due to an osmotic barrier between the fibres and the sap conducting vessels. This barrier prevents sucrose in the sap from diffusing into the fibres, creating a sufficient osmotic pressure difference to keep embolisms stable. In this work we examine the existence of this osmotic barrier using high resolution synchrotron based MicroCT. By taking sections of maple stem we were able to directly image fibre embolisms in freshly cut stem segments. We then perfused segments with either water, or 2% sucrose solution for 2 hours, imaging after perfusion. In this way we observed the effects of either preserving, or removing the osmotic pressure difference on fibre embolisms. We observed a large reduction in fibre embolisms following perfusion with water, while only a small change following perfusion with sucrose solution. Our results thus support that an osmotic barrier does exist between the fibres and vessels.

BI3: Regulating biomaterial uptake across the endothelium: The interplay between biomaterial properties and interactions with the glycocalyx

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Despite significant investment in biomaterials development for drug delivery, few have progressed to clinical use. Notwithstanding elegant biomaterials design, overcoming biological barriers *in vivo* remains a challenge. This is in part attributed to the disparity in the evaluation of biomaterials between *in vitro* and *in vivo* systems.

Fundamental to all mammalian cells are the cell surface anchored glycan macromolecules which form an anionic hydrogel-like matrix, called the glycocalyx. Yet, few have considered the glycocalyx in the design and preclinical testing of biomaterials. With most biomaterial drug delivery systems administered via the bloodstream, the blood vessel wall is a key tissue barrier to traverse on the path to target tissues. Thus, the endothelial cell glycocalyx is an important consideration in the design of biomaterials able to interact with and traverse the blood vessel wall.

We established an *in vitro* blood vessel model in which the glycocalyx is expressed by primary human endothelial cells. This model was used to assess biomaterial-cell interactions including polysaccharides, poly(amino acids), and gold nanoparticles. Confocal microscopy and flow cytometry analyses of the blood vessel model reveal a charge-based extent of association of biomaterials with endothelial cells and their glycocalyx. Specifically, cationic and neutrally charged biomaterials possess higher levels of association with cells expressing a glycocalyx than anionic biomaterials. We developed models of the glycocalyx by tethering glycans to gold substrates via thiol chemistry enabling analysis of biomaterial-glycan interactions using the quartz crystal microbalance. This approach revealed that cationic biomaterials bind anionic glycocalyx components via a condensation mechanism with the affinity of these interactions positively correlated with glycan sulphation. This finding suggests a mechanism of biomaterial transport through the glycocalyx enabling intracellular uptake.

Together this study reveals the interplay between biomaterial properties and interactions with endothelial cells and their glycocalyx toward improved delivery system design and application.

BI4: Light-activated adipose tissue grafts for soft tissue reconstruction

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Autologous fat grafting has potential as a regenerative strategy to repair large contour defects, as needed in breast reconstruction after mastectomy or for scar/burn repair. Clinically, there is a limit on the volume of lipoaspirate which can be utilised to repair a soft-tissue defect. Surgical complications are the result of poor graft vascularisation and are hindered further by the poor structural fidelity of lipoaspirate as a filling material. This study aims to engineer lipoaspirate-derived adipose tissue grafts with biologically and clinically admissible structural and functional properties.

Patient-derived lipoaspirate was crosslinked by establishing covalent dityrosine bonds between extracellular matrix (ECM) proteins within the material, using a visible light photoinitiating system. The degree of crosslinking was tuned and dityrosine bond formation measured using mass spectrometry. To predict patient response, SWATH-MS was used to identify differences in patient ECM and crosslinked grafts were implanted *in vivo* using a subcutaneous mouse model. Graft resorption and functional \vessel infiltration were quantified using micro-CT, where tissue-remodelling was assessed via histology.

The photoinitiating strategy is tailorable, allowing progressive increases in the structural fidelity of lipoaspirate, as measured by a stepwise reduction in fiber diameter, increased graft porosity and reduced variation in graft resorption. There is an increase in dityrosine bond formation with increasing photoinitiator concentration, and tissue homeostasis is achieved ex vivo, with vascular cell infiltration and vessel formation in vivo. The crosslinking approach described here in tunable and functional across different patient samples.

Improving the structural properties of lipoaspirate through minimal manipulation has clinical utility for the delivery of grafts with higher shape fidelity and therefore increased graft survival when implanted. This study provides a conclusive example of the opportunities of simple photocrosslinking technologies for delivering structural support to heterogenous patient-derived materials and to ultimately achieve more desirable patient outcome.

BI5: Decoding the Unknown: Unveiling the Role of an Effector Protein During Myrtle Rust Infection by *Austropuccinia psidii*

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Myrtle rust is caused by the invasive fungus *Austropuccinia psidii*, which is incredibly infectious and physically devastating to plants in the Myrtaceae family. The disease was first detected in New Zealand in 2017 and continues to spread rapidly across the country. *A. psidii* has already caused major declines in Myrtaceae populations worldwide. It threatens many Aotearoa-New Zealand natives, including taonga species such as pōhutukawa (*Metrosideros excelsa*), mānuka (*Leptospermum scoparium*) and rātā (*Metrosideros umbellata*). Localised extinctions of Myrtaceous plants are already occurring in Aotearoa.

Transcriptomic experiments have identified several proteins that are expressed during the first 24–48 hours of infection by *A. psidii* on mānuka. This expression pattern is a signature of their important role in the successful infection of plant cells. In other plant pathogens these 'effector proteins' are known to manipulate the host plant's cellular processes to boost pathogen fitness.

We aim to elucidate the role of the *A. psidii* effector protein, AP1260, during infection through bioinformatic and biophysical analysis. *A. psidii*'s dikaryotic nature has led to the presence of two haplotypes of AP1260, with both being present during infection. Biophysical studies have sought to determine the physical characteristics of both haplotypes in solution. These include analytical ultracentrifugation, circular dichroism, small-angle X-ray scattering, and nuclear magnetic resonance.

Functional analysis of AP1260 uses agrobacterium-mediated transformation of *Nicotiana benthamiana* and yeast-two-hybrid to determine its localisation and potential *in planta* interaction partners. This study represents the first investigation of an *A. psidii* effector protein. Characterisation of AP1260 improves the knowledge of the mechanisms of *A. psidii* infection and may lead to the development of a novel and effective method to treat and control myrtle rust.

BI6: Cancer-associated mutations of tumour suppressor p16^{INK4a} increase the propensity for amyloid fibril formation

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p16^{INK4a} (p16 for short) is a tumour suppressor protein that regulates cell cycle progression by binding to and inhibiting the cyclin-dependent kinases 4 and 6 (CDK4/6). p16 is a 16 kDa, α-helical protein with a single solvent-accessible cysteine residue located on the surface facing away from the CDK4/6 binding site. Our group discovered that this single cysteine in p16 can, under relatively mild oxidizing conditions, form disulfide-dependent homodimers. This dimerization leads to subsequent structural rearrangement into amyloid fibrils. In this amyloid fibril state p16 is unable to perform its normal function as a CDK4/6 inhibitor[1].

