INFORMATION ON RESEARCH PROGRAMS FOR 2020 HONOURS AND POSTGRADUATE STUDENTS

BIOCHEMISTRY AND MOLECULAR BIOLOGY

monash.edu/medicine
# Table of Contents

1. **New Department Research Groups**
   - Croft, Dr Nathan 3
   - Davey, Dr Martin 4
   - Del Borgo, Dr Mark 5

2. **Cell Signalling & Cancer**
   - Bird, Prof Phil 6
   - Cole, A/Prof Tim 7
   - Mitchell, Prof Christina 8
   - Nguyen, Dr Lan 9
   - Papa, Dr Antonella 10
   - Rosenbluh, A/Prof Joseph 11
   - Tiganis, Prof Tony 12
   - Wagstaff, Dr Kylie 13

3. **Education Research**
   - Samarawickrema, Dr Nirma 14

4. **Infection & Immunity**
   - Berry, Dr Richard 15
   - Bird, Prof Phil 6
   - Borg, Dr Natalie 16
   - Caminschi, Dr Irene 17
   - Coulibaly, A/Prof Fasseli 18
   - Fletcher, Dr Anne 19
   - Gras, A/Prof Stephanie 20
   - Huntington, Prof Nicholas 21
   - Jacobson, Dr Kim 22
   - Jans, Prof David 23
   - Knoblich, Dr Konstantin 24
   - Kwok-Schuelein, Dr Terry 25
   - La Gruta, Prof Nicole 26
   - Lahoud, Dr Mireille 27
   - Le Nours, Dr Jerome 28
   - Mathias, Dr Rommel 29
   - Mifsud, Dr Nicole 30
   - Naderer, Dr Thomas 31
   - O’Keeffe, A/Prof Meredith 32
   - Purcell, Prof Tony 33
   - Reid, Dr Hugh 34
   - Rossjohn, Prof Jamie 35
   - Roujeinikova, A/Prof Anna 36
   - Stone, A/Prof Martin 37
   - Tiganis, Prof Tony 12
   - Traven, A/Prof Ana 38
   - Wagstaff, Dr Kylie 13
   - Vivian, Dr Julian 39
   - Zaph, Prof Colby 40
5. Genetics & Development
Beilharz, Dr Traude 41
Boag, Dr Peter 42
Cole, A/Prof Tim 7
Smyth, Prof Ian 43

6. Molecular Cell Biology
Jans, Prof David 23
Lazarou, Dr Michael 44
Ramm, Dr Georg 45
Ryan, Prof Mike 46
Schittenhelm, Dr Ralf 47

7. Diabetes & Obesity
Rose, Dr Adam 48
Ryan, Prof Mike 46
Tiganis, Prof Tony 12

8. Structural Biology
Aguilar, Prof Mibel 49
Borg, Dr Natalie 16
Buckle, A/Prof Ashley 50
Coulibaly, A/Prof Fasseli 18
Cryle, A/Prof Max 51
Davidovich, A/Prof Chen 52
De Marco, A/Prof Alex 53
Dunstone, A/Prof Michelle 54
Ellisdon, Dr Andrew 55
Elmlund, A/Prof Dominika 56
Elmlund, A/Prof Hans 56
Law, Dr Ruby 57
Rossjohn, Prof Jamie 35
Stone, A/Prof Martin 37
Whisstock, Prof James 58
Wilce, A/Prof Jackie 59
Wilce, Prof Matthew 59

Department of Biochemistry & Molecular Biology
http://med.monash.edu.au/biochem/
RESEARCH BACKGROUND

My research is focused on understanding how different aspects of the MHC antigen processing pathway impact upon T cell immunity to pathogens and tumors. I am particularly interested in how the abundance of MHC-bound peptides plays a role in driving the magnitude and efficacy of T cell responses. The Systems Immunology Group utilizes a combination of biochemistry, immunology, proteomics and bioinformatics to take a holistic view of immune processes, striving to develop novel bioinformatics and data repositories that will model and predict the generation of peptide epitopes and their immunogenicity.

The Systems Immunology Group is part of the larger Immunoproteomics Laboratory (led by Prof. Tony Purcell), specializing in using cutting-edge mass spectrometry instrumentation to study MHC-peptide repertoires (collectively termed ‘immunopeptidomes’). Our recent publications include characterization of peptide presentation driving T cell responses to vaccinia virus [1] and influenza virus [2], as well as refining our understanding of peptide splicing [3].

HONOURS PROJECTS

Honours projects are available in a variety of areas, including the following:

Project 1: The glyco-immunopeptidome – mining an untapped resource for immunotherapy

**Project description:** The presentation of post-translationally modified (PTM) peptides by cell surface MHC molecules increases the diversity of targets for recognition by T cells. Our understanding of the range and types of PTMs presented in this manner is largely restricted to small structural changes such as phosphorylation, deamidation and citrullination. Far less understood is the potential presentation of peptides modified by glycosylation [4], one of the most common PTMs occurring on proteins. Given that dysregulated or aberrant protein glycosylation is associated with many disease states including cancer, improving our knowledge of the glyco-immunopeptidome will greatly assist in the identification of novel T cell targets.

