QMB Abstracts (Main Meeting)

Why planning a research career is futile but fun

Harding, J.E.[1]

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Early career researchers are often encouraged to plan their research careers, and seek support to do this. However, the skills acquired during research training often seem irrelevant to this plan, and many aspects of such plans are beyond the individual's control. I will outline some of my own accidental research career and its multiple changes in direction. I will also describe aspects of my new role as President of the Royal Society Te Apārangi.

A research career is a privilege and tremendously rewarding, but often unpredictable. Those who accept this and make the most of opportunities as they arise are most likely to build a fulfilling career.

Biography:

Distinguished Professor Dame Jane Harding is a University Distinguished Professor, and a researcher in the LiFEPATH research group of the University's Liggins Institute. Her training included a medical degree at the University of Auckland, a D Phil at the University of Oxford, and a postdoctoral Fogarty Fellowship at the University of California at San Francisco.

Professor Harding is a paediatrician and practised as specialist neonatologist caring for newborn babies at National Women's Hospital. She was Deputy Vice-Chancellor (Research) at the University of Auckland, with overall responsibility for the University's research activities, and is currently President of the Royal Society Te Apārangi.

Her on-going research concerns the role of nutrition and growth factors in the regulation of growth before and after birth, blood glucose regulation in the newborn, and the longterm consequences of treatments given around the time of birth.

How does research inform health policy and practice in New Zealand, and how can we do better?

Bloomfield A.[1]

[1] School of Population Health, University of Auckland | Waipapa Taumata Rau, Auckland, NZ.

Research, evidence and knowledge are essential inputs into policy and political decision-making in New Zealand. However, policy development is not a linear, predictable process. Researchers need to understand the different drivers, timeframes and expected outcomes of policy development and political decision-making to be best placed to influence these processes.

This presentation draws on the author's more than 20 years' experience in the public service, including through the COVID-19 pandemic, to shed light on how policies decisions are made and implemented. It will include specific health policy examples to illustrate key points, and consider ways that the interface between research and policy-making can be strengthened to help ensure better outcomes.

Biography:

Sir Ashley Bloomfield has 25 years' experience in public health, policy and health leadership, including at the WHO in Geneva. He was New Zealand's Director-General of Health from June 2018 to July 2022 and led the country's health response to the COVID-19 pandemic. He was appointed a Knights Companion of the New Zealand Order of Merit (KNZM) in the 2023 New Year Honours for services to public health. Dr Bloomfield is now a Professor at the University of Auckland's School of Population Health.

Q1: *In situ* structural biology by cryo-electron tomography

Fei Sun
Guangzhou Institutes of Biomedicine and Health (GIBH), Chinese Academy of Science, Guangzhou, China

With the continuous advancement of cryo-electron tomography (cryo-ET) technology, it is now possible to directly resolve the in situ three-dimensional (3D) structures of protein complexes within tissues and cells. Over the past few years, I have led the research team in continuously developing and enhancing the cryo-ET workflow. For sample preparation, we have developed supporting films for electron microscopy grids, a focused ion beam (FIB) technology called D-cryoFIB, and an in situ sample preparation workflow for biological tissues known as VHUT-cryoFIB. In the field of cryo-correlative light and electron microscopy (cryo-CLEM), we have introduced HOPE-cryoCLEM equipment and the improved HOPE-SIM-cryoCLEM equipment. Additionally, we have designed the ELI-TriScope, an integrated system for preparing target cryo-lamellae with real-time fluorescence monitoring. For data collection and processing, we have pioneered a beam tilting-based high-throughput tilt series collection technology, the cryo-ET image alignment algorithm MarkerAuto, and the 3D reconstruction algorithms ICON and FIRT, along with a deep learning-based technology for 3D particle picking called DeepETPicker.

By leveraging these technological advancements, we have elucidated the in situ structure of RyR1 within the triad of mouse skeletal muscle, providing direct evidence of the physical coupling between these proteins. We have also resolved the in situ structure of the doublet microtubule (DMT) in mouse sperm at a resolution of up to 4.5 Å, identifying 36 distinct microtubule inner proteins (MIPs) and highlighting their role in the DMT lumen. Furthermore, we have uncovered the nanometer-resolution structure of the post-fusion state of the Spike protein on the SARS-CoV-2 virus membrane. These in situ structural studies significantly advance our understanding of cellular physiological activities at the molecular level.

Q2: 3D Printing to Engineer Gynecological Tissues

John P. Fisher
Fischell Department of Bioengineering, University of Maryland, College Park, MD

Generating complex tissues has been an increasing focus in tissue engineering and regenerative medicine. With recent advances in bioprinting technology, our laboratory has focused on developing platforms for treating and understanding clinically relevant problems ranging from congenital heart disease to orthopedic trauma. In this presentation, we focus on engineering gynecological tissues, particularly a nipple-areolar complex (NAC), as well as a model of the human placenta. We utilize digital light processing (DLP)-based and extrusion-based additive manufacturing to generate engineered tissues. Both approaches begin with CAD models that are then transferred to printing platforms. Prints use a variety of inks, including synthetic (PEG) and natural (GelMA, ECM) materials. Evaluation of printed constructs assesses critical criteria, including print fidelity, material properties (immediately after printing and at longer time points), cell viability, and cell functionality. Tissue function is assessed both in vitro and in vivo. Our initial studies have generated a portfolio of NAC implant designs via Solidworks, allowing for the rapid development of personalized implants. CAD designs explore the impact of nipple projection height, nipple diameter, areola diameter, and NAC infill patterning upon the implant's properties, particularly on the shape retention over time. NAC physical and biological properties (shape, mechanics, cell seeding efficiency, cell viability / proliferation, matrix production) have been assessed. NAC vascularization strategies have been developed. We have also developed a 3D printed bioengineered placenta model (BPM) to better understand placental biology. Our multicellular, multiphase construct models the role of the placental matrix upon both the barrier functionality of the tissue and the cellular remodeling throughout pregnancy. We are now using the model to better assess the impact of intrinsic and extrinsic factors on placental functionality.

Biography:

Dr. John P. Fisher is the MPower Professor, Distinguished-Scholar Teacher, Fischell Family Distinguished Professor, and Department Chair in the Fischell Department of Bioengineering at the University of Maryland. Dr. Fisher is also the Director of the Center for Engineering Complex Tissue (CECT), which aims to create a broad community focusing on 3D printing and bioprinting for regenerative medicine applications. As the Director of the Tissue Engineering and Biomaterials Laboratory, Dr. Fisher's group investigates biomaterials, stem cells, bioprinting, and bioreactors for regenerating lost tissues, particularly bone, cartilage, and soft tissues. Dr. Fisher's laboratory has published over 225 articles, book chapters, editorials, and proceedings (17,000+ citations / 74 h-index) and delivered over 400 invited and contributed presentations, with support from NIH, NSF, FDA, NIST, DoD, and other institutions.

Q3: Challenges and opportunities in RNA therapeutics: spotlight on cancer

Fox, Archa

The University of Western Australia

RNA biotechnology is a toolbox for new therapeutic development, including mRNA, antisense oligonucleotides, siRNA, microRNA, aptamers, engineered RNA binding proteins and small molecules that target RNA. Here I will present progress on the development of an antisense oligonucleotide that modulates the processing of the long noncoding RNA, NEAT1. Cell nuclei contain many nuclear bodies that form when their components phase separate and condense within permissive local regions within the nucleus. Here we focus on paraspeckles, stress-responsive subnuclear bodies that form by phase separation around the long non-coding RNA NEAT1. In the context of triple negative breast cancer and modelling the process of intravasation, we found that as cells entered moderate confinement, a significant increase in paraspeckle number and size was observed. Increasing paraspeckle abundance using antisense oligonucleotides that alter NEAT1 RNA processing resulted in increases in confined migration likelihood, speed, and directionality, as well as an enhancement of paraspeckle polarization towards the leading edge. In contrast, in the infant cancer neuroblastoma, the same antisense oligonucleotides were associated with reduced proliferation and a differentiation phenotype. Thus, mediators of N

EAT1 lncRNA processing can give different potential effects in different cancer subtypes.

Q4: Models and mechanisms of O-GlcNAc transferase intellectual disability

van Aalten, D. M. F.[1]
[1]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

The O-GlcNAc post-translational modification of intracellular proteins is essential for embryogenesis, development and brain function. Our knowledge of how O-GlcNAcylation regulates protein function and associated pathways is limited. An exciting new inroad into this is our recent discovery that patients with mutations in O-GlcNAc transferase (OGT) suffer from intellectual disability and (neuro)developmental delay (e.g. *PNAS* 2019), in a syndrome that we have defined as OGT-Congenital Disorder of Glycosylation (OGT-CDG, *Eur J Hum Gen* 2020). Although several OGT-CDG mutations have now been reported, it is not understood how these mutations are mechanistically linked to the neuro-developmental deficits seen in the patients.

I will describe common symptoms (intellectual disability, epilepsy, developmental delay) in a cohort of >65 unpublished patients and will present the first example of genome editing in mice to generate three independent lines that carry OGT-CDG mutations. Excitingly, these mice are viable, unlike previously reported Ogt knock-out mice, allowing the first phenotypic characterization of a vertebrate model of OGT-CDG. Notably, these mice show changes in size and weight suggesting developmental delay as observed in patients. Additionally, we observe changes in O-GlcNAc homeostasis to compensate for loss of OGT catalytic activity in the brain. This is associated with microcephaly, behavioural and cognitive defects, including hyperactivity, anxiety, compulsive behaviour and altered spatial working memory – again recapitulating several of the symptoms in OGT-CDG patients. Following on from a range of omics approaches, we have discovered the RNA helicase and intellectual disability protein DDX3X as a potential driver of this syndrome.

Taken together, this is the first example of phenotypes in mouse models of OGT-CDG reminiscent of patient symptoms resulting from pathogenic loss of OGT catalytic activity. These models are an invaluable starting point to gain insight into OGT-CDG etiology, identify underlying mechanisms of the disease and provide a platform for evaluation of potential future treatment strategies.

Q5: 3D printing in medicine: regeneration & medical devices

Cabral, J.D.[1,3], Turner, P.R.[1], Murray, E.[2], Yoshida, M.[3],Thorsnes, Q.[3], Ali, M.A.[3]

[1]Department of Microbiology & Immunology, University of Otago, Dunedin, NZ, [2]Department of Chemistry, University of Otago, Dunedin, NZ, [3]Department of Oral Rehabilitation, Centre of Bioengineering & Nanomedicine, University of Otago, School of Dentistry, Dunedin, NZ

The use of additive manufacturing, or 3D printing has evolved with the development of the appropriate biomaterials to enable researchers to recreate native cellular microarchitecture as next generation tools for a variety of healthcare needs. Tissue engineering, a branch of regenerative medicine, aim is to build functional 3D tissue engineered constructs to replace damaged or diseased tissues or organs. A/P Cabral will present tissue engineered constructs developed by her group using an assortment of biofabrication techniques for regenerative medicine applications;[1-5] as well as discuss ongoing medical device development projects. Her overall journey from chemist to biomedical scientist and bioengineer will be showcased, highlighting the multidisciplinary nature of this dynamic and exciting field.