To further understand the mechanism of p16 amyloid fibril formation and better understand how p16 is inactivated in cancer, we have screened cancer-associated single-residue mutations. These variants have been characterised using thioflavin-T aggregation assays, SDS-PAGE analysis of dimerization, and electron microscopy. These mutations result in a significant increase in the rate of dimer and/or fibril formation. Our analysis has determined some of the key influences in p16 dimer and amyloid fibril formation.[2] We are also exploring the impact of four stabilising mutations on dimer and amyloid fibril formation. This will aid investigation of the short-lived dimer species and overall structure of p16 amyloid fibrils. Overall, this work will lead us to a greater understanding of the mechanism by which these unique aggregate species form and their role in pathogenic processes.

1. Göbl, C.; Morris, V. K.; van Dam, L.; Visscher, M.; Polderman, P. E.; Hartlmüller, C.; de Ruiter, H.; Hora, M.; Liesinger, L.; Birner-Gruenberger, R.; Vos, H. R.; Reif, B.; Madl, T.; Dansen, T. B., *Cysteine oxidation triggers amyloid fibril formation of the tumor suppressor p16(INK4A)*. Redox Biol 2020, 28, 101316.
2. Heath, S.G., Gray, S.G., Hamzah, E.H., O'Connor, K.M., Bozonet, S.M., Botha, A.D., de Cordovez, P., Magon, N.J., Naughton, J.D., Goldsmith, D.L.W., Schwartfeger, A.J., Sunde, M., Buell, A.K., Morris, V.K.,*, Göbl, C.* *Amyloid formation and depolymerization of the tumor suppressor protein p16INK4a is strictly controlled by an oxidative thiol-based mechanism.* Nature Communications 2024, In press

B17: Stumbling upon the ancestral phosphofructokinase

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Phosphofructokinase (PFK) is a key enzyme in the central metabolic pathway of glycolysis. PFKs are categorized according to whether they use ATP, ADP, or pyrophosphate as their phosphate donor. Typically, eukaryotes use allosterically regulated, ATP-dependent PFKs while archaea have non-allosteric, ADP-dependent PFKs. Therefore, it was fascinating when an ATP-dependent PFK was annotated in the genome of the archaeon *Candidatus* Prometheoarchaeum syntrophicum. *Ca*. P. syntrophicum is a member of the newly discovered Asgard superphylum and currently the closest cultured prokaryotic relative of eukaryotes. Thus, we hypothesized that *Ca*. P. syntrophicum PFK (PsyPFK) might shed light on the evolution of phosphofructokinases and phosphate donor specificity.

We determined structures of apo and ligand-bound PsyPFK using X-ray crystallography. As expected, its closest structural homologues were ATP-dependent PFKs. Therefore it was a great surprise when activity assays revealed that PsyPFK had a strict preference for pyrophosphate as its phosphate donor, not ATP or ADP. The surprises kept coming when further assays revealed that PsyPFK is not allosterically regulated—but it is a multi-functional kinase/phosphotransferase. This single enzyme appears to perform physiological roles in glycolysis, gluconeogenesis and the pentose phosphate pathway. Indeed, the catalytic efficiency (k_{cat}/K_M) of PsyPFK is highest for the reaction in gluconeogenesis, which requires an entirely different enzyme in most organisms. Careful analysis of the experimentally determined PsyPFK structures revealed the molecular basis of these unusual biomolecular properties. Ultimately, it appears we have stumbled upon a molecular “living fossil”, with some—and perhaps all—of the properties of the ancestral PFK.

BI8: Engineering thermostable bioplastic degrading enzymes

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Plastics such as polyesters can take centuries to biodegrade in landfills or water bodies, but some organisms in nature are slowly evolving to degrade plastic waste in their environments. Bioplastics can be more susceptible to biodegradation, with the benefit of lower net carbon emissions as they are produced from renewable plant materials. Plastic degrading enzymes produced by such organisms can be repurposed for potential industrial applications, including chemical recycling in a circular economy, environmental remediation, and designing easily biodegradable plastics. However, natural enzymes are often not optimal for industrial use due to their limited activity efficiency and thermal tolerance.

By data mining genomic information from microorganisms living in high temperature environments, we have identified naturally occurring highly thermostable enzymes that degrade the bioplastics polybutylene succinate co-terephthalate (PBAT) and polybutylene succinate co-butylene adipate (PBSA). We then used ancestral protein resurrection to further improve protein thermostability, as well as catalytic activity with model substrates. These enzymes can be further engineered to optimise plastic degradation activity using directed protein evolution aided by emerging Machine Learning and Artificial Intelligence technologies.

BI9: Enzymatic Ligation of Xeno Nucleic Acids With Non-Canonical Bases

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Beyond the five natural nucleotides which comprise DNA and RNA, a range of Xeno Nucleic Acids (XNAs) with alternate chemistries have been chemically synthesised. This includes non-natural bases which have orthogonal hydrogen bonding patterns to canonical A/T(U) and G/C pairs. The increased information content of XNAs containing additional nucleobases has applications such as expanded genetic codes for designer enzymes and therapeutics; however, the present enzymatic toolkit for XNA cloning and manipulation as well as synthesis of long XNA polymers is limited.

We have investigated the capacity of diverse DNA ligases to join segments of XNA containing non-natural bases. These include a range of structurally-divergent DNA binding domains, cofactor usage and temperature optima. Despite having no direct contacts with the nucleobase portion of DNA during the ligation reaction, different ligases show distinct preferences for the nature and position of non-canonical bases in the nucleic acid duplex. We have determined the structure of one of these DNA ligases in complex with XNA which, combined with a range of biophysical characterisations provides insight into the reasons for sub-optimal ligation of non-natural base XNA. Such structural studies will guide the engineering of XNA ligases with superior properties, as well as providing insight into broader enzyme interactions and their dynamics with XNAs.

BI10: A Glimpse into New Zealand’s RNA Technology Development Platform

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RNA technology is nothing short of revolutionary. The groundbreaking discoveries underlying RNA technology have not only led to innovative new vaccines but have also revealed the vast potential of these products to address cancer, metabolic and genetic disorders, autoimmune diseases, as well as biosecurity threats for both animals and plants. The National RNA Platform, currently in its first year, aims to leverage existing expertise and infrastructure while investing in new resources and talent to enhance New Zealand's RNA manufacturing capabilities and broaden our reach to researchers nationwide. In this talk, I will update you on the progress, structure, and current focus points of the National RNA Platform, including some of the projects we have started working on.