This project will encompass mining of existing MHC immunopeptidome datasets—as well as the generation of new datasets in the lab—to identify and characterize the frequency and diversity of glycopeptides presented by MHC class I and II molecules and how these may form novel targets for T cells. In concert, the study will be complemented with a detailed analysis of the whole cell glycoproteome in collaboration with the Analytical Glycoimmunology group headed by Dr Morten Thaysen Andersen, Macquarie University, Sydney. The prospective student will get the opportunity to spend time in Dr Thaysen Andersen’s laboratory and garner invaluable skills in glycomics and glycoproteomics.

References

RESEARCH BACKGROUND

The adaptive arm of immune system uses lymphocytes to generate antibody and memory responses to challenges throughout life. Three lineages of lymphocytes have co-evolved over the last 550 million years: B cells, αβ T cells and γδ T cells. Human γδ T cells remain poorly understood and their exact role in immunity is unclear. However, human γδ T cells are frequently implicated in protective microbial and tumour immunity. γδ T cells are distributed throughout the body and form an extensive immune surveillance network (Figure 1). Our group seeks to explore the role of this network in health and disease.

HONOURS PROJECTS

Project 1. γδ T cell memory responses in tuberculosis infection. Tuberculosis is caused by an infection with the bacterial pathogen Mycobacterium tuberculosis (Mtb). Despite significant efforts to control and eliminate Mtb, it remains a significant global health problem. 90% of acute Mtb infections result in a state of latent Mtb. The student will use 17-colour flow cytometry antibody panels to track the emergence of anti-Mtb γδ T cell responses in longitudinal blood samples from patients either acutely or latently infected with Mtb. The student will then sort γδ T cell subsets and perform cutting-edge γδTCR repertoire sequencing to understand the TCR response to this pathogen.

Project 2. Transcriptional control of γδ T cells in CMV infection. Cytomegalovirus (CMV) is a human herpesvirus that infects over 80% of the population and is a major cause of mortality in immunocompromised individuals. γδ T cells make a dramatic and sustained expansion towards acute CMV infection in transplant patients, the magnitude of which has been correlated with lower morbidity and transplant failure. The student will have access to longitudinal cohorts undergoing CMV activation in different transplant scenarios (lung, kidney and stem cell). The student will undertake state-of-the-art single cell RNA sequencing to map the transcriptional trajectories of CMV-reactive γδTCRs in stem cell transplant patients.

Project 3. Human intestinal γδ T cells in inflammation. Human γδ T cells are enriched at intestinal barrier sites, where microbial infection and chronic inflammation occur. The student will have access to pediatric intestinal biopsies on a weekly basis from Monash Children’s Hospital. The student will use state-of-the-art single cell TCR sequencing and RNAseq to investigate matched blood and tissue samples, allowing the identification of tissue resident γδ T cell populations. Understanding the properties of these tissue resident sentinels will allow the development of new γδ T-cell-based immunotherapeutics.
RESEARCH BACKGROUND
Over the past few years, our lab has developed a research program which has focussed on peptides comprised entirely of β3-amino acids that self-assemble to form fibrous structures. The novel self-assembly displayed by these molecules allows for a level of flexibility in the chemistry that is not typical for peptide material synthesis. We have used synthetic chemistry to introduce function to these fibres that has allowed us to use these materials in a broad range of applications including tissue engineering and drug delivery. More specifically, we have shown that these ‘smart’ materials can assemble themselves into functional hydrogels, that are injectable and are able to release or deliver stem cells, stem cell products and biomacromolecules in a controlled fashion. Through clever peptide design, we believe we can tailor these materials for truly personalised medicines.

HONOURS PROJECTS
1. Charged Hydrogels for the Controlled Release of Therapeutics
Macromolecular therapeutics like growth factors, cytokines and miRNAs are promising drug therapies with limited clinical translation due to difficulties with delivery. We have recently developed a number of bespoke amino acids for the purpose of creating materials that are either highly positively or negatively charged. This project will involve creating materials that are able to release macromolecules in a controlled fashion using these amino acids.

2. Adding Function to Materials Post-Assembly
Currently, peptide materials are often used to encapsulate and deliver stem cells for targeted delivery. To ensure cellular attachment and survival, the materials are often functionalised with peptide epitopes. Functionalisation with larger growth factors and cytokines would provide greater biocompatibility but remains a challenge for most 3D materials. We believe that our materials are ideally suited to post-assembly functionalisation chemistries and would represent a significant advance in the materials chemistry field.

3. Tailoring Hydrogel Porosity for Exosome Release
We have recently shown that different β-peptide monomers can co-assemble to provide fibres and gels with multiple functions. In this instance, the assembly of some β-peptides produce pores that are sub-micron whilst others assemble to produce large pores (front cover in Clinical Science).
The combination of these peptides has the potential to tailor the pore size for the encapsulation and delivery of a variety of stem cell products. This project will focus on the modulation of pore size for the targeted delivery of exosomes in the treatment of heart disease.
RESEARCH BACKGROUND

Serpins and cell death

Serpins are proteins that trap and inactivate proteases, and are present inside cells (intracellular) and in the circulation (extracellular). Some intracellular serpins protect cells against injury by their own proteases. Serpin deficiency or misfolding in humans results in blood clots, immune dysfunction, lung and liver disease, cancer, or dementia.