1. Nelson, V.J., Dinnunhan, F., Turner, P.R., Faed, J.M., Cabral, J. D, *A chitosan/dextran-based hydrogel as a delivery vehicle for human bone-marrow derived mesenchymal stem cells.* Biomed Mater, 2017. **12**(3): p. 035012.
2. Turner, P.R., et al., *Peptide chitosan/dextran core/shell vascularized 3D constructs for wound healing.* ACS Applied Materials & Interfaces, 2020.
3. Turner, P.R., et al., *Melt Electrowritten Sandwich Scaffold Technique Using Sulforhodamine B to Monitor Stem Cell Behavior.* Tissue Eng Part C Methods, 2020. **26**(10): p. 519-527.
4. Yoshida, M., et al., *A comparison between β-tricalcium phosphate and chitosan poly-caprolactone-based 3D melt extruded composite scaffolds.* Biopolymers, 2022. **113**(4): p. e23482.
5. Thorsnes, Q.S., et al. *Integrating Fused Deposition Modeling and Melt Electrowriting for Engineering Branched Vasculature*. Biomedicines, 2023. **11**, DOI: 10.3390/biomedicines11123139.

Biography:

Jaydee Cabral is a Research Associate Professor in the Microbiology and Immunology department at the University of Otago (UoO), NZ. She received her Ph.D. in Biomedical Sciences from Eastern Virginia Medical School and Old Dominion University, USA. She was a lecturer at California State University, Monterey Bay prior to her postdoctoral fellowship in the Chemistry department at the UoO. Her research applies a multidisciplinary approach to the development and characterization of biomaterials, medical devices, and tissue engineered constructs (TECs). She utilizes synthetic chemistry, chemical/physical and microbiological characterization techniques, as well as in vitro tissue culture and mouse models. She was a key researcher in a multi-million-dollar MBIE funded project, "Smart gels for Commercialization," where she contributed to the development of an FDA approved, commercialized post-surgical wound healing gel, Chitogel®. Her laboratory features the only GeSiM 3.1 Bioscaffolder in NZ, a 3D bioprinter with multifunctional capability. Other funding includes an HRC Explorer Grant and a LHR Grant to 3D bioprint vascularized, regenerative living dressings for the treatment of chronic wounds. Other projects are a Catalyst Seed funded grant to 3D print nipple-areolar complexes for mastectomy patients, and a Translation grant to develop a medical device to treat precancerous cervical lesions.

Q6: Dietary supplementation of male mice with inorganic, organic or nanoparticle selenium preparations: a comparison of metabolic activity and fertility parameters

Mojadadi A.,[1] Au A.,[1] Shao J-Y.,[1] Ortiz-Cerda T.,[1] O'Neil T.,[1] Bell-Anderson K.,[2] Andersen J.W.,[53] Salah W.,[1] Ahmad G.,[1], Harris H.H.[3] and Witting P.K.[1,*]

[1] Redox Biology Group, Charles Perkins Centre, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, 2006
[2] Charles Perkins Centre, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW, 2006.
[3] Discipline of Chemistry, The University of Adelaide, SA 5005, Australia

Background: Selenium (Se) is important for development and reproduction. However, there is no consensus on the chemical form/dose of dietary Se for maintaining physiological homeostasis. We examined dietary inorganic-Se, organic-Se and nanoparticle elemental-Se forms on metabolic activity, gut microbiome and male fertility in mice.

Methods: Male C57BL/6 mice (n=8/group) were fed control (AIN93G) or Se-enriched diets: sodium selenite (NaSe, 5.6ppm), methylselenocysteine (Met, 4.7ppm), diphenyl diselenide (DDS, 14.2ppm), or nano-elemental-selenium (NanoSe, 2.7ppm) with Se levels ascertained by inductively-coupled plasma mass spectrometry. Metabolic parameters were for 3 weeks using Promethion metabolic cages and body composition determined by Echo-MRI. At 4 weeks testes, sperm, blood and stool were assessed for fertility parameters, immune-histopathology, and microbiota.

Results: NaSe, Met, and NanoSe (not DDS)-enriched diets enhanced physical activity and energy expenditure at Week 1. However, by week 3, these proclivities returned to control levels, indicating a transient metabolic effect. At 4 weeks, testicular weight, sperm count, and motility trended to increase for all Se *vs* control diets except for the DDS-diet, where all measures were markedly lower. Testosterone increased significantly in mice supplemented NaSe, Met, and NanoSe; testicular glutathione peroxidases1/4 increased while superoxide dismutase and catalase showed a slight increase in NanoSe group indicating a selective antioxidant action. All Se diets promted marked changes in gut microbiome linked to enhanced immune status.

Conclusion: Dietary NaSe, Met, and NanoSe transiently increased metabolism and altered microbiome. NanoSe and NaSe both enhanced reproductive, and redox protein activities in testis with NanoSe showing greatest promise as a safe bioactive forms of Se.

Biography:

Professor Paul Witting is an academic working at the Discipline of Pathology, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney. Witting is a career biochemist with an interest in metals and micronutrients in health and disease. His research lab employs advanced imaging, mass and biochemical technologies together with analytical pipelines that probe the role for metals in pathophysiology using experimental models of disease.

Q7: Redox-controlled amyloid formation and disassembly of the tumour suppressor protein p16

Sarah G. Heath[1], Shelby G. Gray[2], Emilie M. Hamzah[2], Karina M. O'Connor[1], Stephanie M. Bozonet[1], Alex D. Botha[1], Pierre de Cordovez[1], Nicholas J. Magon[1], Abigail J. Schwartfeger[2,5], Margaret Sunde[3], Alexander K. Buell[4], Christoph Göbl[1,5], Vanessa K. Morris[2,5]

[1] Mātai Hāora – Centre for Redox Biology and Medicine, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ, [2] School of Biological Sciences, University of Canterbury, Christchurch, NZ, [3] School of Medical Sciences and Sydney Nano, The University of Sydney, Sydney, Australia, [4] Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Lyngby, Denmark, [5] Biomolecular Interaction Centre, University of Canterbury, Christchurch, NZ.

p16^{INK4A} (also known as p16) is a critical regulator of the mammalian cell-cycle, and thus is found to be mutated in many cancers. We have found that under oxidizing conditions, the single cysteine amino acid of p16 forms an intermolecular disulfide bond.[1,2] Formation of the disulfide-linked dimer leads to a dramatic structural rearrangement of the protein, from an all alpha-helical structure, to beta-sheet-based amyloid fibrils. Conversion to amyloid fibril structures prevents p16 from carrying out its normal function as a kinase inhibitor, and therefore p16 amyloid formation may be a novel regulatory mechanism of p16 activity. Furthermore, p16 amyloid fibrils are disassembled by addition of reducing agent, and the native function of p16 can be recovered, making the p16 oxidation state a fully-reversible functional switch. We find that multiple cancer-related mutations show increased amyloid formation propensity whereas mutations stabilizing the fold prevent transition into amyloid. These findings provide insight into a novel disulfide-linked amyloid structure, as well as highlighting their potential role in cancer and cell division mechanisms.

1. 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.* (2024*) Amyloid formation and depolymerization of the tumor suppressor p16INK4a are regulated by a thiol-dependent redox mechanism.* Nature Communications. In press.

2. 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 (202). *Cysteine oxidation triggers amyloid fibril formation of the tumor suppressor p16(INK4A)*. Redox Biology. 28: 101316

Biography:

Vanessa Morris is a senior lecturer at the University of Canterbury in Christchurch, New Zealand, where she leads a protein biophysics and structural biology research group focused on studying the structures, mechanisms and interactions of aggregating proteins. She obtained her PhD from the University of Sydney before carrying out postdoctoral research at the Technical University of Munich and the University Health Network/University of Toronto, and then starting her current position in 2019. Vanessa's group studies amyloid proteins involved in disease, including Alzheimer's disease and cancer, as well as those with important biological functions in a variety of organisms, using a range of methods including NMR spectroscopy and electron microscopy.

Q8: One ring to rule them all: redox biology and the emergence of novel therapeutic strategies

Hampton, M.B.[1]

[1]Mātai Hāora – Centre for Redox Biology and Medicine, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ.

Redox signalling pathways enable cells to respond to environmental oxidative stress and endogenous oxidants generated by various metabolic processes. Signal transmission appears to occur via transient oxidation of specific cysteine residues in key regulatory proteins. Diseases associated with sustained oxidative stress can perturb these sensitive signalling pathways, but the design of protective strategies has been limited by our understanding of how these pathways operate. It is hypothesized that redox signalling is controlled by a family of thiol peroxidases called peroxiredoxins that are exquisitely sensitive to oxidation by hydrogen peroxide. While peroxidase activity is mediated by dimer subunits, peroxiredoxins form complex decameric rings. These rings may be scaffolds for enabling the interaction of peroxiredoxins with specific intracellular proteins. Compounds that disrupt these protein-protein interactions provide new strategies for either protecting cells from pathological oxidative stress or promoting stress in cancer cells and pathogens.

Biography:

Professor Mark Hampton was an undergraduate at the University of Canterbury before completing his PhD at the University of Otago in 1995. He had postdoctoral positions at the Karolinska Institute and Harvard before returning to the Christchurch campus of the University of Otago in 1999. He is currently a research professor and leads the Mātai Hāora Centre for Redox Biology and Medicine.

Mark's research focuses on the fundamental mechanisms by which oxidative stress impacts signalling pathways in cells, and how modulation of these processes might impact health and disease. Current projects include investigations of how disruption of redox homeostasis can promote the killing of cancer cells and pathogenic microbes, and how oxidative stress and mitochondrial dysfunction are associated with the biological processes underlying human ageing.

Q9: Genome wide essentiality screens to define therapeutic vulnerabilities in an isoniazid-resistant catalase mutant of *Mycobacterium tuberculosis*

XinYue Wang[1], William J Jowsey[1], Chen-Yi Cheung[1], Caitlan Smart[1], Hannah Klaus[1], Noon E Seeto[1], Natalie JE Waller[1], Michael T Chrisp[1], Amanda L Peterson[5], Akua Ofori-Anyinam[6,7], Emily Strong[8], Brunda Nijagal[5], Nicholas P West[8], Jason H Yang[6,7], Peter C Fineran[1,2,3,4], Gregory M Cook[1,2,9], Simon A Jackson[1,2], Matthew B McNeil[1,2#]

[1]Department of Microbiology and Immunology, University of Otago, New Zealand

[2]Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, New Zealand.

[3]Genetics Otago, University of Otago, Dunedin, New Zealand

[4]Bio-Protection Research Centre, University of Otago, Dunedin, New Zealand.

5Metabolomics Australia, Bio21 Institute, The University of Melbourne, Melbourne, Australia

[6]Center for Emerging and Re-emerging Pathogens; Public Health Research Institute; Rutgers New. Jersey Medical School; Newark, NJ 07103; USA

[7]Department of Microbiology, Biochemistry, and Molecular Genetics; Rutgers New Jersey Medical School; Newark, NJ 07103; USA

[8]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, 4072, Australia.

[9]School of Biomedical Sciences, Queensland University of Technology, Translational Research Institute, Woolloongabba, Queensland 4000, Australia

Mycobacterium tuberculosis is the primary causative agent of Tuberculosis (TB) and a leading cause of infectious disease morbidity and mortality. Drug susceptible strains of *M. tuberculosis* are curable, yet this requires the use of four drug regimens for six months. Treatment options for drug-resistant strains of *M. tuberculosis* are woefully inadequate with long treatment times and significant toxicities.

Combination therapies are necessary for the treatment of *M. tuberculosis* to target cells in different physiological states. Combination therapies are favoured for their ability to increase efficacy, delay the development of resistance and reduce toxic side effects. Yet, identifying the most synergistic drug interactions using conventional experimental procedures is an extremely resource intensive process. We hypothesized that high-throughput genetic interaction screens would allow for thousands of potential drug combinations to be screened in a single experiment.