BI11: High-throughput Optimization of Protein Expression and UPR-mediated Cell Stress Monitoring in *Pichia pastoris* via Biosensors

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Pichia pastoris is a valuable host organism for recombinant protein production. This study investigates the application of synthetic unfolded protein response (UPR) biosensors in *P. pastoris* to enhance early-stage evaluation of expression and strain engineering strategies for improved protein secretion. While a previously developed synthetic minimal UPR biosensor effective in *Saccharomyces cerevisiae* showed compromised performance in *P. pastoris*, a UPR sensor utilising a minimal *P. pastoris* KAR2 promoter exhibited superior performance. Notably, *P. pastoris* displayed higher resistance to tunicamycin-induced stress compared to *S. cerevisiae*, highlighting unexplored strain-specific differences with significant industrial relevance.

In addition to monitoring cell stress triggered by the UPR, this study adapted the yeast G-protein coupled receptor (GPCR) biosensor system, initially developed for *S. cerevisiae*, to quantify protein secretion in *P. pastoris*. This system measures the concentration of a pheromone peptide fused to the protein of interest, enabling high-throughput screening of genetic constructs. Using Combinatorial Golden Gate cloning, we assembled 35 combinations of signal peptides and peptide tags. The biosensor effectively established relationships between combinations of peptide tags and signal peptides for protein secretion.

Our findings demonstrate that synthetic UPR biosensors are powerful tools for early-stage screening of expression strategies in *P. pastoris*, leading to more efficient production processes. The application of high-throughput GPCR biosensors facilitates the optimization of signal peptides and expression cassettes, potentially transforming industrial protein production by improving yield and reducing screening times. The combination of both biosensors, supported by the co-expression of a library of chaperones, will help identify optimal conditions for achieving the highest expression levels with minimal cell stress.

BI12: Understanding and incorporating Māori world views and expectations in gene technologies: implications for synthetic biology

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Over the last three decades there has been a significant corpus of knowledge at the interface of gene technologies and te ao Māori (the Māori world) has developed. This has consisted of political activism, published opinions, academic research on Māori perspectives and the development of multiple tikanga-informed frameworks for conducting gene technology-based research. However, this corpus is often ignored by gene technology researchers, who instead continue practices of institutional and systemic racism including false allyship and expecting Māori researchers to prioritise professional requirements over cultural norms derived from tikanga. This presentation will exemplify this by describing a recently published meta-analysis of Māori perspectives of gene editing and contrasting this with early steps in the development of new legislation covering gene technologies. Finally, I will describe some recent developments whereby Māori have developed their own entities that have implications for synthetic biology research and application in Aotearoa New Zealand.

BI13: LUCY in the sky with diamonds: developing platforms for protein crystallisation in microgravity

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Microgravity protein crystallisation can be a valuable tool for generating high-resolution protein structures, with a recent meta-analysis indicating that over 90% of all crystals grown in the microgravity environment of Low Earth Orbit (LEO) exhibit superior quality over control experiments conducted on Earth. Despite the high-quality crystals and resulting structural data microgravity experimentation can provide, executing microgravity protein crystallisation is currently orders of magnitude more challenging than analogous experiments on Earth, with costs and extended experimentation timelines cited as leading reasons preventing most potential researchers from utilising microgravity. To address these challenges, our team is developing fully automated protein crystallisation facilities which will enable high-throughput screening and real-time monitoring of protein crystallisation, comparable to terrestrially-based services. Our partnership with world-leading Axiom Space will ensure regular, frequent, and cost-effective missions to both the International Space Station (ISS) in the near term and the first commercial space station from 2026. This talk will outline the success of New Zealand's first mission to the ISS earlier this year testing our prototype facility and a range of protein experiments from researchers around Aotearoa and will also provide an overview of our team's upcoming plans for additional researchers to become involved in microgravity research.

BI14: A conserved second-sphere residue in lytic polysaccharide monooxygenases controls copper-site reactivity

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Lytic polysaccharide monooxygenases (LPMOs) are monocopper enzymes able to cleave glycosidic bonds in a range of recalcitrant polysaccharides including cellulose and chitin. This activity makes these enzymes attractive candidates for engineering, utilising their powerful monocopper active sites to evolve new activities. Despite rapid expansion in LPMO research, the mechanism of these enzymes remains largely unknown, with few mutagenesis studies performed on residues in the catalytic centre. A previous study identified a residue in *Sm*AA10A (an AA10 family LPMO from *Serratia marcescens*), as playing a pivotal role in the peroxygenase reaction through positioning the oxygen co-substrate and the hydroxyl radical intermediate. This residue is universally conserved in all LPMOs, as either a negatively charged glutamate (typical for AA10 LPMOs) or a neutral glutamine (typical for AA9 LPMOs).

We investigated the role of this residue in *Nc*AA9C, an AA9 family LPMO from *Neurospora crassa*. Using site directed mutagenesis, the conserved glutamine residue in *Nc*AA9C was mutated to three different amino acids (glutamate, aspartate or asparagine). Our results show that the nature and distance of the headgroup to the copper fine tune LPMO functionality and copper reactivity[1]. The presence of a glutamate or aspartate close to the copper lowered the redox potential and decreased the ratio between reduction and reoxidation rates by up to 500-fold. The glutamate mutant also exhibited a lower redox potential and higher oxidase activity. All mutants showed increased enzyme inactivation, likely due to changes in confinement of radical intermediates and displayed changes in a protective hole-hopping pathway. Taken together, these results provide insights into how second sphere residues fine-tune the functionality of the LPMO copper site.

1. Hall, K. R., Joseph, C., Ayuso-Fernández, I. *et al*., *A conserved second sphere residue tunes copper site reactivity in lytic polysaccharide monooxygenases.* J. Am. Chem. Soc. 145: 18888-18903.

BI15: A thirty-year journey designing and synthesising nucleoside analogues as drugs

Evans, G.B.

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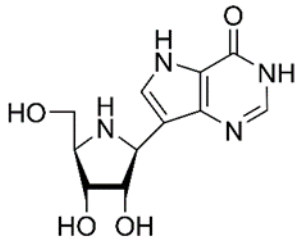
Nucleoside analogues have a long history as drugs used to mainly treat cancers and viruses. Beginning in 1994 and up until the present day, I have been part of a team focussed on the design of nucleoside analogues as potent enzyme inhibitors and drugs. Most notably, Mundesine has progressed to market for the treatment of peripheral T-cell lymphoma while Ulodesine and Galidesivir have progressed to Phase II clinical trials, the latter for the treatment of SARS-CoV-2. As part of my seminar I'll talk about some of the chemistry used to make these compounds as well as some of our other less successful drug candidates. I'll also discuss aspects of the journey to commercialise these compounds and Ferrier's efforts to build a drug discovery ecosystem here in New Zealand.