HONOURS PROJECTS

1. SerpinA1 is an extracellular protein produced in the liver and released into the circulation to protect the lung from neutrophil proteases. Mutation and misfolding of SerpinA1 results in the failure of hepatocytes to release it into the circulation, and the retained protein aggregates in the ER, leading to hepatocyte death, and resulting in liver and lung disease. To study this process, we have made transgenic zebrafish expressing wild type or mutant human SerpinA1 (Fig 1). Proteomic and transcriptomic analyses have identified metabolic pathways perturbed in fish expressing mutant SerpinA1. Current studies are aimed at manipulating key genes in these pathways to ameliorate serpin misfolding and increase its release, and identify targets for human therapeutic development. Zebrafish projects are co-supervised by A/Prof. R. Bryson-Richardson (School of Biological Sciences).

2. Serpinb6 is an intracellular serpin produced by leukocytes and epithelial cells. Surprisingly, mutation of Serpinb6 causes adult-onset hearing loss in humans. Using knockout mice we have shown that Serpinb6 deficiency causes inner ear degeneration, hair cell death, and hearing loss (Fig 2). We think that the hearing loss seen in Serpinb6-deficient individuals results from failure to protect cells of the inner ear from a protease released by noise trauma. We wish to identify the protease, its location, and produce antibodies that inhibit it.

In these studies we use advanced techniques in molecular cell biology. These include recombinant protein production and analysis, gene manipulation, RNA interference, proteomics, transcriptomics, bioinformatics, cell culture and confocal imaging, and the analysis of model organisms.
RESEARCH PROJECT BACKGROUND:
The endocrine system controls cell-cell communication and coordinates almost all our daily activities. Abnormalities in hormones, receptors and cell signalling pathways underpin many common diseases such as cancer, diabetes, high blood pressure and obesity. We are studying the actions of two important steroid hormones, cortisol (a glucocorticoid) and aldosterone (a mineralocorticoid) that are secreted by the adrenal gland and regulate important aspects of systemic physiology and homeostasis, in humans and other mammals. Cortisol has many homeostatic roles in a wide range of tissues both during embryogenesis, particularly the developing lung. Premature babies have underdeveloped lungs and require treatment with synthetic glucocorticoids. Glucocorticoids exert their effects by binding to the intracellular glucocorticoid and mineralocorticoid receptors, GR and MR respectively. Both are members of the nuclear receptor super-family of ligand-dependent nuclear transcriptional regulators. Research projects below will utilize a range of molecular, biochemical and genetic techniques in both cell-based and animal systems to investigate these cell signalling pathways and their specific roles.

2020 HONOURS/PhD PROJECTS:

1. Glucocorticoid-regulated pathways in the preterm lung and the development of Selective Glucocorticoid Receptor (GR) Modulators (SGRMs):
Lung dysfunction in adults and from premature birth is a major cause of morbidity and mortality. Systemic hormones such as retinoic acid, glucocorticoids play an important role in embryonic lung development. We have a number of mouse gene-knockouts that interrupt the cell signalling of these hormones. These include mouse knockout lines of GR, HSD1 and RARα genes. These mice develop perinatal lung dysfunction and will be used to investigate the specific molecular and cellular role each hormone/receptor pathway plays during fetal respiratory development. We are utilizing the Cre-recombinase/loxP gene recombination system in mice to produce cell-type-specific gene knockouts in the developing lung. This will identify specific endocrine actions of these pathways in mesenchymal, epithelial and endothelial cell compartments. Novel steroid-like compounds are being developed that have potent selective effects via the GR in specific tissues such as the liver, brain and respiratory system. These compounds bind to the GR and modulate interactions in the nucleus of cells to allow regulation of particular sets of down-stream target genes. This aspect of the project will test a range of new SGRM compounds in lung cell lines, lung explants cultures and in vivo with mice for potential clinical use.

2. The Short-Chain Dehydrogenase Reductase (SDR) Enzymes: Roles in metabolism and cancer:
This project will investigate SDR enzymes such as 11bHSD3/1L, a third member of the 11bHSD enzyme sub-family. This enzyme is absent in rodents and we will study its expression pattern in tissues, cellular localisation using specific antibodies and substrate specificity in samples from non-human primates, the sheep and in available human tissue samples and human cell lines.