To achieve this we have combined pooled multiplexed CRISRP-interference (CRISPRi) transcriptional repression with deep sequencing. Using this platform we screened all genetic interactions between >50 possible drug targets. Each gene is either the target of an existing antibiotic or has been previously identified as a high-priority drug target. This presentation will provide updates on our the development of our experimental platform, results from pooled interaction screens, and our current work validating high value generalists target that have synergistic interactions with a large number of functionally diverse antibiotics.

Biography:

Senior Research Fellow at the University of Otago investigating drug resistance and drug development in Mycobacterium tuberculosis.

Q10: Discovery of antimycobacterial drugs and regimens in GIBH

Tianyu Zhang
Guangzhou Institutes of Biomedicine and Health (GIBH), Chinese Academy of Science, Guangzhou, China

In the report, I will briefly introduce tuberculosis and the bottle neck in discovery and development of new anti-tuberculosis drugs. This includes progress in screening and evaluation techniques for anti-tuberculosis drug activity, primarily based on our own studies. These techniques encompass high throughput drug screening methods both in vitro and in vivo, using autoluminescent mycobacteria invented by us; innovative concepts for in vitro drug screening such as negative selection, simulating physiological nutrition condition screening, and non-replicating screening; rapid methods for testing compound activities against clinical isolates; a new inhalation administration model developed by us; and the latent infection tuberculosis model, among others. Additionally, I will discuss the devices we have developed for conducting these specialized experiments. Furthermore, I will present the research and development progress of our team's anti-mycobacterial drugs, with particular emphasis on TB47, which will enter clinical trial soon. Finally, I will describe the novel regimens containing TB47 and the new therapeutic vaccine candidate, rdrBCG.

Q11: Building a better Bedaquiline, development of TBAJ-587 and TBAJ-876.

Hamish Sutherland,[1,2] Adrian Blaser,[1] Peter Choi,[1,2] Daniel Conole,[1,2] William Denny,[1,2] Brian Palmer,[1] Amy Tong,[1] Sophia Tsang,[1] Scott Franzblau,[3] Christopher Cooper,[4] Manisha Lotlikar,[4] Anna Upton.[4]

[1]Auckland Cancer Society Research Centre, School of Medical Sciences, University of Auckland, Private Bag 92019, Auckland, 1142, New Zealand. [2]Maurice Wilkins Centre, University of Auckland, Auckland, 1142, New Zealand. [3]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL, 60612, USA. [4]TB Alliance, 40 Wall St, New York, 10005, USA

Tuberculosis (TB) is the deadliest infectious disease around the world, claiming over a billion lives in the past two hundred years, including 1.3 million in 2020. Bedaquiline is a diarylquinoline compound that targets ATP synthase in *mycobacterium tuberculosis*, the causative agent of TB. Although clinically effective against drug-resistant tuberculosis, Bedaquiline has a cardiac liability, resulting in increased risk of death, as well as greater than desired tissue accumulation.

A research collaboration between TB Alliance and medicinal chemists at the Auckland Cancer Society Research Centre led to the synthesis of over 900 analogues of Bedaquiline[1,2,3], this resulted in derivatives with greatly improved pharmacological properties, two of these compounds (TBAJ-876 and TBAJ-587) have progressed to clinical trial. TBAJ-876 is currently in phase II clinical trial, while TBAJ-587 is currently in phase I clinical trial. This presentation will outline the development of these second-generation analogues of Bedaquiline.

1. H. S. Sutherland, A. S. T. Tong, P. J. Choi, A. Blaser, D. Conole, S. G. Franzblau, M. U. Lotlikar, C. B. Cooper, A. M. Upton, W. A. Denny, B. D. Palmer, *Bioorg. & Med. Chem.*, **2019**, *27(7)*, 1292-1307.
2. A. Blaser, H. S. Sutherland, A. S. T. Tong, P. J. Choi, D. Conole, S. G. Franzblau, C. B. Cooper, A. M. Upton, M. Lotlikar, W. A. Denny, B. D. Palmer, *Bioorg. & Med. Chem.*, **2019**, *27(7)*, 1283-1291.
3. H. S. Sutherland, A. S. T. Tong, P. J. Choi, A. Blaser, S. G. Franzblau, C. B. Cooper, A. M. Upton, M. Lotlikar, W. A. Denny, B. D. Palmer, *Bioorg. & Med. Chem.*, **2020**, *28*, 115213.

Biography:

I am a medicinal chemist at the Auckland Cancer Society Research Centre (ACSRC), working on infectious disease and cancer projects for the previous 23 years. I am currently the principal investigator for the collaboration between TB Alliance and the Auckland Cancer Society Research Centre, during this long standing collaboration with TB Alliance I have worked as part of a team on projects developing the clinical candidates TBA-354, TBAJ-587 and TBAJ-876, as well as working on a number of early stage medicinal chemistry projects.

I completed my PhD in chemistry at the University of Auckland under the supervision of Associate Professor Paul Woodgate, followed by postdoctoral research at the University of Waterloo, Ontario, Canada with Professor Russell Rodrigo.

Q12: Where did all the antibodies go? Exploring the biological basis of immunity gaps to endemic respiratory pathogens following Covid-19 restrictions in Aotearoa New Zealand

Nikki Moreland[1,2], Natalie Lorenz[1,2], Reuben McGregor[1,2], Tiaan van Rooyen[1], Aimee Paterson[1], Lauren Carlton[1,2], Ciara Ramiah[1], Prachi Sharma[1], Alex James[3], Richard Charlewood[4]

[1]Department of Molecular Medicine and Pathology, The University of Auckland, [2]Maurice Wilkins Centre for Biodiscovery, The University of Auckland, [3]Department of Mathematics, University of Canterbury, [4]The New Zealand Blood Service, Auckland

New Zealand, like many countries, reported markedly reduced community transmission of non-vaccine preventable respiratory pathogens after March 2020 as a consequence of COVID-19 restrictions. Subsequent to the relaxation of these restrictions, surges in infections caused by pathogens transmitted via respiratory routes such as respiratory syncytial virus (RSV), and Streptococcus pyogenes (Group A Streptococcus, Strep A) have been documented, often exceeding pre-pandemic levels. This large-scale study investigated whether reduced pathogen exposure during the pandemic resulted in immunity gaps to endemic pathogens that may have contributed to these surges. It utilised samples from long term plasma donors collected by the NZ Blood Service between January 2020 and September 2023 and involved screening of >4500 individual donations. Building on our prior assay systems for Strep A and SARS-CoV-2, a new multi-pathogen bead-based assay comprised of major antigens from RSV (glycoproteins G and F), Strep A (Streptolysin-O, SpnA, DNaseB and SpeA), endemic human coronaviruses (hCoV; NL63 and HKU1 spike), and SARS-CoV-2 (spike) was developed for detection of pathogen specific antibodies (IgG). Paired analysis showed that the total IgG level was significantly reduced by 7-16% for all antigens from RSV, Strep A and hCoV between 2020 and 2023. In contrast, IgG for SARS-CoV-2 spike increased exponentially in these donors over the same time period. The reduced IgG to respiratory pathogens observed in healthy adults in New Zealand likely reflects reduced exposure to these endemic agents during the pandemic, and may have contributed to the pronounced surges in these pathogens in 2023.

Biography:

Associate Professor in Immunology and Principal Investigator of the Maurice Wilkins Centre for Biodiscovery

Q13: Withdrawn

Q14: Biosensing of tuberculosis volatile biomarkers using insect odorant receptors

Carraher, C[1,2], Di Brewster[1], Wendy Huo[1], Mark Agasid[1], Jonathan Good[1] and Andrew V. Kralicek[1]
[1]Scentian Bio Ltd, Auckland, New Zealand
[2]Plant and Food Research, Auckland, New Zealand

Current diagnostics for tuberculosis (TB) are slow, expensive, and often reliant on highly trained personnel which limit their use, especially in areas with the highest disease burden. Recent studies have identified that volatile organic compound (VOC) biomarkers for TB diagnosis exist in breath, skin and urine samples, opening the door to the rapid non-invasive diagnosis of TB patients. Unfortunately, current VOC sensing approaches are not available in a portable format for in-field real-time diagnosis of TB patients. Here we present an insect Odorant Receptor (OR) based biosensing approach for the detection of TB biomarker VOCs. We validate this approach using three Drosophila melanogaster odorant receptors: DmOr42a and DmOr69a for the breath TB biomarkers 2-butanone and nonanal, respectively, and DmOr35a for the skin TB biomarker hexyl butyrate. We overexpress, purify and reconstitute each receptor with DmOrco into liposomes. We then use fuse each DmOrX/Orco liposome with planar lipid bilayers to enable ion channel measurements. By directly monitoring the change in electrical current through the planar lipid bilayer we can detect binding of each biomarker VOC to their respective receptor. Furthermore, we show with DmOr69a/Orco that it is possible to detect a VOC biomarker at the nM (ppB) concentration, providing the sensitivity required for disease diagnosis in biological samples. This study is the first step towards the development of an OR based electrochemical biosensor for the rapid point-of-care diagnosis of TB and other disease states which have unique volatile signatures.

Q15: Reconstructing complex gut microbiomes in the “-ome” era.

Phil B. Pope[1,2], Thea Os Andersen[2], Carl M. Kobel[2], Velma Aho[2], Ove Øyås[2], Torgeir R. Hvidsten[2], Arturo Vera Ponce de Leon[2], Magnus Ø. Arntzen[2], Live H. Hagen[2], Simon McIlroy[1], Gene W. Tyson[1], Sabina Leanti La Rosa[2]

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As humanity expands pressure is being applied to food production systems (FPS) to develop nutritious, efficient and sustainable practices, such as optimizing health benefits from food, improving feed conversion and animal welfare and mitigating harmful byproducts such as greenhouse gases (GHG). One promising route to FPS advancement is using deeper understanding of the intimate genetic and physiological connection between animals and their microbiota to devise microbiome-based interventions that control key phenotypes of interest. However, first we must unlock critical and poorly understood microbiota and their biological pathways that control digestion as well as identify exploitable interactions that exist within the complexity of gut microbiomes. Our research seeks to combine high-resolution genome-guided meta-omics technologies with enzymology, bacteriology, bioinformatics and phenotyping of relevant digestive eco-systems from human and production animals (pigs, fish and ruminants). Herein we highlight how such an integrated approach can be used to visualize the stimulatory effects that distinctive dietary fibres have upon known model microorganisms within a complex endogenous microbiome. We further reveal the metabolic influence of uncharacterized bacterial and eukaryotic populations that are surprisingly conserved across diverse dietary conditions and host species. Importantly, as we develop with the technological advancements, we are actively "retrofitting” our analyses to include high-throughput cultivation strategies to bring keystone microbes into the lab for their first time, with the long-term objective being: to understand, monitor and ultimately manipulate host-microbiome interactions.

Biography:

As a microbial ecologist and physiologist, Prof Phil B. Pope has greater than 15 years' experience using multi-omic tools to deconvolute the intimate genomic and physiological relationships between microbial populations within complex microbiomes that are integral to gut function, health and nutrition of animals. Phil has a BSc from Griffith University (Queensland, Australia) majoring in physical mathematics, with honours in environmental microbiology (2003); a PhD in metagenomics from Griffith University (2007); and postdoc experience at CSIRO with Professor Mark Morrison (2007-2010).