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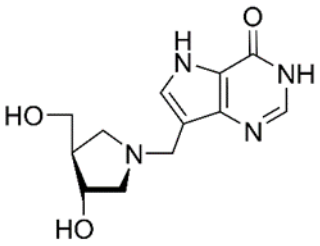

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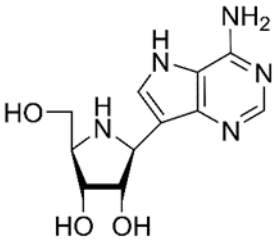

Galidesivir

BI16: Structural and molecular basis of choline uptake into the brain by FLVCR2

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Choline is an essential nutrient that the human body needs in vast quantities for cell membrane synthesis, epigenetic modification, and neurotransmission. The brain has a particularly high demand for choline, but how it enters the brain has eluded the field for over fifty years. The MFS transporter FLVCR1 was recently determined to be a choline transporter, and while this protein is not highly expressed at the blood-brain barrier (BBB), its relative FLVCR2 is. Previous studies have shown that mutations in human *Flvcr2* cause cerebral vascular abnormalities, hydrocephalus, and embryonic lethality, but the physiological role of FLVCR2 is unknown. Here, we demonstrate both *in vivo* and *in vitro* that FLVCR2 is a BBB choline transporter and is responsible for the majority of choline uptake into the brain. We also determine the structures of choline-bound FLVCR2 in the inward- and outward-facing states using cryo-electron microscopy to 2.49 and 2.77 Å resolution, respectively. These results reveal how the brain obtains choline and provide molecular-level insights into how FLVCR2 binds choline in an aromatic cage and mediates its uptake. Our work could provide a novel framework for the targeted delivery of neurotherapeutics into the brain.

BI17: From Bench to Body: Translating a Medical Device Innovation into an Implantable Prototype

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Everything works in theory, but translating a medical device from research to an implantable prototype presents numerous challenges. This presentation details the journey of converting benchtop research into an implantable spinal fusion sensor, intended for a pilot sheep study. The sensor array, consisting of 64 individual sensors, was developed using MEMS (micro-electro-mechanical systems) technology. Once fabricated, the sensors were manually extracted from a silicon wafer and mounting into a miniature titanium enclosure integrated with a spinal rod assembly. This process utilised cyanoacrylate gel adhesive and an alignment recess for precise positioning.
To enable wireless functionality, essential electronics such as the coil antenna, capacitance-to-digital converter, and components for wireless power and communication were integrated into the enclosure and lid. The assembly process included the use of foam tape, soldered joints, and connectors. An acrylic lid, chosen for its electromagnetic transparency, was secured using medical-grade epoxy, ensuring a waterproof seal for the implant.

Each stage of development was accompanied by rigorous testing to ensure robustness, reliability, and functionality. However, despite meticulous planning, the project faced significant challenges, including issues with wireless communication, and achieving a hermetic seal for the acrylic lid. These obstacles led to delays of over a year, underscoring the unpredictable nature of transitioning from research to practical application.

Key lessons from this endeavour highlight the importance of a comprehensive framework for risk analysis, detailed testing protocols, realistic timeframes, and budget allocations with contingencies. Effective communication with external, international suppliers proved critical, as unresolved issues quickly escalated. This case study emphasises that while theoretical knowledge is crucial, practical implementation will require adaptability, thorough planning, and collaborative problem-solving.

The findings underscore the significance of structured methodologies and proactive communication in successfully translating a medical device technology from the lab to a functional, implantable prototype.

BI18: Human gut organ-on-a-chip for studying host-pathogen interactions

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Complex *in-vitro* models, such as organ-on-a-chip, are new and innovative technologies leveraging the interface between engineering and biology. These 3-dimensional (3D) cell culture models are rapidly expanding our knowledge of human physiology and are emerging as the tools of choice to study cellular responses to microorganisms and chemicals. The benefits of these new models include the ability to control fluid and gas movement, apply tensile stretch forces, observe increased cellular complexity, mix different types of cells and, in some systems, linking multiple organs together. For many bacterial pathogens of humans, the intestine is the site of infection and pathogenesis. ESR is using a 3D intestinal organ-on-a-chip to study enteric pathogens, particularly genotype-phenotype associations, cellular interactions, and immune responses. We have established a model using the Emulate 3D organ-chip with microfluidics and peristaltic-like stretch with cultured Caco-2 and HUVEC cell lines. With this model we observe that epithelial cells differentiate with apical and basolateral orientation, complete barrier function, and villus-like structures within 7 days of culture. We used this model to investigate the pathogenesis of *Campylobacter jejuni,* the leading cause of food- and water-borne illness in Aotearoa New Zealand. The organ-chips were infected with *C. jejuni* clinical strains 81-176 (ST-42) or NCTC11168 (ST-15) at <1 MOI (multiplicity of infection) for up to 5 days corresponding with the human incubation period. Results suggest both *C. jejuni* strains can invade Caco-2 cells in the organ-chip without killing the cells or disrupting the barrier integrity. These are the first studies to report the infection of a Caco-2/HUVEC intestinal-chip with clinical *C. jejuni* strains in the Emulate system and begin to uncover important insights into the biomolecular interactions between host and pathogen.

BI19: High-throughput compound screening at the Biomolecular Interaction Centre

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In this presentation, I will showcase the advanced capabilities of the Biomolecular Interaction Centre (BIC) for screening small molecule binders. An example project will be discussed, where we successfully identified a compound that disrupts a protein complex. Various methodologies, including dynamic scanning fluorimetry, will be examined in detail. Additionally, I will highlight the newly established Southern BioNMR Centre and its capacity for high-throughput ligand screening. I will explore other nuclear magnetic resonance (NMR) applications, emphasizing our ability to perform specialized and automated measurements on proteins, metabolites, and natural products.

BI20: Biofabrication of Advanced Hydrogel and Tissue Spheroid Fusion Models for Regenerative Medicine and Disease Modelling

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Biofabrication technologies, including 3D bioprinting, bioassembly and volumetric bioprinting, offer the potential to engineer constructs that replicate the complex 3D organization of native tissues via automated hierarchical placement of cell-laden bioinks, tissue modules, and/or bioactive factors. Photo-polymerisation combining light and photo-initiators to crosslink cell-laden hydrogels and bioinks has been widely employed in 3D bioprinting of. Despite rapid advances in biofabrication technologies, development of individual bioinks for each biofabrication technique and specific tissue niche is required, limiting rapid innovation and clinical translation. Our overall goals are to address major bottlenecks that still remain in designing materials that are both cell-instructive and compatible with high resolution additive manufacturing and 3D biofabrication techniques. Bottom-up spheroid biofabrication strategies offer flexibility to precisely arrange cellular modules to mimic the zonal structure of tissues, manipulate cell signalling through spatial presentation of different biomolecules, as well as uncouple the reliance on narrow biofabrication windows.