3. Novel Roles of Mineralocorticoid Receptor (MR) Signalling in vivo:
The adrenal steroid aldosterone regulates systemic fluid and solute homeostasis in the kidney/distal colon via genomic actions in the nucleus via the activated MR. We have made novel tissue-specific mouse knockouts of the MR, and also both dimerization and LBD mouse mutants to explore novel genomic & non-genomic actions in non-epithelial cells/tissues such as macrophages, cardiomyocytes, vascular endothelial cells, the lung, and specialised neurones in the brain. (in collaboration with Prof Peter Fuller & Dr Morag Young at MIMR-PHI, Clayton).
RESEARCH BACKGROUND

Cells respond to changes in their microenvironment by activation of complex signalling cascades. The phosphoinositide 3-kinase (PI3K) signalling pathway is involved in a number of cellular processes such as cell growth, survival, migration and differentiation. PI3K is a proto-oncogene in up to 30% of all human cancers. Additionally, deregulation of the PI3K pathway occurs in many other human diseases including diabetes and muscular dystrophy, as well as in developmental disorders.

Our laboratory focuses on a family of PI3K regulatory enzymes that are critical regulators of the PI3K pathway and regulate many cellular functions. Mutation or altered expression of inositol polyphosphate phosphatases has been detected in human diseases such as Marinesco-Sjogren, Joubert and Lowe syndromes, breast cancer, insulin resistance, leukaemia and degenerative neuropathies.

HONOURS PROJECTS

Role of PI3K regulatory enzymes in development and cancer
Contact: Christina.Mitchell@monash.edu, Lisa.Ooms@monash.edu, Michele.Davies@monash.edu
Regulation of PI3K signalling is crucial for embryonic development and physiological homeostasis. PI3K signalling is deregulated in developmental disorders and in many human diseases, including cancer, diabetes and retinopathies. We have shown that loss or inactivation of PI3K regulatory enzymes leads to defects in embryonic development. We have also shown that loss of these enzymes affects tumour initiation, progression and metastasis in mouse cancer models. Recent clinical trials using PI3K inhibitors have indicated the potential of these drugs for targeted anti-cancer therapy, therefore identifying cancers with amplified PI3K signalling is of critical importance for optimising treatment strategies. These projects will utilise animal models, and genetic manipulation in cell culture, to investigate the role of PI3K regulatory enzymes in development and cancer. Overall these projects aim to identify and characterise novel therapeutic candidates for PI3K-driven disease and development disorders.

Skeletal muscle disease; identification of causes and novel therapies
Contact: Christina.Mitchell@monash.edu, Meagan.McGrath@monash.edu
Skeletal muscle homeostasis is essential for human health and mobility. The human muscular dystrophies and myopathies describe a broad range of debilitating human muscle diseases, with affected individuals often suffering significant muscle weakness, loss of mobility and in severe cases, early mortality. This project will utilise transgenic, knockin and knockout mouse models to identify and characterise the molecular mechanisms of human muscle disease, with particular emphasis on fundamental muscle processes including autophagy, regeneration/repair, metabolism and muscle stem cell function. This research aims to understand the causes of human muscle disease, to uncover potential treatment strategies for sufferers.
RESEARCH BACKGROUND
When the human genome sequence was published in 2003, it was called the “book of life”. But in reality the book of life has turned out a vocabulary of life, and ~20 years later we are still struggling to work out the grammar that makes the book comprehensible. Like in a language, knowing the words (i.e. the genes in this metaphor) is an absolute prerequisite yet it does not allow us to understand what is being said without knowing the grammar, which gives context and meaning to the words. Therefore, a grand challenge of the post-genomic era is to work out the grammar that structures and interprets the relationships between genes and their biological meaning in order to allow us to understand how the biological functions of a living cell are specified. This has instigated a new and exciting research paradigm called "systems biology", which aims to obtain a holistic, systems-level view of biological processes.

The primary interest of the Nguyen Lab is to employ systems biology approaches to address fundamental questions in cell signalling and in cancer, through an integration of computational modelling, bioinformatics and wet-lab experiments. Students will benefit from a highly interdisciplinary research environment and develop mixed skillsets. Depending on background and interest, students can select projects that are primarily lab based, or computational, or containing both aspects. Below are example projects but other projects are also available. If interested, candidates are strongly encouraged to contact Lan for further discussion.

HONOURS PROJECTS

Project 1. Overcoming adaptive resistance to anti-cancer drugs using a systems biology approach
Triple-negative breast cancer (TNBC) is highly aggressive but patients have no targeted treatment options beside toxic chemotherapies. Like ants who are very good at seeking alternative ways to travel when their path is blocked, TNBC cells manage to find escape paths to bypass drug treatment. Combining computer simulations and lab work, this project aims to predict these escape paths and identify ways to block them by combining multiple drugs, which will lead to new treatments for this disease. This project employs a range of state-of-the-art biochemical and cellular assays to investigate the question experimentally. The candidate students are not required to have computational background, and can primarily spend time in the wet-lab if preferred. However, they will have opportunities to work alongside a modeler in the lab and will thus uniquely benefit from a highly cross-disciplinary research environment and the practice of systems biology, a paradigm which is being increasingly embraced by industry and academia.