Phil moved to Europe in 2010 as a Marie Sklodowska-Curie Incoming fellow with renowned enzymologist Prof. Vincent Eijsink and started his group with an ERC starting grant in 2014. Phil was awarded a Novo Nordisk Fonden Ascending fellowship in 2019 and also makes central scientific and management contributions to large national and European collaborative efforts (ERA-Net and Horizon 2020 projects) that are focused on animal-microbiome interactions.

In 2024, Phil relocated to the Queensland University of Technology and is a group leader at the Centre for Microbiome Research. Phil was recently awarded an Australian Research Council funded Future Fellowship "HoliCow" which aims to expand our fundamental understanding of the rumen microbiome by coupling omics technologies with high throughput cultivation.

Q16: Antibody binding to rumen methanogens is not a hurdle for development of an anti-methanogen vaccine

Khanum, S.[1], Roberts, J. M.[2], Heathcott, R. W.[1], Bagley, S.[1], Wilson, T.[1], Gupta, S. K.[1], Kirk, M. R.[1], Janssen, P. H.[1], and Wedlock, D. N.[1]

[1]AgResearch, Palmerston North, NZ,[2]Flowjoanna, Palmerston North, NZ.

The rumen is a complex ecosystem where microbes ferment indigestible feed material to nutrients that ruminants can use. Resident methanogenic archaea convert hydrogen, a fermentation by-product, to methane and so contribute to about 30% of global anthropogenic methane emissions. Development of a vaccine to mitigate this methane source would be aided by a better understanding of antibody interactions with methanogens. However, this is challenging due to the complexity of rumen microbial populations and the presence of plant material. We developed a spectral flow cytometry method to identify and quantify the F_{420} (8-hydroxy-5-deazaflavin)-containing methanogens in rumen contents from sheep and cattle based on the intrinsic autofluorescence of coenzyme F_{420}. A methanogen-specific signature was defined using cultured methanogen cells. This enabled better differentiation between methanogens autofluorescence and fluorescence of plant material in the rumen contents. This methodology was applied to quantify the interaction of antibodies with rumen methanogens. Antibodies against *Methanobrevibacter ruminantium* M1, against a mixture of five strains of methanogens (Mix5) or against mixtures of methanogen surface proteins were produced in sheep. Anti-M1 antibodies bound to the majority (91%) of cultured M1 cells. Antibodies against Mix5 or mixtures of methanogen proteins cross-reacted with 20-25% of the resident methanogens in rumen contents. We also developed antibody-assisted immunomagnetic capture technology (ICT) to determine antibody specificity and cross-reactivity to methanogens in rumen content samples. Magnetic beads coated with anti-methanogen antibodies produced against individual methanogen strains bound strongly to cells of that strain and also showed a degree of cross-reactivity with other cultured methanogens in the presence of rumen fluid, and with resident methanogens in rumen contents. These methods are being used to study the interaction of antibodies with surface antigens of rumen methanogens, to understand antigen cross-reactivity and the efficiency of antibody binding, and to evaluate antigens for a broad-spectrum anti-methanogen vaccine to reduce methane.

Q17: Rumen methanogen inhibitors

Muetzel S.[1]

[1]AgResearch, Palmerston North, NZ

Enteric methane contributes 40% to the national greenhouse gas inventory. In the rumen, the production of methane is the principal pathway which removes hydrogen generated during the fermentation of plant carbohydrates to acetic acid. The hydrogen is used by methanogens, a subgroup of organisms in the domain Archaea, reducing carbon dioxide or methyl-groups to methane. There are two principal ways to inhibit methane production. Hydrogen sinks are compounds that have a higher affinity to hydrogen than methanogens do, therefore starving the methanogens of their substrate. The second approach is to selectively target methanogens in the rumen without inhibiting rumen bacteria which convert plant matter into short chain fatty acids and microbial biomass, the principal source of energy and protein of the ruminant. The targets for methanogen inhibitors are specific methanogen cell wall proteins or the enzymes in the final steps of the methane pathway which are common to the hydrogenotrophic, methylotrophic and acetoclastic methanogen pathways.
However, many methane inhibitors only have a short lifespan as the bacterial community adapts and metabolises the compounds. Intraruminal compound turnover is the principal determinant of the compound concentration in the rumen. Effective inhibitors increase ruminal hydrogen concentration and hinder the regeneration of NAD. Consequently, bacteria shift away from hydrogen producing fermentation pathways to more hydrogen neutral or hydrogen consuming pathways. However, the fate of the hydrogen not captured in methane remains unknown, as only 12-25% is accounted for in known metabolites. Currently, several international efforts are underway to identify these missing metabolites as they could have long-term negative effects on the host animal. On the other hand, methane inhibition may open up a pathway to induce reductive acetogenesis and recapture some of the energy lost during fermentation for productive purposes.

Biography:
I graduated from the University of Hohenheim as a Agricultural Scientist. I started my work working in vitro on the impact of secondary plant components on rumen plant fibre degradation in Hohenheim. In between I spend a year in the United States to learn culture independent microbial identification methods at Northwestern University. I did a post doc for three years on rumen biohydrogenation at the Rowett Research Institute and joined AgResearch as a scientist in 2008. Here I developed three automated in vitro systems for gas and methane measurements. My work focuses on hydrogen management in the rumen ranging from national enteric methane inventory and early life interventions to commercial product development both in vitro and in vivo.

Q18: Condensed tannins in grazed pastures: Mitigating greenhouse gas emissions and boosting animal health

Roldan, M.B.[1], Cousins, G.[2], Woodfield, D.R.,[2], Caradus, J.R.[3], Kaur, R.[2], Voisey, C.[1]

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Livestock farming contributes approximately 50% of NZs total greenhouse gases through methane and nitrous oxide emissions from ruminants. Consumption of plant-based condensed tannins (CTs) are proven to reduce methane, nitrous oxide emissions as well as urinary nitrogen, while simultaneously enhancing animal health and productivity. However, integrating CTs into animal nutrition within NZ's existing pastoral farming systems remains a key implementation challenge.
CTs are largely absent in the foliage of important temperate pasture species, although present in the flowers and seed coats of white clover. Previous attempts at enhancing CT expression in white clover leaves by mutagenesis and conventional breeding have not yielded successful results. In this research, white clover was genetically engineered to induce CT expression in leaves by inserting TaMYB14-1, an R2R3 MYB transcription factor from a related species, hare's foot clover. TaMYB14-1 upregulates the genes involved in CT synthesis in leaves. The stability of the CT trait has been confirmed over several generations with CTs primarily composed of prodelphinidin and procyanidin, having a mean degree of polymerisation greater than 10 units. Recurrent selection resulted in mean CT levels exceeding 1.5% dry matter (DM).
In vitro studies demonstrate that CTs effectively bind and release proteins at the physiological pH of the rumen and intestine of ruminants, respectively. White clover leaves containing CTs >2.0% DM significantly reduced methane emissions (by 19%) and ammonia production (by 60%) under laboratory conditions. Additionally, CTs significantly reduced frothy foam and biofilm formation ($P<0.01$), which are indicators of bloat incidence in animals. This development has the potential to enhance animal health in addition to reducing methane, ammonia, and nitrous oxide emissions. Further, this suggests that the commercial release of such a white clover cultivar could provide valuable contributions toward sustainable animal productivity with enhanced environmental benefits.

Biography:
Dr. Marissa Roldan is a senior scientist at AgResearch Ltd based at the Grasslands campus in Palmerston North, NZ. After completing her PhD at Massey University in 2007, she joined a research team at AgResearch that focuses on enhancing forage traits using various molecular, physiological, and biochemical techniques. Being a molecular biologist, she is passionate about improving the environmental and animal health benefits of forages using molecular tools. Since 2015, she has been leading a cutting-edge research programme that uses genetic modification technology to develop forages that synthesizes condensed tannins (CTs) in leaves aiming to mitigate greenhouse gas emissions from livestock and improve animal nutrition and health.

In her presentation, she will highlight their research on enhancing CT expression in white clover leaves and the studies conducted so far to show the environmental and health benefits of condensed tannins.

Q19: Modulating hydrogen flow to reduce emissions and increase productivity of ruminants

Greening, C
Department of Microbiology, Biomedicine Discovery Institute, Monash University, Australia

Rumen microbiota enable ruminants to grow on carbohydrate-rich feed, but also produce methane, driving 5% of global greenhouse gas emissions and leading to a loss of gross energy content. The methanogenic archaea responsible for these emissions use molecular hydrogen (H_2), produced during bacterial and eukaryotic carbohydrate fermentation, as their primary energy source. Through integrating studies at the protein, cellular, and community levels, my group is resolving the microbes controlling the production and consumption of H_2 in ruminants. Here I will show that diverse microbes control the production and consumption of hydrogen in the rumen. Variations in these pathways explain differences in methane yield between breeds, diets, and gastrointestinal compartments. Moreover, following administration of methanogenesis inhibitors, shifts in the composition and gene expression of H_2-cycling microbes reduces emissions but limits productivity gains. We also provide evidence that alternative hydrogenotrophs, including acetogenic and respiratory bacteria, can prosper in the rumen and effectively compete with methanogens for H_2 especially following methanogenesis inhibition. These findings emphasise the importance of microbiota-wide analysis for optimising methane mitigation strategies and identify promising strategies to simultaneously reduce emissions while increasing animal production.

Q20: Using molecular phenotypes to meet global emissions targets in ruminant livestock.

Rowe, S.J.[1], Bilton, T.P.[1], Alemu, S [1]., Clarke, S.M [1] ., McRae, K.M.[1], Perry, B.[1], Jonker, A. [2], Janssen, P.H.[2], Henry H.M.[1], Hickey, S.M.[3], Johnson, P.L.[1] and McEwan, J.C.[1]

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Enteric microbial fermentation of feed in ruminant livestock accounts for more than 11% of all global anthropogenic greenhouse gas emissions. Genetic selection of low emitting livestock is the only mitigation strategy that has been implemented on a national scale. We have shown that individuals vary in the composition of the microbial communities that they harbour, and so the dominant fermentation pathways and the resultant amount of methane also vary. These pathways in turn, control energy sources for the animal and have a downstream effect on metabolic processes such as tissue deposition and milk composition. All of these processes offer candidates for molecular phenotypes that have potential as predictors of methane emissions and other feed related traits. We describe a program of work to determine the impact of genetic selection for methane emissions, correlated changes in downstream metabolites, and discuss the utility of a range of biological and spectral predictors of methane emissions in livestock breeding schemes for the reduction of environmental impact. Results show that breeding is an effective long-term strategy to mitigate agricultural greenhouse gases and that substantial progress in the reduction of enteric methane can be achieved whilst simultaneously improving the efficiency and productivity of ruminant livestock.

Q21: Breeding as a tool for reduced methane emissions in dairy cattle

Lorna McNaughton[1], Rhiannon Handcock[2], Olivia Spaans[1], Peter Van Elzakker[3] and Richard Spelman[1]

[1]LIC, Research and Development, Hamilton, NZ, [2] DairyNZ, Hamilton, NZ, [3]CRV, Hamilton, NZ.