This talk discusses alternative strategies to engineer highly tuneable hydrogel platforms that 1) promote a specific cell-instructive niche using visible light-activated crosslinking in gelatin-based micro-gels, bioresins and high-throughput modular spheroids, and 2) are printable across multiple biofabrication technologies, including extrusion-bioprinting, lithography-bioprinting & microfluidic-based bioprinting.

We further discuss experience in developing hybrid tissue constructs and convergence with 3D spheroid bioassembly platforms for probing multicellular spheroid fusion, extracellular matrix (ECM) formation and stem-cell niche, offering new paradigms for high-throughput screening and disease modelling in healthy and osteoarthritic spheroid fusion models. This work demonstrates advances in development of cell instructive universal bioink platforms for 3D bioprinting and regenerative medicine, and advances scalable, modular approaches for bioassembly of functional tissues towards clinical translation.

1. X Cui, et al (2022). 3D bioassembly of cell-instructive chondrogenic & osteogenic hydrogel microspheres containing allogeneic stem cells for hybrid biofabrication of osteochondral constructs. Biofabrication. 14, 034101.
2. G Lindberg, et al (2021). Probing Multicellular Tissue Fusion of Cocultured Spheroids - A 3D-Bioassembly Model. Advanced Science. 8(22), 2103320.

BI21: Biomimetic leaf surfaces – A platform technology to study bio-interactions

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Leaf surfaces feature diverse microenvironments for microbial colonizers, such as bacteria and fungi, with the spatial and temporal variability of leaves modulating plant-microbe and microbe-microbe interactions. Artificial surfaces that mimic leaf complexity greatly benefit the study of microorganisms residing on plant leaf surfaces[1]. Ranging from very simple nutrient agars to very complex silicon-based casts that aim to replicate leaf surface topography[1], surrogate surfaces aid in deconstructing leaf surfaces into their individual aspects.

In this paper I will introduce our efforts to develop biomimetic leaf surfaces as a platform technology to study bio-interactions, in particular related to bacterial colonization and invasion by pathogenic rusts. To date, this has involved *Arabidopsis thaliana*[2-5], as well as wheat, poplar, eucalyptus and mānuka[6], incorporating properties such as leaf topography or hydrophobicity, with the goal to enable colonizer survival in the absence of a living plant host. Characterizing agarose, polydimethylsiloxane (PDMS) and gelatin[7], we determined PDMS to be one of the most suitable materials for leaf replicas, in particular when diffusion of water and nutrients to the surface was optimized by addition of fillers[3]. Increasing permeability demonstrated the possibility of delivering fructose to the surface, thus allowing division and distribution of bacteria to be affected[5]. Such leaf replicas have since also enabled us to culture biotrophic rusts, normally considered un-culturable on artificial substrates, in-vivo[6], as well as helped to demonstrate that RNAi can be used to inhibit infections by these rusts[8].

1. Doan, H.K., Leveau, J.H.J. (2015). Phytopathology 105:1036-1042.
2. Soffe, R., et al. (2019). Sci. Rep. 9:14420.
3. Bernach, M., et al. (2019). Jpn. J. Appl. Phys. 58:SDDK01.
4. Soffe, R., et al. (2022). Small 2002035.
5. Bernach, M., PhD Thesis. 2024, University of Canterbury: Christchurch.
6. Sale, S., PhD Thesis. 2024, University of Canterbury: Christchurch.
7. Soffe, R., et al. (2019). PLoS ONE 14:e021810.
8. Degnan, R.M., et al. (2022), Mol. Plant Pathol. 24:191-207.

BI22: Honey, I shrunk the lead discovery process: using new technologies for the rapid discovery of polypeptide-based inhibitors

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Small molecules and antibodies are the two prevalent classes of therapeutic molecules on the market today. However, new technologies are opening the door to other modalities, including cyclic peptides isolated from massive library screens as well as proteins hallucinated by artificial intelligence. In this talk, I will describe recent efforts in my lab to discover lead molecules that target proteins involved in the regulation of gene transcription – a class of proteins that are emerging as desirable therapeutic targets for various disorders.

Our work with cyclic peptides demonstrates that this class of molecules can obtain unparalleled specificity through surprising mechanisms of action, and our fledgling efforts with AI suggest that this strategy can yield inhibitors that successfully target protein domains that are unlikely to be amenable to traditional small-molecule strategies. Most pleasingly, both of these approaches are readily accessible to labs that don’t have an army of medicinal chemists at their disposal.

BI23: An integrated bioanalytical and biophysical approach to study carbon metabolism in *Mycobacterium tuberculosis*

Huang, E.Y.-W.[1], Kwai, B.X.C.[1,2], Wu, H.[1], Zhou, J.[1], Bhusal, R.P.[2], Anh Nguyen, L.T.[1], Yu, C.X.[1], Vasku, G.[1], Jiao, W.[3], Sperry, J.[2], Bashiri, G.[4], Maher, M.J.[1], Coussens, A.K.[5], Leung, I.K.H.[1,2]

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Isocitrate lyase (ICL) and isocitrate dehydrogenase (ICD) are metabolic enzymes that are crucial for the virulence and survival of *Mycobacterium tuberculosis* during infection. These enzymes regulate the flow of carbon through the tricarboxylic acid (TCA) cycle and the glyoxylate shunt, enabling the pathogen to balance energy production with the growth of biomass when nutrients are limited. In this talk, I will discuss the strategy that our group uses to study the functions and mechanisms of these enzymes. Specifically, by using an integrated bioanalytical and biophysical approach, we found that the TCA cycle and the glyoxylate shunt signal and interact with each other, as well as with their upstream pathways, through metabolites to maintain homeostasis. Our work provides new insights into the currently unknown mechanisms that enable mycobacteria to survive in environments deprived of carbon-based nutrients and should offer opportunities for the development of new therapeutics to treat tuberculosis.

1. Huang, E.Y.-W.; Kwai, B.X.C.; Bhusal, R.P.; Bashiri, G.; Leung, I.K.H. *Mycobacterium tuberculosis* Rv1916 is an Acetyl-CoA-Binding Protein. *ChemBioChem* **2023**, *24*, e202300162. DOI: 10.1002/cbic.202300162
2. Kwai, B.X.C.; Collins, A.J.; Middleditch, M.J.; Sperry, J.; Bashiri, G.; Leung, I.K.H. Itaconate is a Covalent Inhibitor of the *Mycobacterium tuberculosis* Isocitrate Lyase. *RSC Med. Chem.* **2021**, *12*, 57–61. DOI: 10.1039/D0MD00301H
3. Bhusal, R.P.; Jiao, W.; Kwai, B.X.C.; Reynisson, J.; Collins, A.J.; Sperry, J.; Bashiri, G.; Leung, I.K.H. Acetyl-CoA-Mediated Activation of *Mycobacterium tuberculosis* Isocitrate Lyase 2. *Nat. Commun.* **2019**, *10*, 4639. DOI: 10.1038/s41467-019-12614-7
4. Bhusal, R.P.; Patel, K.; Kwai, B.X.C.; Swartjes, A.; Bashiri, G.; Reynisson, J.; Sperry, J.; Leung, I.K.H. Development of NMR and Thermal Shift Assays for the Evaluation of *Mycobacterium tuberculosis* Isocitrate Lyase Inhibitors. *Med. Chem. Commun.* **2017**, *8*, 2155–2163. DOI: 10.1039/C7MD00456G