Project 2. Optimization of drug scheduling for cancer treatment
Frequent resistance to single-drug treatment has led cancer scientists to look for cocktails of drugs that can beat resistance to single drug alone. Though, this is often at the cost of increased toxicity. We found that the ways drugs are combined can have a huge effect on their ability to kill cancer cells. This project takes a deep dive into exploring various scheduling strategies for combined drugs, with the goal to maximise treatment benefit by: enhancing efficacy while reducing toxicity. This project employs a range of state-of-the-art biochemical and cellular assays to investigate the question experimentally. The candidate students are not required to have computational background, and can primarily spend time in the wet-lab if preferred. However, they will have opportunities to work alongside a modeler in the lab and will thus uniquely benefit from a highly cross-disciplinary research environment and the practice of systems biology, a paradigm which is being increasingly embraced by industry and academia.

Project 3. Modelling the PI3K-Akt-mTOR network to discover novel cancer therapies
The PI3K-Akt-mTOR signalling network plays a pivotal role in the regulation of cell growth and proliferation, and is highly complex with multiple feedback loops, pathway crosstalk, upstream regulators and downstream functions. Its frequent aberrations in cancers makes this network an important therapeutic target, and indeed many targeted drugs have been developed directed at its components. However, the clinical success in inhibiting PI3K, Akt, mTORC1 and/or mTORC2 has been disappointing due to the emergence of drug resistance. This project will employ an integrative systems approach to gain systems-level understanding of the network dynamics in cancer cells before and after drug treatment. This knowledge together with model simulations will help design new therapeutic strategies that exploit network vulnerabilities and are capable of overcoming resistance. Predictions will then be tested experimentally in the wet lab.
RESEARCH BACKGROUND
Cancer is a complex disease that evolves over time and becomes progressively more malignant by acquiring multiple genetic alterations. The PI3K-Akt-mTOR cascade is a key intracellular signalling pathway that mediates several biological processes including cell growth, proliferation and metabolism. PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a major tumour suppressor that inhibits PI3K pathway activation and is frequently mutated in a range of human cancer and cancer syndromes.

Through a combination of in vitro studies and in vivo analyses, we use novel mouse models of human cancers to investigate how loss of PTEN functions alters normal cell behavior to promote cell survival and cancer progression, with a special focus on breast cancer.

Moreover, we aim to characterise the mechanisms of action of PTEN and to define its role in suppression of tumourigenesis beyond its functional interaction with the PI3K pathway.

The final goal of our studies is to ultimately identify new therapeutic targets regulated by PTEN and propose novel treatment modalities of human diseases associated with PTEN mutations.

HONOURS PROJECTS

1. Validation of candidate Pten-targets: We have performed phosphoproteomics of primary cells derived from two knock-in mouse lines harbouring loss-of-function and cancer-associated mutations in Pten. These mutations differentially affect the lipid, or the lipid and protein Pten phosphatase activities and as a result their expression induces activation of distinct signalling pathways. This project aims to validate the activation status of newly identified Pten-regulated pathways and entails the execution of a number of experiments including: i) expansion, treatment, and purification of human and mouse cell lines; ii) protein analyses; and iii) gene expression profiling.

2. Mammary gland morphology and tumour onset of mutant mice: We have generated new mouse models expressing mutant version of Pten in combination with additional oncogenic mutations and this has resulted in rapid mammary tumour formation. To characterise tumour onset and assess how alterations in the mammary gland development contributed to this phenotype, we plan to collect fat pads from experimental and control mice and study branching morphogenesis and evolution of epithelial buds over time. This projects requires working with animal models, processing of mouse tissues, and a number of immunostaining techniques.
RESEARCH PROJECT BACKGROUND:
Our limited ability to systematically study the function of genes and how they are deregulated in cancer has limited our ability to treat and understand cancer. To address this bottleneck we have been at the forefront of functional genomics and have developed an array of tools mostly based on CRISPR technology that enable high throughput cost efficient studies of genes and how they function in normal cells or during disease progression. Projects in the lab use unbiased CRISPR based genetic screens to identify drug targets in cancers with defined genomic alterations. Our ultimate goal is to develop cancer therapies that will be tailored to specific patients based on defined genomic features.

2020 HONOURS/PhD PROJECTS:
1. Identification of genes that induce breast cancer risk: Breast cancer is a leading cause of death in women. At least 35% of breast cancer cases are due to genetic familial mutations however, we currently understand only a small number of these mutations. Identifying these mutations is particularly significant since these could be used for development of drugs that reduce breast cancer risk or could treat patients with breast cancer. This project will use state of the art CRISPR based technologies aimed at identifying the genes that induce breast cancer risk.

2. The role of circular RNA in cancer: Since the discovery of RNA 150 years ago, we have gained good understanding of mRNA and only recently have we begun to uncover the roles of other RNA types. One such RNA that has been previously overlooked since it is difficult to detect using conventional sequencing strategies is circular RNA (circRNA). CircRNAs are highly abundant and initial studies have demonstrated important functions for some circRNAs. Yet, functional studies of circRNAs are limited due to the absence of tools to systematically perturb circRNAs without affecting the function of the linear RNA. This project will use our currently developed CRISPR based approaches to inhibit circRNAs and identify circRNAs that are important for cancer progression.