Methane emissions are a loss of energy to the animal and contribute to climate change, with 89% of New Zealand's methane emission coming from agriculture, mostly from enteric fermentation[1]. Breeding for animals that emit less methane can reduce the impact of cattle production on the environment[2]. Feed intake impacts methane production, making it important to measure intake alongside methane emissions. Unlike most economically important dairy traits that can only be measured on lactating females, methane production and feed intake can also be measured on sires and are more easily measured in young animals. Since 2021 genotyped bulls (6-15 months of age) entering the progeny test schemes of LIC and CRV are housed for 35-40 days while their methane emissions and feed intakes are measured. Rumen fluid samples are collected for microbiome analysis, as part of a project to develop proxies for measuring methane production. Genetic analysis of 834 animals resulted in a heritability for methane production, adjusted for feed intake, of 0.12; confirming there is genetic variation that is independent of feed intake. A large dairy herd was inseminated with semen from sires with divergent methane breeding values (25 high-methane and 25 low-methane) to validate the use of methane measurements in bulls as a phenotype for breeding lactating cattle with reduced methane production. The resulting 398 daughters were born in 2023. An extensive phenotyping programme of the daughters will see their methane and feed intake measured as young animals (9 – 15 months of age) and as first lactation heifers alongside measurement of traits like milk production and fertility.

1. Ministry for the Environment. (2022). *New Zealand's greenhouse gas inventory 1990 - 2020*.
2. de Haas, Y., Veerkamp, R.F., de Jong, G. and M.N. Aldridge. 2021. Selective breeding as a mitigation tool for methane emissions from dairy cattle. Animal. 15(1). 100294.

Biography:

Lorna is a senior scientist within LICs Research and Development group where she leads a small team focused on field trials to collect data for genetic evaluation. Her first encounter with methane research was way back in 2004, in a project recording methane emission in 600 lactating dairy cattle as part of a broader QTL discovery project. In the intervening years she has worked across projects ranging from female reproduction and heifer liveweight targets to breeding for facial eczema tolerance. Since 2018 her focus has been setting up and running a trial to measure methane emissions and feed intake in dairy bulls and their daughters.

Q22: Fast-tracking climate change adaptation in farmed Chinook salmon

Symonds, J.E.[1], Walker, S.P.[1], Waddington, Z.[2], Mota-Velasco J.C.[3], Diaz Gil, C.[3], Looseley, M.[3], Dodds, K.G.[4], Clarke. S.M.[4]

[1]Aquaculture Group, Cawthron Institute, Nelson, NZ, [2]The New Zealand King Salmon Co. Ltd, Nelson, NZ, [3]Xelect Ltd., Horizon House, St Andrews, Fife, Scotland, UK, [4]AgResearch, Invermay Agricultural Centre, Mosgiel, NZ.

New Zealand is the largest producer of premium farmed Chinook salmon with plans to expand into higher energy open ocean sites. Industry priorities include improving resilience and summer survival as oceans warm. To achieve these goals, the industry needs to implement climate change adaptation plans, including future adaptive breeding strategies that look beyond business as usual.

Since 2016 the objective of our research has been to work closely with commercial breeding programmes to develop genetic evaluation pipelines for key priority traits using pedigree families from these programmes. Through a series of tank trials with tagged and genotyped families we have demonstrated the potential to select for traits such as growth, condition factor, feed intake, feed efficiency and improved spinal health. This included comparison of similar phenotypes assessed in the same families in tanks and sea pens. As part of the pathway towards producing climate adapted Chinook salmon, a tank based temperature challenge model has been established. To date this model has successfully tested over 230 pedigree Chinook salmon families. Heritabilities for time to death at elevated temperature were high (0.34 to 0.48) suggesting that selection for improved thermotolerance is possible.

The next stage is to develop an improved phenomics to genomics pipeline for New Zealand Chinook salmon to enable genomic selection and gene discovery for thermotolerance and other resilience traits. Building on the established genotyping-by-sequencing pipeline, a recently funded MBIE Endeavour programme “Fast-tacking climate change adaptation in finfish” will generate a functionally annotated reference genome for New Zealand Chinook salmon, alongside transcriptomics and methylomics resources. These multi-omics resources will be applied to analyse multiple resilience phenotypes generated through commercial on-farm studies and tank-based challenges for multiple environmental stressors. Ultimately salmon farmers will apply the genomic breeding values generated to produce climate adapted Chinook salmon.

Biography:

Jane Symonds is a Senior Scientist and Team Leader in the Aquaculture Group, Cawthron Institute, Nelson, New Zealand (NZ), and a Senior Adjunct Researcher at the University of Tasmania. Jane’s focus is the application of research to enhance sustainable commercial aquaculture production, including Chinook salmon farming and selective breeding. Jane has overseen a wide range of projects, including salmon genomics, behaviour, health, physiology, microbiomics, data science and selection of thermotolerant and feed efficient Chinook salmon.

Dr Symonds has over 30 years of experience in aquaculture research. In the 1990s she was the Broodstock Manager for the New Zealand King Salmon Co. Ltd and developed the first family-based salmon selective breeding programme in NZ. Since then, Dr Symonds has developed and led multiple, large scale aquaculture R&D programmes in NZ and Canada. In Canada, as Director of Aquaculture at the Huntsman Marine Science Centre, Jane helped establish a large-scale Genome Atlantic project that developed breeding and genomic resources for Atlantic cod aquaculture. Back in NZ, Jane led the breeding and genetic technologies research for marine finfish and abalone, at NIWA’s Bream Bay Aquaculture Park from 2007-2015.

Q23: The climate crisis, and its effect on perennial plants, requires the use of new breeding technologies

Allan, A.C.[1,2]
[1]Plant & Food Research, Mt Albert, Auckland, New Zealand
[2]School of Biological Sciences, University of Auckland, Auckland, New Zealand

Plants should be considered as a third of the solution to the climate crisis, as they fix CO2 and make all our food (directly or indirectly). Moving to a more plant-based economy requires both new crops and enhanced climate-resistance of existing crops.

New Zealand's horticultural sector is based on temperate perennials. Over the winter, dormant buds need low night temperatures (termed winter chill) in order to switch on flowering in spring. Therefore, warmer winters mean less flowers/fruit for key high value crops. New cultivars must be bred with lower winter chill requirements. However, can genetic gain be quick enough in crops which have long generation cycles (seed-plant-seed)? New Breeding Technologies (NBTs) use molecular methods that quickly provide step changes in traits. We are using NBTs to make woody perennials more floral, which can both speed up breeding and rapidly improve the plants response to climate change.

The question remains of how NZ will respond to such plants, which have no additional DNA and harbour only new variants of genes which are identical to "natural" variants already in the environment. In most countries (but not NZ) these resulting plants are not regulated. NZ must quickly decide if NBTs will play a part in our response to a changing climate.

Biography:
Andrew Allan studied biochemistry and cell biology at the University of Canterbury, then received his PhD in Biochemistry at Cambridge University. Post-Doc's in Edinburgh and Israel followed, in projects studying plant stress responses. In 1997 he began work at HortResearch (which became Plant and Food Research) studying fruit responses to stress, and joined the University of Auckland as a lecturer in 2003. He is the Programme Leader of a MBIE-Endeavour project which aims to understand the effect of the warming climate on flowering in perennial plants. He is a full Professor with the University of Auckland.

Q24: Human organ-on-a-chip: Advanced next-generation *in vitro* cell models

Powell, J.L. [1], Advanced Cell Systems team[1].

[1]Institute of Environmental Science and Research (ESR), NZ

Rapid advances in bioengineering and microfabrication are revolutionizing the field of *in vitro* human cell models. Over the past two decades, cell models have evolved from growing single human cancerous cell lines on flat plastic surfaces, to 3-dimensional (3D) multi-cellular organ-like structures using primary human cells. Advanced systems incorporate hardware for microfluidics and stretch to simulate vascular/lymphatic flow and breathing/peristalsis respectively. These new 3D microphysiological systems (MPS) are designed to mimic the complex physiological functions of human tissues and organs, including multi-organ interactions. MPS models have the potential to be used across research disciplines for improving our understanding of human cell and organ responses, particularly to external stimuli such as pathogenic microorganisms or toxic chemicals. Some of these new models, such as the liver organ-chip, are as good, or even better, than animals at predicting liver toxicity which has led to them being embraced by the pharmaceutical industry and are being considered by Regulator's as part of medicine authorisations. When combined with multi-omics technologies, these models become powerful tools for unlocking new knowledge and avenues for research in human health and disease. ESR is among a small number of groups in Aotearoa New Zealand developing and using MPS in research. We are using gut, liver, and lung-chips to investigate issues of public health importance in Aotearoa New Zealand communities including food safety, infectious diseases, toxicology, drug development, and pharmacology.

The impacts of embracing MPS technologies will be improved understanding of human cellular and molecular interactions to microorganisms and chemicals, increased safety of products, exploring individual cellular/organ responses with applications to precision health, and a further reduction in the use of animals in research.

Biography:

Dr Jan Powell is a Science Leader with ESR's Environment group. Jan's research focuses on understanding how hazards (microorganisms, chemicals, pharmaceuticals, particles etc) interact with human cells and the potential effects of these hazards on human health. She is leading ESR's Advanced Cell Systems (ACS) programme which uses state-of-the-art and innovative organ-on-a-chip technology to emulate human organ systems. The organ-on-a-chip technology has broad research applications across infectious diseases, pharmacology, toxicology and environmental health. Prior to joining ESR Jan's career spanned academia, the pharmaceutical industry, and public health. She was on faculty at the University of Maryland, Baltimore, USA for 10 years with teaching, administrative, and research responsibilities, followed by 7 years at Shire, an international pharmaceutical company, located near Boston MA. There she worked in Research & Development, holding senior roles in Discovery Research, Nonclinical Development, and Translational Research. Currently Jan is also a member of the management team of Upstream Medical Technologies, a NZ start-up. Jan earned her BSc, MSc and PhD in microbiology from the University of Otago and her MPH in epidemiology from the Johns Hopkins University Bloomberg School of Public Health in Baltimore. She is an inventor on three granted and one pending patent families.

Q25: Developing Capillary Microfluidics for Commercial Applications

Daniel Mak[1,2], Claude Meffan[3], Julian Menges[1,2], Azadeh Hashemi[1,2], Rhys Marchant-Ludlow[1], Volker Nock[1], Renwick Dobson[2]

[1]Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand, [2] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [3]+Department of Micro Engineering, Kyoto University, Address, Kyoto, Japan.

Channel-based capillary microfluidics, also known as capillarics, has a wide array of applications in point-of-care and other diagnostic settings and have the potential for significant impact through commercialisation [1,2]. The self-powered liquid movement of capillarics minimises end-user requirements and simplifies device operation, with most flow control encoded by channel geometry. Last year, we presented our capillary Field Effect Transistor (cFET), which provides flexible flow control in capillarics by restricting flow through channels via a feedback system [3,4,5]. Here, we will show a new advancement on the cFET, reversible operation, that uses capillaric circuitry to further automate flow switching and further expands the library of capillaric flow control. Additionally, we will detail some of the work we are doing to scale up our production in preparation for the application of capillaric devices in diagnostic settings. Our goal is to develop devices that can be applied in a variety of industries that are rapid, affordable, and easy-to-use. We have started this by investigating the use of capillary microfluidics in the wine industry and are actively searching for new areas where our device can make an impact.

[1] A. Olanrewaju et al., Lab Chip, 2018
[2] C.D. Chin, V. Linder, S.K. Sia, Lab Chip, 2012
[3] J. Menges et al., Lab Chip, 2021
[4] C. Meffan et al., Microsyst. Nanoeng., 2022
[5] D. Mak et al., Proc. Transducers, 2023

Biography:

Ren is a Principal Investigator of the Biomolecular Interaction Centre based at the University of Canterbury (New Zealand) and until recently served as its director. His research focuses on the molecular interactions critical to biological function and has made valuable contributions through publications that span biochemistry and aligned disciplines such as engineering. He works with a group of six staff and 19 students on four general themes:

- *How cells transport nutrients across lipid membranes*
- *Engineering new food systems*
- *Understanding and engineering enzymes*
- *Diagnostic assay and device development*
- *Transcriptional regulation*
- *Molecular interactions in plant/fungal infections (myrtle rust)*

This work is funded by the Marsden Fund, Tertiary Education Commission, Ministry of Business, Innovation and Employment, and industry. He worked at the University of Melbourne as a CR Roper Research Fellow prior to joining University of Canterbury in 2012.