BI24: Capturing filamentous protein complexes of TIR domains by using "small molecule glues"

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TIR (Toll/interleukin-1 receptor) domains are found in proteins involved in immunity pathways in organisms ranging from humans and plant to bacteria. Bacterial TIR domain-containing proteins have been shown to contribute to pathogenicity and anti-viral activity of bacteria. During bacterial infection, some TIR domain-containing proteins act as virulence factors to inhibit immune responses by interfering with Toll-like receptor signalling. Other bacterial TIR domain-containing proteins are involved in bacterial anti-viral defence [1, 2]. Many of TIR domain-containing proteins have been shown to have NAD+ cleavage activity and relevant to host cell death and bacterial anti-phage defence system.

Here, we report our studies on two bacterial TIR domain-containing proteins: AbTir and PumA. AbTir (Acinetobacter baumannii TIR domain-containing) is one of the few bacterial proteins that has been reported to produce a variant of cyclic ADPR (ADP ribose) after NAD+ cleavage. We determined the crystal structure of AbTir TIR domain in its monomeric form and the chemical structure of the cyclic ADPR it produces (termed 2'cADPR) [3]. Furthermore, we found that addition of a "small molecule glue" (3AD), which is an NAD+-mimic inhibitor of AbTir NAD+ cleavage activity induces the formation of AbTir TIR domain filaments, and determined the filament structure using Cryo-EM, which revealed large conformational differences from its monomeric form. PumA from the multi-drug resistant pathogen Pseudomonas aeruginosa PA7 is essential for its virulence [4]. We show that PumA also has NAD+ cleavage activity and forms filaments upon incubation with 3AD. Structural and functional studies currently underway aim to determine the structure of these filaments and investigate the role these assemblies play in bacterial virulence and anti-viral defence.

1. Chan, S. L., Low, L. Y., Hsu, S., Li, S., Liu, T., Santelli, E., Le Negrate, G., Reed, J. C., Woods, V. L., and Pascual, J. (2009) *Molecular mimicry in innate immunity: crystal structure of a bacterial TIR domain.* Journal of Biological Chemistry 284, 21386-21392.
2. Essuman, K., Summers, D. W., Sasaki, Y., Mao, X., Yim, A. K. Y., DiAntonio, A., and Milbrandt, J. (2018) *TIR domain proteins are an ancient family of NAD+-consuming enzymes.* Current Biology 28, 421-430. e424.
3. Manik, M. K.*, Shi, Y.*, Li, S.*, Zaydman, M. A., Damaraju, N., Eastman, S., ... & Kobe, B. (2022). *Cyclic ADP ribose isomers: Production, chemical structures, and immune signaling*. Science, 377(6614), eadc8969 (equal first authors).
4. Imbert, P. R., Louche, A., Luizet, J. B., Grandjean, T., Bigot, S., Wood, T. E., Gagné, S., Blanco, A., Wunderley, L., and Terradot, L. (2017) *A Pseudomonas aeruginosa TIR effector mediates immune evasion by targeting UBAP 1 and TLR adaptors.* The EMBO journal 36, 1869-1887.

BI25: Cell Membrane-Mimicking Liposomes as an Anti-Virulence Therapy for *Acinetobacter baumannii* and *Staphylococcus aureus*

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Acinetobacter baumannii and *Staphylococcus aureus* are two important causes of antibiotic-resistant bacterial infections. *A. baumannii* and *S. aureus* secrete toxic virulence factors that disrupt host cell membranes to release nutrients, combat the immune system, and enhance invasion through tissue layers. Neutralisation of their toxins would diminish the ability of these bacteria to persist in the host and reduce the severity of infection. Sphingomyelin-containing liposomes mimic the eukaryotic cell membrane to bind pore-forming and lipid-degrading toxins. These liposomes have shown broad spectrum efficacy against various pathogens but remain untested against *A. baumannii*. We determined their efficacy against *A. baumannii* under mono- and polymicrobial conditions alongside *S. aureus*. Cultures of A549 (lung epithelial) cells were infected with these bacteria and treated with liposomes composed of sphingomyelin, a combination of sphingomyelin and cholesterol, or both types simultaneously. LDH cytotoxicity assays were performed to determine the extent of cell death caused by the bacteria. Mass spectrometry was conducted on samples of liposomes incubated with secreted bacterial products to identify the toxins bound by different types of liposomes. Treatment with both types of liposomes reduced the cell death caused by infection by approximately 50% following infection with *A. baumannii* alone or in a polymicrobial infection with *S. aureus*. Mass spectrometry identified membrane-targeting toxins such as hemolysins from *S. aureus* and phospholipase D from *A. baumannii* bound to the liposomes. These results indicate that liposomes have promise as adjunct therapy to enhance the treatment of infections caused by *A. baumannii* and *S. aureus*. The binding of secreted toxins could protect host tissues and immune cells from damage, restrict the spread of infection and improve the clearance of invading bacteria. Such anti-virulence therapy may become an important tool to combat antimicrobial-resistant pathogens that urgently require new treatment strategies.

BI26: The enigmatic Lig E, an extracellular DNA ligase

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In addition to catalysing the penultimate step of DNA replication by joining Okazaki fragments, DNA ligases have central roles in repair pathways and genome diversification. Bacteria host a particularly rich diversity of DNA ligases with many bacterial genomes encoding multiple ATP-dependent isoforms in addition to their replicative NAD-dependent enzymes.

One of the most enigmatic groups are the minimal Lig E DNA ligases which are widely distributed among proteobacteria. Lig Es possess N-terminal leader sequences which localise them to the periplasm and are found in the genomes of many naturally-competent biofilm-forming species including the notorious antibiotic resistant pathogen *Neisseria gonorrhoeae*. Deletion of Lig E in *N. gonorrhoeae* causes defects in biofilm formation and cell adhesion in suspension cultures, as well as impacting the gonococcus' ability to colonise and penetrate epithelial tissue. However. this structural role for Lig E in biofilm formation may not be the whole story: here we report our latest findings exploring a possible role for Lig E in DNA uptake and the development of assays to investigate this.

BI27: Is now the time for a Rubiscuit or Ruburger? Increased interest in Rubisco as a food protein.