3. Development of new therapeutic molecules: PROTACs are new and exciting type of inhibitors that work by by recruiting a ubiquitin ligase and degrading a protein of interest. This project will develop new approaches to identify PROTACs that inhibit cancer growth and could be used for cancer treatment.
PROJECTS ON OBESITY & TYPE 2 DIABETES
Excess body weight is a major and leading factor in overall disease burden worldwide and if left unabated could lead to falls in overall life expectancy, particularly in developed nations such as the United States and Australia. In 2010 overweight and obesity were estimated to cause some 3.4 million deaths worldwide. Obesity is a key contributor to a myriad of human diseases including non-alcoholic fatty liver disease (NAFLD) and cancer. Moreover obesity is the single most important contributor to the development of type 2 diabetes, a major cause of obesity-associated morbidity and mortality.

The environmental, epidemiological and socioeconomic factors underlying the development of obesity and type 2 diabetes are multifactorial and complex. Their increasing prevalence indicates that dietary and lifestyle interventions alone are unlikely to be effective in combating obesity and type 2 diabetes, and underscores the need for a better understanding of their aetiology and the development of novel therapeutic approaches.

Projects are available to explore both Central Nervous System (CNS) and peripheral mechanisms contributing the development of obesity, type 2 diabetes and their complications.

PROJECTS ON OBESITY, LIVER DISEASE & LIVER CANCER
Obesity is a leading factor in the development of liver disease, with >85% of overweight individuals developing NAFLD. NAFLD encompasses a broad spectrum of liver conditions ranging from simple steatosis, to the more severe and progressive non-alcoholic steatohepatitis (NASH), a condition that results in fibrosis and if left unresolved, cirrhosis (late-stage liver disease) and/or liver cancer. Obesity-associated NASH is currently the third leading cause for liver transplantation and is expected to soon surpass hepatitis C as the principal cause for liver transplantation and HCC in the developed world.

Projects are available to determine the mechanisms by which obesity drives the development of NASH, fibrosis and liver cancer.

PROJECTS IN IMMUNO-ONCOLOGY
Therapies that enhance the immune response to tumours have revolutionised the management of cancer. However, most tumours do not have a high mutational burden and are therefore not ‘visible’ to the immune system or evolve alternate immunosuppressive mechanisms to escape immune-surveillance. As a consequence, such tumours remain largely unresponsive to current class-leading immunotherapies, including those targeting immune checkpoints. Chimeric antigen receptor (CAR) T cell therapy has emerged as an exciting immunotherapy approach for ‘non-immunogenic’ cancers, as it does not rely on endogenous anti-tumour immunity. CAR T cells are autologous T cells engineered to express a CAR specific for a tumour antigen. CAR T cells targeting CD19 have transformed the treatment of acute lymphoblastic leukemia, with clinical response rates of up to 90%. However, CAR T cells remain ineffective in solid tumours.

Projects focussed on the development of next generation CAR T cell therapies for solid tumours are available.
RESEARCH BACKGROUND

During cellular stress many proteins transition into and out of the nucleus to mediate transcriptional changes, facilitate DNA repair, trigger cell cycle arrest and if the damage is high enough to stimulate apoptosis. The paradox being that under cellular stress conditions, the normal pathways that facilitate protein movement into and out of the nucleus do not function. We have identified a novel nuclear transport pathway that continues to function under cell stress conditions, the first such pathway to be defined. Our laboratory uses a broad range of specialised state-of-the-art techniques including advanced live cell confocal microscopy techniques such as single molecule tracking using pair correlation microscopy, fluorescence recovery after photobleaching and in vitro reconstituted nuclear transport assays, as well as cell stress assays, immunoprecipitation, immunofluorescence, DNA repair assays and a range of protein-protein interaction assays.

HONOURS PROJECTS

Project 1: The role of nuclear transport factors in cellular stress.
During cellular stress conditions the nuclear transport proteins that normally mediate nuclear transport are mislocalised and rendered non-functional due to a collapse in the cellular Ran gradient. We have identified a novel nuclear transport pathway that appears to play a role in both the collapse of the Ran gradient in response to stress and the recovery of it afterwards. Project 1 will examine this pathway in detail, assessing the contribution of nuclear transport proteins and the response to various stress conditions. Techniques will include, quantitative confocal laser scanning microscopy, advanced single molecule microscopic techniques, tissue culture, KO stem cells, protein-protein interaction studies, western blotting, siRNA and immunofluorescence.

Project 2: Identifying a novel stress-responsive nuclear transport pathway. Our laboratory has recently identified a second a potential nuclear transport pathway that is active under stress. Project 2 will define this novel transport pathway for the first time and its role in the stress response. Techniques will include tissue culture, KO stem cells, protein-protein interaction studies, western blotting, siRNA and immunofluorescence.
Biochemistry Education Research
Dr Nirma Samarawickrema
Nirma.samarawickrema@monash.edu

RESEARCH BACKGROUND

Biochemistry Education Research uses quantitative and qualitative approaches to research into effective teaching, assessment and curriculum design that leads to enhanced learning. Our findings are used to improve curricula, authentic assessments and student learning. Adopting this strong evidence based approach ensures quality in teaching and learning.