Q26: Lab-on-a-Chip devices for biomechanical studies on living cells

Nock, V.[1], Onal, S.[1,2], Franke, C.[1,3], Alkaisi, M.M.[1]

[1]MacDiarmid Institute, Department of Electrical and Computer Engineering, University of Canterbury, Christchurch, NZ, [2]Istituto Italiano di Tecnologia, Napoli, 80125, Italy, [3]Robert Bosch GmbH, Renningen, Germany.

Biophysical cues and mechanical forces are known to direct cell behaviour and tissue development [1]. This is also the case for diseases such as cancer, for which evidence continues to emerge that not only genetic mutations, but also altered mechanobiological profiles of the cells and microenvironment play key roles [2]. Biomechanical forces regulate tumour microenvironment by solid stress, matrix mechanics, interstitial pressure and flow. Compressive stress by tumour growth and stromal tissue alters the cell deformation, and recapitulates the biophysical properties of cells to grow, differentiate, spread or invade.

Mechanical compression in particular has been gaining increasing interest due to a growing number of microdevice-based platforms providing force application at single cell resolution [3]. In this seminar I will discuss ongoing efforts to develop such lab-on-a-chip platforms, which in our case are comprised of an integrated, actively modulated actuator for the application of compressive forces on cancer cells [4]. I will demonstrate how the actuator can be used to perform sequential cyclic cell compression at physiological pressure levels, as well as end-point mechanical cell lysis [5]. Further, I will describe a unique non-contact SKOV-3 cancer cell alignment we were able to observe on the platform, and how microstructuring and protein-patterning can be used to study the response of ovarian cancer cells to confinement in polyacrylamide gels [6].

1. Ingber, D.E. (2005). Proc. Natl. Acad. Sci. USA 102:11571.
2. Evans, J.J., M.M. Alkaisi, P.H. Sykes (2019). Cell Biochem. Biophys. 77:293–308.
3. Onal, S., M.M. Alkaisi, and V. Nock (2022). iScience. 25:105518.
4. Onal, S., M.M. Alkaisi, and V. Nock (2021). Front. Phys. 9(280):654918.
5. Onal, S., M.M. Alkaisi and V. Nock (2023). PLOS ONE. 18(1):e0279896.
6. Franke, C., PhD Thesis in Electrical and Electronic Engineering. 2022, University of Canterbury: Christchurch.

Biography:

Volker Nock is a Professor and Rutherford Discovery Fellow in the Department of Electrical and Computer Engineering. He received the Dipl.-Ing. degree in Microsystem Technology from the Institute for Microsystem Technology (IMTEK) at the Albert-Ludwigs University of Freiburg, Germany, in 2005. In 2009 he received the Ph.D. degree in Electrical and Electronic Engineering from the University of Canterbury in 2009. Up to his appointment as a lecturer in Electrical and Computer Engineering in 2012, he was a MacDiarmid Institute and Marsden Research Fellow.

Prof. Nock is a Principal Investigator with the Biomolecular Interactions Centre and theme leader with the MacDiarmid Institute and UC Biosecurity Innovations. From 2017 to 2020, he co-directed of the Biomolecular Interaction Centre. In 2019 he was awarded a 5-year Rutherford Discovery Fellowship to work on the "Electrotaxis and protrusive force generation in fungal and oomycete pathogens – Pathways to new biocontrol strategies".

His research interests include micro- and nanofabrication, surface patterning, microfluidics and Lab-on-a-Chip devices.

Q27: Drug discovery and development in the post FDA modernisation act 2.0/3.0 era.

Alastair G Stewart
ARC Centre for Personalised Therapeutics Technologies, Department of Biochemistry and Pharmacology, University of Melbourne, Vic 3010, Australia

The use of microphysiological systems (MPS) in the drug regulatory pathway has been provided with legislative support in the USA, in the form of the FDA modernisation Act 2.0. The provision of a mandate to the FDA to consider non animal methods for qualification of drug efficacy, safety and toxicology, where scientifically appropriate, marks a major change from established practice. Rare genetic diseases are a particular area of focus for these new MPS technologies. The increment in non-animal method usage for regulatory purposes is likely to be gradual, and to favour efficacy studies over those dealing with safety and toxicology. Efficacy studies are not subject to specific guidances and therefore present a lower barrier to adoption than safety/toxicology. Safety/toxicology models are said to be being used by major Pharma for internal risk management, rather than in regulatory approval submissions. The technologies that are deployed in the MPS field will be discussed, highlighting opportunities for further refinement and qualification of non-animal methods. The application of some of these technologies to the development of casein kinase 1 delta/epsilon inhibitors will also be reviewed.

Q28: Unveiling novel genetic pathways in non-model plants through advanced sequencing technologies

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Recent advances in sequencing technologies have enabled investigation whether canonical molecular pathways characterised from model organisms are evolutionarily conserved across non-model organisms. In my research career, I have used molecular tools and high-throughput sequencing to uncover how non-model organisms, including some endemic Aotearoa/New Zealand plant species, differ markedly in what were thought to be 'core' genetic pathways of plants, advancing ecological and evolutionary research in Aotearoa. In my doctoral research, I specialised in developing bioinformatic pipelines to analyse transcriptomic and physiological responses in masting plants and discovered evidence for the presence of a novel 'summer memory'. My combined study of ecological data modelling and associated molecular analysis showed the effect of temperature change on mast flowering phenology, which will improve masting predictions under the influence of global climate change. Further, my work on genomic analysis of perennial ryegrass cultivars discovered novel paralogs of vernalisation responsive gene(s) and genetic variation among them, information that can assist in developing a non-flowering variety. My current research focuses on the evolution of the flavonoid biosynthesis pathway and associated stress tolerance mechanisms in a major but little studied lineage of land plants – hornworts. Through phylogenetic, structural, and functional analyses, I determined a genetic loss of the entire toolkit required for flavonoid production in the hornwort lineage, making them the only group of land plants that do not produce flavonoids, even under stressful conditions. This study of hornwort molecular and functional biology provides data to address a substantial gap in understanding early land plant stress responses, thereby enhancing our understanding of hornwort ecology and their vulnerability to environmental change.

Q29: New Strategies to Modify Peptide Scaffolds and Selectively Target Pathogenic Bacteria

Cameron, A.J.[1], Na, T.U., Northrop, B.H., Brimble, M.A, Allison, J.R., Hardie Boys, M.T., Pletzer, D., Campbell, G., Ferguson, S.A., Cook, G.M., Sander, V., Davidson, A.J., Tsai, C.J., Proft, T.

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Peptides therapeutics are now at the cutting-edge of modern drug development. Supported by continued advancements in methodology, rapid growth of the field has now provided over 80 FDA approvals. Recent peptide blockbusters include liraglutide and dulaglutide which exemplify the possibilities for new life-changing treatments offered by peptides.

In an on-going effort to discover new methods to modify biomolecules, we have developed an efficient new chemical approach to site-selectively lipidate or modify peptides with a diversity of commercially available thiols. The peptide core can be produced synthetically or semi-synthetically through a chemoenzymatic approach. This talk discusses two applications of this approach to modify peptides towards improved strategies for treating bacterial diseases.

We recently implemented our lipidation approach to modify the polymyxin lipopeptide scaffold, a last-line of defence antibiotic that is severely nephrotoxic. Using our efficient late-stage approach to introduce thiols as new lipid moieties, we discovered a new analogue of this critical medicine which retained exceptional potency towards multi-drug resistant Gram-negative pathogens in planktonic and biofilm forms, but was *ca*. 10-fold less nephrotoxic in a kidney organoid model. The unique partnering of a chemo-enzymatic synthesis with a chemo-selective lipidation approach not only offers a rapid approach to drug discovery, but also a highly cost-effective route to prepare new drugs.

Recurring infection by Group A Streptococcus (GAS) can cause severe autoimmune complications such as acute rheumatic fever (ARF) and rheumatic heart disease (RHD), which are responsible for significant health inequities in New Zealand. We proposed that the persistent internalised (intracellular) bacteria could be eliminated by developing cell permeable peptide-based antibiotics. Applying our peptide modification platform we 'stapled' *ca.* thirty different antimicrobial peptide sequences. Monitoring efficacy *in vitro* with a bioluminescent strain, our efforts afforded numerous cell-permeable stapled-peptides that could eradicate intracellular GAS. This approach offers potential for a novel treatment modality.

Summary of Abstracts for the Poster Session - QMB

No.	Title	Presenter	Institutions
Q30	Attention-based Graph Neural Network Models for Predicting Cardiotoxicity of Molecules	Nguyen, B.P.	School of Mathematics and Statistics, Victoria University of Wellington, Wellington, NZ.
Q31	Decoding the Unknown: Unveiling the Role of an Effector Protein During Myrtle Rust Infection by Austropuccinia psidii	Sullivan, J.V.	Biomolecular Interaction Centre, School of Biological Sciences, University of Canterbury, Christchurch, NZ,
Q32	High-throughput Optimization of Protein Expression and UPR-mediated Cell Stress Monitoring in Pichia pastoris via Biosensors	Martinez, J.P.O.	CSIRO, Advanced Engineering Biology FSP, QLD, Australia
Q33	Optimization of propidium monoazide treatment to quantify viable bacteria in effluent samples via next-generation sequencing	Gatenby, S.	Livestock Improvement Co-operation
Q34	Gene expression changes across the brain-pituitary-gonad-axis of tāmure/snapper (Chrysophrys auratus) during gametogenesis	Modern, S.J.	School of Biological Sciences, University of Auckland, Auckland, NZ; Plant and Food Research, Nelson, NZ, 3Institute of Marine Science, Leigh Marine Laboratory, University of Auckland, Leigh, NZ
Q35	Development of Norovirus targeting aptamers for electrochemical biosensing	Rachmadi, A. T.	Institute of Environmental Science and Research Ltd (ESR), Kenepuru Science Centre, PO Box 50348, Porirua, 5240, New Zealand
Q36	A rapid, highly sensitive and specific detection platform for Clostridium perfringens based on the RPA-CRISPR/Cas12a system	Ma, C.	Tāwharau Ora | School of Veterinary Science, Massey University, Palmerston North, NZ; Food System Integrity team, AgResearch Ltd, Hopkirk Research Institute, Massey University, Palmerston North, NZ,
Q37	Whole genomic sequencing reveals	Whitelaw, B.	Department of

	the complex invasion history of Rattus rattus in New Zealand, complicating our understanding of recent movement dynamics.		Anatomy, University of Otago, Dunedin, 9054, New Zealand
Q38	Functional amyloid formation of tumour suppressor p16 protein during S-phase of the cell cycle	O'Connor, K.M.	Mātai Hāora – Centre for Redox Biology and Medicine, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ
Q39	Impacts of established sterilisation and novel crosslinking techniques on marine fish skin collagen extracts for biomedical applications	Leonard, A.R.	1The New Zealand Institute for Plant and Food Research Limited, Nelson Research Centre, Nelson, New Zealand. Department of Oral Rehabilitation, Centre for Bioengineering & Nanomedicine, University of Otago, Dunedin, New Zealand
Q40	ChuA from pathogenic Escherichia coli selectively acquires heme from host haemoglobin	Daniel Fox	1Department of Biochemistry and Pharmacology, Bio21 Institute, The University of Melbourne, Parkville, Victoria, Australia 2Infection and Immunity Program, Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia
Q41	Determining How Lectin-Like Antibiotics Kill Pseudomonas Using Proteomics and Phenotypic Analysis	Samuels, I.G.	Department of Biochemistry & Pharmacology, The University of Melbourne, Parkville, 3010, Australia.