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Much of the research on Rubisco focuses on increasing crop yields, with the goal of enhancing plant production to feed a growing global population. Since its identification as the most abundant protein in leaf material, Rubisco has also been considered a direct source of dietary protein. Its nutritional and functional properties are comparable to many animal proteins and superior to many other plant proteins. Despite this potential, challenges in efficiently extracting and isolating Rubisco have limited its widespread use as a global food source. However, with rising consumer demand for plant-based proteins, there is growing interest in the potential of leaf protein. We are investigating some of the challenges associated with purification of Rubisco at a large scale, as well as testing the functional aspects of the purified protein.

BI28: Soil to solution: Progress towards a phage-based solution to American foulbrood in honeybees

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The European honeybee (*Apis mellifera*) is arguably the most economically valuable pollinator of crops worldwide. American Foulbrood (AFB) is a devastating disease of honeybee larvae caused by the bacterial pathogen *Paenibacillus larvae*. AFB is the most serious disease of honeybees and is found on every continent that has honeybees. The use of antibiotics or any substance to treat or mask AFB is prohibited under New Zealand law and infected hives must be destroyed. The ABAtE (Active Bacteriophages for AFB Elimination) project aims to discover and develop bacteriophages as a preventative measure against AFB. Previous work by Yost and colleagues in the USA has demonstrated that bacteriophages can protect hives against AFB infection when applied in advance of exposure[1]. I will describe my PhD work in which a community science approach was used to undertake sampling in a short period of time. From these samples 26 novel *P. larvae* bacteriophages were discovered, sequenced, and annotated. I will present phylogenetic data on these bacteriophages and how they have added to our understanding of the global distribution of *P. larvae* phages. I will discuss the 93% host range coverage of *P. larvae* found in NZ and the testing of cocktails in-vitro, including the observation of potential phage-phage antagonism. This project provides the groundwork for an innovative approach to naturally protect beehives against AFB and could be a solution used globally to protect the apiculture industry.

1. Yost, D. G., Tsourkas, P., & Amy, P. S. (2016). *Experimental bacteriophage treatment of honeybees (Apis mellifera) infected with Paenibacillus larvae, the causative agent of American Foulbrood Disease.* Bacteriophage, 6(1), e1122698.

BI29: Understanding the ubiquitination and characterisation of the atypical kinase RIOK3

Ormsby, J.J.L.[1], Horne, C.R.[2], Warrender, A.K.[3], Murphy, J.M.[2], Lodmell, J.S.[4], Komander, D.[2], Dobson, R.C.J.[1]

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The Rift Valley Fever Virus (RVFV) is an RNA virus identified as a category A pathogen by the National Institute of Allergy and Infectious Diseases (NIAID) because of its devastating effects on both livestock and human populations. In ruminants, RVFV causes "abortion storms," in which up to 100% of infected pregnant animals abort their pregnancies. Within Africa, the Rift Valley Fever Virus has had a high economic impact of up to $470 million in losses. Globally, RVFV is listed by the World Health Organization as a virus that is likely to cause the next global pandemic.

The right open reading frame kinase (RIOK) family consists of three proteins: RIOK1, RIOK2, and RIOK3. Both RIOK1 and RIOK2 are conserved from archaea to humans, but RIOK3 is exclusively found in multi-cellular eukaryotes, including humans. During infection with the Rift Valley Fever Virus, the protein RIOK3 is required for creation of the antiviral interferon (IFN) response within the human body. RIOK3 is an atypical serine/threonine kinase. However, the biological function of RIOK3 is largely unknown, making it a ‘dark’ kinase.

RIOK3 is hypothesised to bind ubiquitin via a domain in the N-terminus of the protein; however, any further detail is unknown. Structural studies employing native and intact mass spectrometry, cryo-EM, small-angle x-ray scattering and circular dichroism spectroscopy reveal new insights into RIOK3's conformation in solution. Interaction studies using analytical ultracentrifugation, crosslinking mass spectrometry, kinase activity assays and surface plasmon resonance identify potential ubiquitin binding sites and the specific ubiquitin chains preferred by RIOK3. Through this research, we aim to elucidate the structure and function of RIOK3, providing new knowledge on its ubiquitination mechanisms.

BI30: Cancer-associated mutations of tumour suppressor $p16^{INK4a}$ increase the propensity for amyloid fibril formation

Gray, S.G.[1,3], Heath, S. G.[2], Hamzah, E.M.[1], Botha, A.D.[2], O'Connor, K.M.[2], Bozonet, S.M.[2], Morris, V.K.[1,3], Göbl, C.[2, 3]

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$p16^{INK4a}$ (p16 for short) is a tumour suppressor protein that regulates cell cycle progression by binding to and inhibiting the cyclin-dependent kinases 4 and 6 (CDK4/6). p16 is a 16 kDa, α-helical protein with a single solvent-accessible cysteine residue located on the surface facing away from the CDK4/6 binding site. Our group discovered that this single cysteine in p16 can, under relatively mild oxidizing conditions, form disulfide-dependent homodimers. This dimerization leads to subsequent structural rearrangement into amyloid fibrils. In this amyloid fibril state p16 is unable to perform its normal function as a CDK4/6 inhibitor[1].

To further understand the mechanism of p16 amyloid fibril formation and better understand how p16 is inactivated in cancer, we have screened cancer-associated single-residue mutations. These variants have been characterised using thioflavin-T aggregation assays, SDS-PAGE analysis of dimerization, and electron microscopy. These mutations result in a significant increase in the rate of dimer and/or fibril formation. Our analysis has determined some of the key influences in p16 dimer and amyloid fibril formation.[2] We are also exploring the impact of four stabilising mutations on dimer and amyloid fibril formation. This will aid investigation of the short-lived dimer species and overall structure of p16 amyloid fibrils. Overall, this work will lead us to a greater understanding of the mechanism by which these unique aggregate species form and their role in pathogenic processes.

1. Göbl, C.; Morris, V. K.; van Dam, L.; Visscher, M.; Polderman, P. E.; Hartlmüller, C.; de Ruiter, H.; Hora, M.; Liesinger, L.; Birner-Gruenberger, R.; Vos, H. R.; Reif, B.; Madl, T.; Dansen, T. B., *Cysteine oxidation triggers amyloid fibril formation of the tumor suppressor p16(INK4A)*. Redox Biol **2020,** 28, 101316.
2. Heath, S.G., Gray, S.G., Hamzah, E.H., O'Connor, K.M., Bozonet, S.M., Botha, A.D., de Cordovez, P., Magon, N.J., Naughton, J.D., Goldsmith, D.L.W., Schwartfeger, A.J., Sunde, M., Buell, A.K., Morris, V.K.,*, Göbl, C.* *Amyloid formation and depolymerization of the tumor suppressor protein p16INK4a is strictly controlled by an oxidative thiol-based mechanism.* Nature Communications **2024,** In press

BI31: Role of nucleophosmin 1 oligomeric state in acute myeloid leukemia

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Nucleophosmin 1 (NPM1)-mutated acute myeloid leukemia (AML) has been recognized as a distinct hematopoietic cancer by the World Health Organisation. NPM1 is a critical multidomain nucleolar protein that participates in the formation of nucleolus through multivalent interactions with ribosomal RNA and other nucleolar proteins. These interactions lead to liquid-liquid phase separation (LLPS) of the nucleolus, an organelle important for ribosome biogenesis and assembly, and is crucial for its proper cellular function. NPM1 is active in its pentameric form whereas its monomeric form is intrinsically unstable. The protein domains consist of a hydrophobic N-terminal oligomerization domain (OD), a central intrinsically disordered region (IDR), and a C-terminal nucleic acid binding domain (NBD). In 30% of AML cases, NPM1 contains a frameshift mutation at its C-terminal domain. Although, the functions of both the N-terminal and C-terminal regions of NPM1 are well-studied, the impact of protein oligomerization of AML-mutated NPM1 and its role in AML tumorigenesis remains unclear.