HONOURS PROJECTS

Building Assessment Literacy: Exploring Opportunities Across Large Courses (Nirma Samarawickrema)
Monash University recognises that engaging students in assessment processes is central to the student learning experience. For students to develop assessment literacy they need to understand the purpose of, and process associated with, assessment tasks, to an extent that this translates into life-long learning. The proposed study will explore the development of assessment literacy in selected courses of the University through an evaluation of: (1) student and staff perspectives; and (2) course material. The finding of the study will inform learning designs that will ensure a better learning experience for students.

An evaluation of active learning approaches adopted in the Biochemistry workshops (Nirma Samarawickrema)

This study evaluates the case study approach adopted in the Biochemistry workshops designed to connect theory to practice as well as develop graduate attributes.

Please contact Nirma.Samarawickrema@monash.edu for further information.
RESEARCH BACKGROUND

Although the human immune system is uniquely equipped to provide protection against infections, many viruses have developed sophisticated strategies to thwart this immune surveillance. For example, herpesviruses are a group of ubiquitous and potentially deadly human pathogens that encode an arsenal of immunoevasin proteins that function to subvert immune recognition. Our research is focused primarily on one such herpesvirus member, cytomegalovirus (CMV), which has become a paradigm for immune evasion. Our research is focused on identifying new CMV-encoded immunoevasins, defining their molecular targets and characterising their mode of action.

HONOURS PROJECTS

Project 1. Cryo-electron microscopy of viral Fc Receptor decoys.
A major mechanism by which Natural Killer (NK) cells kill infected cells is antibody-dependent cellular cytotoxicity (ADCC). Here, NK cells use Fc receptors to recognise immunoglobulins that coat the surface of infected cells. However, CMV has evolved to thwart this process via the expression of a variety of Fc receptor decoy molecules that bind to immunoglobulin G and prevent ADCC. This project will investigate the mechanistic basis of the viral Fc receptor:immunoglobulin interaction and include structural determination of the complex using cutting edge cryo-electron microscopic techniques.

Project 2. Ligand recognition by natural killer (NK) cell receptors
NK cells recognize self, foreign and tumour derived ligands using a variety of germline encoded receptors. This project will focus on an enigmatic NK cell receptor, 2B4, that recognises CD48. In humans, this interaction is critical for efficient NK cell cytotoxicity and tumour clearance. In support of an important role for 2B4 in the control of viral infections, certain CMV species have recently been identified to encode soluble CD48 homologs that abrogates host 2B4:CD48 interactions. Remarkably, the viral CD48 decoys bind to 2B4 with much higher affinity than the endogenous ligand. This project will investigate recognition of these viral and host ligands by 2B4.

RESEARCH BACKGROUND

RNA viruses are a significant cause of global mortality and economic burden. RNA viruses such as Influenza A, killer of >40 million people in 1918, retain the ability to mutate rapidly, which increases their chance of developing into new and highly virulent strains. The retinoic acid inducible gene I (RIG-I) like receptors (RLRs) are instrumental to our innate immune defence against RNA viruses including influenza A/B, flaviviruses such as hepatitis C, paramyxoviruses such as respiratory syncytial virus, rhabdoviruses such as rabies, and retroviruses such as HIV. RLRs detect viral products within the cell and mount a protein-signalling cascade that results in the rapid production of type I interferons and pro-inflammatory cytokines, key mediators of anti-viral immunity. This immune response is often compromised by RNA viruses that exploit host immune signalling components to enhance their replication and spread within the host. It is critical to understand the molecular basis of RLR function, and in particular the detailed mechanism of viral-RLR antagonism. Our research will advance knowledge of host- and microbial-dependent regulation of innate immunity, and build on this to enable new strategies to combat microbial infections.

Techniques: Tissue culture, cellular biology and imaging, viral infections/transfections, molecular biology, protein expression and purification, biochemical characterisation, biophysical characterisation (eg analytical ultracentrifugation), structural techniques (eg X-ray crystallography, SAXS).

HONOURS PROJECTS

1. Understanding anti-viral immunity
Our immune response must be dynamically and rapidly controlled. This is partly achieved by ubiquitination, which alters the fate of the tagged protein. E3 ligases are enzymes pivotal to ubiquitination. These projects are focused on how particular E3 ligases function in innate immunity.

2. Viral immune evasion mechanisms
These projects are aimed at understanding how viral proteins hijack our immune system to aid their replication.

The crystal structure of the parainfluenza virus type 3 haemagglutinin-neuraminidase protein bound to the influenza drug Relenza (shown in purple)
RESEARCH BACKGROUND

Dendritic cells drive immunity and tolerance but we still do not fully understand how. Our group focuses on studying dendritic cells (DC) by analysing the cell surface receptors they express with the view that these receptors contribute to specialised functions. Ultimately the knowledge that we acquire is directed at generating better and safer vaccines.

Our research approach is exemplified by our work with Clec9A: we have identified a molecule critical to the function of a certain DC subset and then exploited this molecule as a means to deliver cargo to DC and thereby creating better vaccines.