Q30: Attention-based Graph Neural Network Models for Predicting Cardiotoxicity of Molecules

Nguyen, B.P.[1]

[1]School of Mathematics and Statistics, Victoria University of Wellington, Wellington, NZ.

Concerns about toxicity frequently impede drug discovery efforts. Many promising molecules fail to advance to the later stages of drug development due to stringent toxicity assessments. This challenge significantly raises the cost, time, and effort required to discover new therapeutic molecules. Furthermore, negative side effects have led to the withdrawal or re-evaluation of many drugs already on the market. Among the various types of toxicity, drug-induced heart damage is a severe side effect that is frequently associated with several medications, particularly those used in cancer treatments. Despite the proposal of several computational approaches to determine the cardiotoxicity of molecules, their performance and interpretability remain limited. In our research, we proposed a more effective computational framework for predicting the cardiotoxicity of molecules using an attention-based graph neural network. Experiments showed that the proposed framework outperformed the other methods. To help researchers evaluate the cardiotoxicity of molecules, we also created an easy-to-use online web server that includes our model.

Biography:

Dr. Binh Nguyen is a Senior Lecturer in Data Science at Victoria University of Wellington (VUW). In the past, he has won several awards for his work on the development of innovative medical devices and the application of data science to improve healthcare. Since joining VUW, he has been focusing on developing novel methods based on machine learning to solve emerging problems in health informatics, bioinformatics, and drug discovery. He has been funded with a total of more than NZ$1.3 million for doing research in AI-aided drug discovery.

Q31: Decoding the Unknown: Unveiling the Role of an Effector Protein During Myrtle Rust Infection by *Austropuccinia psidii*

Sullivan, J.V.[1], Currie, M.J.[1], Eccersall, S.E.[1], Hambrook, N.M.[1], Frampton, R.A.[2], Sethi, A.[3], Panjikar, S.[3], Morris, V.K.[1], Smith, G.R.[2], Meisrimler, C.N.[1], Dobson, R.C.J.[1]

[1]Biomolecular Interaction Centre, School of Biological Sciences, University of Canterbury, Christchurch, NZ, [2]The New Zealand Institute of Plant and Food Research Ltd., Lincoln, NZ, [3]The Australian Nuclear Science and Technology Organisation, The Australian Synchrotron, Clayton, Victoria, AU.

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.

Biography:

Jovarn is a PhD. student at the University of Canterbury, working under Professor Renwick Dobson and Dr. Claudia Meisrimler. His research is on characterising effector proteins identified from Austropuccinia psidii during myrtle rust infection. Using many biophysical techniques including, analytical ultracentrifugation, small-angle x-ray scattering, and nuclear magnetic resonance, as well as functional techniques including yeast-two-hybrid.

Q32: High-throughput Optimization of Protein Expression and UPR-mediated Cell Stress Monitoring in Pichia pastoris via Biosensors

Martinez, J.P.O.[1], Vanhercke, T.[2], Speight, R.[1], Scott, C.[3]

[1]CSIRO, Advanced Engineering Biology FSP, QLD, Australia [2] CSIRO, Advanced Engineering Biology FSP, ACT, Australia [3] CSIRO, Agriculture and Food, ACT, Australia

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 utilizing 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.

Q33: Optimization of propidium monoazide treatment to quantify viable bacteria in effluent samples via next-generation sequencing

Gatenby, S.[1], Neely, C.[1], Couldrey, C.[1]

[1]Livestock Improvement Co-operation

One of the challenges in understanding microbiomes and metagenomes using DNA sequencing, is the inability to distinguish between DNA extracted from viable and dead bacteria. Propidium monoazide (PMA) is a cell membrane-impermeable, photo-reactive dye that offers a promising solution. PMA functions by binding the DNA of compromised (dead) cells with a high affinity upon photoactivation, leaving the bound DNA incapable of being amplified by PCR. As PCR is common step in the preparation of DNA sequencing libraries, PMA may also be used to prevent the sequencing of DNA from dead cells. The aim of this study is to optimise two methodological factors (photoactivation time and concentration of PMA) to quantify viable cells in dairy farm effluent.

Effluent samples were inoculated with either live or boiled (dead) *S. aureus* in the log phase of growth. A variety of parameter combinations were tested in triplicate: PMA concentration (10 µM, 20 µM, and 55 µM), and photoactivation time (25 min, 45 min, and 60 min). DNA was extracted from all samples and the amount of *S. aureus* was measured using commercial qPCR testing. Samples from the most optimal parameters were sequenced to examine a wider range of species in the effluent.

Results from the qPCR showed 60 min photoactivation with 55 µM PMA gave the greatest reduction of non-viable *S. aureus* cell amplification. However, 20 µM PMA with 60 min photoactivation gave a comparable reduction, with reduced impact on live cells. The sequencing results were in alignment with the qPCR, with 20 µM PMA and 60 min photoactivation resulting in no sequence reads of non-viable *S. aureus*, and the lowest impact on live cells. These results indicate the potential of PMA to discriminately sequence DNA extracted from live cells.

Biography:
I am a research associate from Livestock Improvement Co., early in my science career, having just finished my MSc(research). Our main focus is sequencing the microbiome of milk, and my area of expertise is optimising the extraction of DNA/RNA for sequencing.

Q34: Gene expression changes across the brain-pituitary-gonad-axis of tāmure/snapper *(Chrysophrys auratus)* during gametogenesis

Modern, S.J.[1,2], Wylie, M.J.[2], Radford, C.[3], Wellenreuther, M.[1,2]

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Teleost fishes exhibit diverse reproductive strategies which often vary significantly even between closely related species. Most reproductive processes involving gametogenesis occur in the gonad; however, reproduction is controlled via an endocrine network called the brain-pituitary-gonad (BPG) axis. Few studies to date have investigated the entire BPG-axis using omics techniques, especially in teleosts.

We investigated the hypothesis that genes expressed in various tissues exhibit distinct, tissue-specific signatures that are associated with different sexes and stages of gametogenesis. We sampled captive tāmure/snapper (*Chrysophrys auratus*) (n=105) throughout their first (maiden) reproductive cycle. RNA-Seq was performed on brain, pituitary, and gonad samples. Histology was used to stage samples according to gametogenic progression and to investigate temporal transcriptomic changes. Five stages for females and four for males were defined based on gonadal morphology and the literature. Additionally, plasma hormone levels implicated in BPG-axis signalling are currently being analysed by ELISA.

In total, 869 Gb of raw reads were obtained across all samples and tissues. After pre-processing, 3.7 billion of the raw reads were aligned to the snapper genome and gene-level expression was quantified. Principal component and differential gene expression analysis revealed significant differences between sexes and gametogenic stages. Significantly more differentially expressed genes (DEGs) were found in the gonads than in other tissues. For example, over 11,000 DEGs were discovered between male and female gonads, while only 73 were found between male and female pituitaries.

Investigations of candidate genes involved in sex determination and reproductive maturation in other teleosts revealed important similarities and differences with our data and the literature on other teleost species. These findings offer new insights into the roles of key hormones and genes in teleost reproduction. Furthermore, our transcriptomic analysis provides novel insights into the biological pathways and gene regulatory mechanisms involved in producing mature gametes in snapper.

Biography:

My current PhD project focuses on the molecular and endocrine mechanisms which direct the reproductive cycle in the teleost fish Tāmure/Australasian snapper. This project captured my attention as it promised to combine the areas of work I had pursued in my career since graduating – namely molecular and data-driven biology with the areas of work which I focused on during my undergraduate/master's degree, marine reproductive biology. I enjoy using my skills in data analysis to investigate the reproductive biology of snapper. Fish have very varied and often quite unique reproductive biology. Snapper undergo juvenile sex inversion, with some turning from females to males in adolescence. I find using molecular techniques to investigate the biology behind reproduction fascinating.

Life has taken me in unusual directions before. I graduated just before the pandemic and took the unexpected opportunity to start working in molecular laboratories working with COVID. During the pandemic my work ranged from routine qPCR diagnostic testing to data science/software engineering where I was helping to track variants and the nation-wide patterns in COVID-19. This work was very different to my previous studies; however, it provided me with the skills and experience to begin my current work studying snapper.

Q35: Development of Norovirus targeting aptamers for electrochemical biosensing

Rachmadi, A. T.[1], Dunham, H.[1], Raymond, O.[1], Hewitt, J.[1]

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Noroviruses are the leading cause of non-bacterial acute gastroenteritis worldwide. Noroviruses are highly stable in the environment which can cause widespread outbreaks through contaminated water, food, and person-to-person transmission infecting all age groups[1]. While most reported outbreaks of norovirus occur in enclosed settings, the impact of norovirus contamination of the environment and food has significant impact on human health and has financial consequences in markets globally. Due to this there is a need to quantitatively detect norovirus rapidly and accurately to prevent infections. The current standard qPCR method for norovirus detection requires a specialised lab, equipment and expertise. Nucleic acid aptamers that target the virus capsid have been proposed as an alternative detection method. Aptamers are short sequences of single stranded (ss)DNA or RNA that act as chemical receptors for specific target molecules such as viruses and can be integrated into diagnostic technologies to create biosensors for in-field testing needs. Norovirus aptamers that have been reported in the literature[1] are often generated to individual capsid proteins or virus like particles (VLPs) which do not perfectly mimic the live virus. We herein present validation of the binding interaction of existing norovirus aptamers for binding to live norovirus. These were the SMV-25 (truncated), the M6-2 and the APTL1 aptamers. The M6-2 and APTL-1 aptamers exhibited good binding to murine norovirus (norovirus GV), with the APTL-1 aptamer also exhibiting low to moderate binding of norovirus GII. We also present preliminary *in vitro* selection of new aptamers for norovirus GI and GII. Decreasing PCR cycles were required for amplification across each successive *in vitro* selection round indicating evolution of the oligonucleotide library towards norovirus binding sequences.

1. Schilling-Loeffler, K., Rodriguez, R., Williams-Woods, J. (2021) Target Affinity and Structural Analysis for a Selection of Norovirus Aptamers. *Int. J. Mol. Sci. 22*, 8868. https://doi.org/10.3390/ijms22168868

Q36: A rapid, highly sensitive and specific detection platform for *Clostridium perfringens* based on the RPA-CRISPR/Cas12a system

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[1]Tāwharau Ora | School of Veterinary Science, Massey University, Palmerston North, NZ, [2]Food System Integrity team, AgResearch Ltd, Hopkirk Research Institute, Massey University, Palmerston North, NZ, [3]Food Science and Engineering, College of Science and Engineering, Jinan University, Guangzhou, China, [4]Animal Health and Solutions team, AgResearch Ltd, Hopkirk Research Institute, Massey University, Palmerston North, NZ.

Clostridium perfringens (*C. perfringens*) is an important foodborne pathogen that can cause a range of enteric diseases in both humans and animals. *C. perfringens* can be isolated using culture methods and confirmed using biochemical analysis and nucleic acid-based detection methods. However, these methods are time-consuming, complicated, and have low sensitivity. Hence the combination of recombinase polymerase amplification (RPA), and CRISPR/Cas12a system was utilised in this study to develop a rapid, sensitive, and specific platform to detect *C. perfringens*.