Here, we focus on new insights about the oligomeric state of the most mutated variant of NPM1 in AML (Mutation A: 70-80% of cases). Using a combination of biophysical approaches, we analyze and compare the monomer-oligomer equilibrium of NPM1 wild type and NPM1 Mutation A. We hypothesize that the change in the monomer-pentamer equilibrium due to AML mutations will have a substantial effect on the binding capabilities of NPM1. In turn, this may have a direct correlation to the formation and regulation of biomolecular condensates in the nucleolus of AML cells. Our work will pave the way for the development of a new high-throughput platform where we can use biomolecular condensates as a platform for screening and development of novel therapies against AML in the future.

BI32: *Shigella flexneri* Type III secretion system (T3SS) effector proteins exploit the human exocyst complex to promote cell-to-cell spread.

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Shigella flexneri is a gram-negative bacterium responsible for life-threatening gastroenteritis in children under five worldwide. *Shigella* internalises into host colonic epithelium and exploits host actin polymerisation to gain actin-based motility to form protrusions that mediate spread. The mechanism of *Shigella* protrusion formation is not well understood. *Shigella* possesses a T3SS that injects ~25 effector proteins into the host cytosol, the effectors are essential for *Shigella*'s intracellular lifecycle.

Our group revealed that *Shigella*'s subversion of host exocytosis to mediate cell-to-cell spread is T3SS-dependent[1]. However, the mechanism by which *Shigella*'s T3SS effectors subvert host exocytosis remains unsolved. Exocytosis is the delivery of vesicles to the plasma membrane, we hypothesised that exploitation of host exocytosis promotes expansion of the plasma membrane for efficient *Shigella* protrusion formation. Using an exocytic probe, *Shigella* mutants deleted for T3SS genes were screened to identify effectors that induce exocytosis in protrusions. IpgD was one of multiple effectors identified to induce exocytosis in protrusions. Another group reported IpgD-dependent Arf6 activation for efficient *Shigella* internalisation[2]. The host GTPase Arf6 interacts with exocyst proteins and may promote exocytosis. To determine if Arf6 is involved in IpgD-dependent *Shigella* spread, mammalian cells were subjected to siRNA-mediated Arf6 depletion or SecinH3 (inhibitor of Arf6 activation) treatment and infection with wild-type or Δ*ipgD Shigella*. These experiments revealed that IpgD-dependent Arf6 activation is required for induction of host exocytosis, protrusion formation, and spread to occur efficiently. Collectively, these findings demonstrate that the *Shigella* T3SS effectors co-opt the host exocyst to promote efficient cell-to-cell spread.

1. Herath TUB, Roy A, Gianfelice A, Ireton K (2021). *Shigella flexneri subverts host polarized exocytosis to enhance cell-to-cell spread.* Mol Microbiol. 116(5):1328-1346.
2. Garza-Mayers AC, Miller KA, Russo BC, Nagda DV, Goldberg MB (2015). *Shigella flexneri regulation of ARF6 activation during bacterial entry via an IpgD-mediated positive feedback loop*. mBio. 3;6(2):e02584.

BI33: Discovering novel prodrug activating enzymes for multiplex targeted cell ablation

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Nitroreductases, which can convert the prodrug metronidazole to a toxic and cell-entrapped form, are widely used for cell ablation in zebrafish (*Danio rerio*). This is achieved by expressing a nitroreductase in target tissues under the control of tissue-specific promoters, to specifically sensitise those tissues to metronidazole. As zebrafish embryos are translucent, cell ablation and regeneration can be monitored via co-expressed fluorescent protein reporters. This enables the effect of bioactive compounds on cellular function to be studied via administration of metronidazole in nitroreductase-expressing transgenic zebrafish.

To expand the utility of nitroreductase-mediated ablation, we are developing a 'multiplex' system to address multiple cell types in the same model. Our goal is to express unique nitroreductases in different tissue types, with each nitroreductase being able to specifically activate a distinct nitroaromatic prodrug. Although our lab has previously engineered non-overlapping pairs of nitroreductases that function in *Escherichia coli* and cultured vertebrate cells, these enzymes were not tolerated in transgenic zebrafish.

This project aims to discover alternative multiplex nitroreductase/prodrug pairs by generating a large library of bacterial nitroreductases using a functional metagenomic approach. For this, bespoke high-expression metagenome libraries have been generated in *E. coli* using total DNA extracted from soil and gut samples. By plating these libraries on media supplemented with the nitro-antibiotic niclosamide, which is detoxified by nitroreduction, we enable positive selection for clones expressing nitroreductase enzymes. We have developed an Oxford Nanopore pipeline to enable rapid parallel sequencing of these clones. In ongoing work, the recovered nitroreductases are being counter-screened for activity against a panel of prodrugs to identify promising pairs for multiplexed ablation applications. Lead pairs will ultimately be tested for functionality in vertebrate cell lines and ultimately in diverse zebrafish models.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
BI28	Soil to solution: Progress towards a phage-based solution to American foulbrood in honeybees	Danielle Kok	School of Biological Sciences, University of Canterbury, New Zealand
BI29	Understanding the ubiquitination and characterisation of the atypical kinase RIOK3	Jacqueline Ormsby	School of Biological Sciences, University of Canterbury, New Zealand
BI30	Cancer-associated mutations of tumour suppressor p16INK4a increase the propensity for amyloid fibril formation	Shelby Gray	School of Biological Sciences, University of Canterbury, New Zealand
BI31	Role of nucleophosmin 1 oligomeric state in acute myeloid leukemia	M. Sadananda Singh	Biomolecular Interaction Centre and the School of Biological Sciences, University of Canterbury, New Zealand
BI32	*Shigella flexneri* Type III secretion system (T3SS) effector proteins exploit the human exocyst complex to promote cell-to-cell spread	N. Lee	Department of Microbiology and Immunology, University of Otago, Dunedin, NZ
BI33	Discovering novel prodrug activating enzymes for multiplex targeted cell ablation	Thomas Skurr	School of Biological Sciences, Victoria University of Wellington, Wellington, NZ