The RPA method was developed and optimised to target a conserved gene in *C. perfringens*. RPA products were subsequently used to develop the CRISPR/Cas12a based assay. Three different CRISPR RNAs (crRNA) targeting the RPA-amplified region were screened. The selected crRNA was then used to optimise other parameters of the RPA-CRISPR/Cas12a assay. The sensitivity of the RPA-CRISPR/Cas12a assay was examined and compared to the RPA method alone. The specificity of the developed assay was evaluated using the DNA isolated from different toxin type strains of *C. perfringens* and 52 pathogens that could contaminate different food sources.

Both RPA and RPA-CRISPR/Cas12a were conducted at 38°C. The limit of detection of the optimised RPA-CRISPR/Cas12a method was 1 fg/µL (equivalent to 0.3 genome copies) of *C. perfringens* genomic DNA, which was significantly higher than the RPA alone, and this process took only 1 h. DNA from different toxin type strains of *C. perfringens* showed positive signal in the RPA-CRISPR/Cas12a platform, but DNA from other pathogens did not produce any signal, indicating that the newly developed platform is highly specific for *C. perfringens*.

These results indicate that the developed RPA-CRISPR/Cas12a assay can detect *C. perfringens* rapidly, with high sensitivity and specificity. This platform shows promise for rapid and reliable identification of *C. perfringens* in the food industry.

Biography:

PhD student based at Hopkirk Research Institute and Massey University in Palmerston North. My research focuses on developing advanced diagnostic method for detecting foodborne pathogens.

Q37: Whole genomic sequencing reveals the complex invasion history of *Rattus rattus* in New Zealand, complicating our understanding of recent movement dynamics.

Whitelaw, B.L.[1], Wellenreuther, M.[2,3], Russell, J.C[3], Collins, C.J.[1]
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European ships introduced ship rats (*Rattus rattus*) to Aotearoa New Zealand in the 1800s, causing severe harm to native vertebrate and invertebrate populations. These invasive mammals pose a significant threat to indigenous bird species, which are treasured as taonga by NZ Māori. Current rodent elimination methods, primarily using poison and traps, face challenges in scaling up on the mainland due to refuge populations that enable survivors to re-establish. Gene-drive technology offers a promising solution by introducing infertility genes to spread throughout target populations. However, the successful application requires a deep understanding of population dynamics, which is crucial for informing modelling efforts. To achieve this, we conducted whole genomic resequencing of 147 *R. rattus* samples collected across New Zealand, resulting in 68,000 SNPs. Our analysis revealed no clear genetic structure between the north and south islands, with all samples falling into the same genetic cluster. Increased genetic diversity around major ports in Auckland and Northland suggests multiple introduction events, aligning with previous predictions. Studying genetic signals in invasive species like the ship rat involves challenges such as deciphering historical events such as multiple introductions, selective sweeps, and bottlenecks against current population dynamics. Our study employs a comprehensive genomic approach to explore both historical genetic diversity and localised movement patterns.

Biography:

Brooke embarked on their academic journey with a bachelor's degree in Wildlife Conservation Biology, initially drawn to the field through a fascination with venom evolution in octopods. Their early research using proteomics to study octopus venom laid the foundation for their doctoral studies. During their PhD, Brooke specialised in annotating the genome of the southern blue-ringed octopus, employing advanced comparative genomics techniques to illuminate its genetic makeup relative to other octopods. Currently, as a postdoctoral fellow at the University of Otago and part of a collaborative effort led by Genomics Aotearoa, she has returned to their conservation roots. They are leveraging their expertise in genetics and bioinformatics to conduct whole-genome resequencing of approximately 200 Rattus genomes. This research aims to unravel Norway and ship rats' genetic structure and migration patterns across Aotearoa. By deciphering the genetic diversity within these rat populations, the team seeks to provide crucial insights that can inform the development of effective management strategies for invasive species in New Zealand.

Q38: Functional amyloid formation of tumour suppressor p16 protein during S-phase of the cell cycle

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

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p16 is one member of the INK4 tumour suppressor family, so-called for their function as inhibitors of cyclin-dependent kinase (CDK) 4. p16 binds CDK4 (and 6) and together with cyclin D1 acts to prevent phosphorylation of retinoblastoma protein to keep the cell in a quiescent state. Mutation of p16 protein or altered expression occurs in ~50% cancers.

A recently discovered loss of function of p16 has been described in which oxidation of a critical cysteine residue results in homodimerization and altered interaction with CDK4/6[1]. Furthermore, p16 homodimers can aggregate into functionally inert amyloids. This is the first known example of oxidatively-driven amyloid formation. Other amyloids are often disease-associated, forming spontaneously and irreversibly.

Here, we present evidence for the formation of p16 amyloids during early S-phase in synchronised cell lines. Amyloid p16 is detected distinctly from monomeric p16 via filter trapping of fractionated cell lysates on nitrocellulose membrane followed by antibody-mediated detection. Amyloid p16 levels are low in populations of thymidine-blocked cells at the G1/S checkpoint, but accumulate within 30 minutes of entering the cell cycle before diminishing as cells progress through S-phase. Furthermore, p16 amyloid detection peaks concomitantly with phosphorylation of retinoblastoma protein, suggesting release of CDK4-inhibition via the structural rearrangement of monomeric p16. We have also observed re-localization of ectopically expressed GFP-tagged p16 within minutes of exposure of cells to low levels of hydrogen peroxide.

Together, our data suggests a potentially functional response of p16 to oxidation that might be exploited by various types of cancers to dysregulate the p16-CDK4/6-Rb axis in favour of cell proliferation.

1. Heath, S.G., Gray, S.G., Hamzah, E.M., O'Connor, K.M., Bozonet, S.M., Botha, A.D., . . . Gobl, C., Amyloid formation and depolymerization of tumor suppressor p16(INK4a) are regulated by a thiol-dependent redox mechanism. Nat Commun, 2024. 15(1): p. 5535.

Biography:

Dr Karina O'Connor is investigating a novel amyloid formation mechanism ignited by oxidation.

Q39: Impacts of established sterilisation and novel crosslinking techniques on marine fish skin collagen extracts for biomedical applications

Leonard, A.R.[1,3] M.H. Cumming[1], J.D. Cabral[2,3] M.A. Ali[3]

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The International Organisation for Standardisation's (ISO) approved medical sterilisation techniques used to treat mammalian collagens are known to modify collagen structure, altering its biophysical properties and affecting subsequent cellular responses. However, the effects of these techniques on marine-sourced collagens are largely unknown. To address this, we assessed the sterilisation efficacy of gamma irradiation, ethanol, ultraviolet light, and supercritical CO_2, on both native and denatured hoki fish-skin collagen. Biomedical use of fish collagen requires modification to stabilise the collagen structure and ensure the necessary mechanical integrity for *in vitro/in vivo* applications. Therefore, a novel crosslinking technique was also tested to identify whether the carbohydrate species on the backbone of collagen molecules could be used as a potential crosslinking site.

Three bacterial inoculate species were used to test sterility assurance levels of 10^{-6} for each treatment, the minimum ISO requirement for medical-grade sterility. Galactose and glucosylgalactose sugars on the collagen backbone were oxidised using sodium periodate, triggering Schiff-base formations with free amines on lysine residues to form stable imine crosslinks. We then analysed all sterilised and modified collagens using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), circular dichroism, Fourier-Transform Infrared Spectroscopy (FTIR), solubility, and rheology to determine the effect of the treatments on structural and physicochemical properties of the collagen. Each treated collagen was then also tested for cytotoxicity using the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

By comparing the characteristics of each treated collagen extract, we identified the most appropriate sterilisation technique for collagen biomaterials used in soft tissue engineering applications and assessed the success of the novel crosslinking technique. The advantages and disadvantages of each sterilisation technique on these marine collagens will be discussed, along with the suitability of the novel carbohydrate crosslinking technique and its potential applications for biomedical applications.

Biography:

Alexandria Leonard is a PhD candidate at the University of Otago in the Department of Oral Rehabilitation and the Marine Products group at the New Zealand Institute of Plant and Food Research. Research interests include marine biomaterials and biofabrication, biochemical conjugations, polymer chemistry, rheology, and fluid dynamics.

Q40: ChuA from pathogenic *Escherichia coli* selectively acquires heme from host haemoglobin

Daniel Fox[1,2], Rhys Grinter[1,2]

[1]*Department of Biochemistry and Pharmacology, Bio21 Institute, The University of Melbourne, Parkville, Victoria, Australia*

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Iron is an essential nutrient for most bacteria. However, iron limitation is a major barrier for pathogenic microbes due to host nutritional immunity, where iron is sequestered by human iron-containing proteins such as transferrin or in heme bound by proteins such as haemoglobin. This sequestration makes iron inaccessible to bacteria, and to infect the host, pathogenic bacteria must steal it using TonB-dependent transporters (TBDTs) present in the Gram-negative bacterial outer membrane. The TBDT ChuA from pathogenic *Escherichia coli* binds host haemoglobin in order to scavenge heme. In this study, we solved the crystal structure of ChuA in complex with heme extracted from human haemoglobin to a resolution of 3.1Å, identifying residues required for coordination of heme. In addition, we modelled the
ChuA-haemoglobin complex using AlphaFold and identified a hydrophobic haemoglobin binding region in the extracellular binding loops of ChuA, which is contiguous with the heme binding region. Based on these data, we have developed a putative mechanism defining initial ChuA-haemoglobin interaction and subsequent heme extraction. To test this model, we generated a panel of ChuA mutants in key residues from this region and validated their importance for binding haemoglobin and heme extraction using growth assays, and further purified them and characterised their ability to bind haemoglobin. Finally, using RFDiffusion, we directed the design of 10,000 de novo proteins to bind at the ChuA:haemoglobin binding interface, and selected and tested 96 of the best candidates for their ability to bind ChuA and block growth on haemoglobin, highlighting their potential as therapeutic targets.

Q41: Determining How Lectin-Like Antibiotics Kill *Pseudomonas* Using Proteomics and Phenotypic Analysis

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The emergence and global spread of antimicrobial resistance (AMR) is a significant threat to human, plant, and animal health. Central to the AMR problem are Gram-negative bacteria, which exhibit intrinsic resistance to many antibiotics due to their impermeable outer membrane (OM). A promising new avenue for antibiotic development targets the β-barrel assembly machinery (BAM), a conserved and essential protein complex present in all Gramnegative bacteria. BAM facilitates the insertion of β-barrel outer membrane proteins (βOMPs) into the OM. These β-OMPs are crucial for functions like nutrient uptake, signalling, adhesion, and acting as virulence factors in pathogenesis. The surface-exposed nature of BamA makes it an attractive target for developing novel antibiotics, specifically lectin-like bacteriocins (Llps). Llps are bactericidal proteins naturally secreted by Gram-negative bacteria to eliminate competitors, and they are hypothesised to disrupt OM protein assembly by specifically binding to and disrupting the BAM complex. Utilising *Pseudomonas spp.,* a ubiquitous and opportunistic pathogen, as a model organism, we aim to uncover the molecular mechanism of Llp killing. This will be achieved through a combination of phenotypic and proteomic analyses to determine the molecular effects of Llp treatment on cellular homeostasis. This project will provide comprehensive insights into the cytotoxicity of Llps produced by *Pseudomonas spp*. In addition, it will generate knowledge that will allow us to exploit the natural defences of Gram-negative bacteria to develop new therapeutic strategies to combat multidrug resistant pathogens.