We have also discovered a receptor that plays an important role in recognising certain types of DNA. Since modified oligonucleotides (DNA) are used as adjuvants in vaccines, it is important to understand how this receptor (DEC-205) interacts with DNA and what the consequences of this interaction are. Importantly, by maximising the efficiency with which DEC-205 captures DNA, we can design DNA with superior adjuvant properties.

HONOURS PROJECT

Characterising the immunostimulatory properties of CpG to harness tumour immunity.

There are an array of synthetic oligonucleotides (CpG) that have been used as vaccine adjuvants. We seek to understand which of these oligonucleotides are captured by DEC-205 and depend on this receptor to induce potent inflammatory immune responses. In this project we will assess the ability of several oligonucleotides to induce inflammatory cytokines (IL-6, IL-12, IL-1b, IFN-a/b) in normal and DEC-205 deficient (DEC205-/-) mice.

Recently, one of our CpG oligonucleotides was shown to promote the development of tissue-resident memory cells (Trm), a new subset of memory CD8 T cells. Trm function to survey the tissue that they are initially recruited to, and provide local protection against re-infecting pathogens. When our new CpG oligonucleotide is given as an adjuvant, it preferentially drives Trm formation in the liver and lung. Trm are also thought to play an important role in tumour immunity. In this project we will compare the capacity of various CpG oligonucleotides to induce immunity against tumours, analyse the T cells infiltrate and establish whether our new CpG oligonucleotide induces tumour Trm. We will also compare the ability of different types of CpG oligonucleotides to induce anti-tumour immunity alone or in conjunction with checkpoint inhibitors.
RESEARCH BACKGROUND

The Structural Virology laboratory aims at understanding the assembly and replication of viruses combining molecular virology and structural biology approaches. Our research produces 3-D molecular models of viruses and viral proteins to provide functional insights and design novel antiviral therapeutics.

**Project Areas**

1. Structure determination and engineering of beneficial viruses
2. Exploring the world of giant viruses using structural biology
3. Towards rapid structure determination of human viral pathogens by cryo-electron microscopy (cryo-EM)

HONOURS PROJECTS

Structure determination and engineering of beneficial viruses

Viruses are best known for the diseases they cause in human, animals and plants. This historical focus on a limited number of pathogenic viruses has occulted for long the fact that most viruses do not cause disease in animals and plants. Indeed life thrives despite – or perhaps thanks to – staggering numbers of viruses, which outnumber cellular organisms by an order of magnitude. Thus viruses represent a formidable evolutionary force as vectors of genetic exchange and constant selective pressure. This is true at every scales from the microbiota in our guts to the microbial communities in large ecosystems such as seas and oceans. This provocative view of viruses led to the recognition that some of them could be our allies in research, biotechnology and health.

This project will investigate the structure and function of insect viruses that have novel applications to human health as vaccines and production systems for therapeutics. Despite the beneficial impact and biomedical potential of these viruses, their engineering has been impeded by the lack of molecular understanding of essential processes, specifically the self-assembly of the viral capsid and its trafficking in/out of the cell. The Honours project will aim at filling this gap by (i) generating 3D model of the infectious particle using integrated structural biology approaches and (ii) elucidating key aspects of viral morphogenesis using in vitro assembly assays and imaging.

**Fields of research:** molecular virology; structural biology; biochemistry; immunology (vaccine)

**Techniques:** molecular biology (cloning, site directed mutagenesis), protein biochemistry (protein expression & purification; biophysical characterisation) and structural biology (X-ray crystallography; cryo-EM).

*Details on Projects 2 & 3 can be obtained upon email request to A/Prof Coulibaly.*
RESEARCH BACKGROUND

We work on understanding how structural cells, called fibroblasts, influence the immune system. We and others have shown that fibroblasts are fundamental to healthy immune function, directly maintaining T and B cell survival, and directing their migration and interaction with antigen presenting cells, while limiting their autoimmune potential. Our newest area of study explores how they influence the anti-tumour immune response, an exciting and clinically relevant topic at the forefront of cancer immunology. Cancer-associated fibroblasts (CAFs) are not cancerous, but they support and expand alongside the cancer (see: human colorectal images below). The CAF load in a tumour strongly predicts poor outcomes for patients, and in some tumours CAFs even strongly outnumber the tumour cells, yet our understanding of their function is still in its infancy. Our new work shows that human CAFs directly impair T cell activation and is working towards development of a new class of anti-cancer drugs that target CAFs, to improve the success rate of immunotherapies.

Our lab is passionate, hard-working, creative, collaborative and fun, and we are committed, friendly supervisors who are enthusiastic about your career development, wherever you head. We have extensive experience in honours supervision.

HONOURS PROJECTS

We have been studying and profiling human fibroblast samples, and we have more exciting genes to look at than to study than people to study them, so we are looking for a talented student to select one or more genes of interest and explore their function. The projects are flexible but systematic in approach, to maximise the chance of the student generating interesting, publishable outcomes.

Project 1. How do CAFs interfere with anti-tumour immunity? Using primary CAF cultures and human leukocytes, supported by mouse models of cancer, a candidate gene will be chosen and tested for its function in CAFs. Candidates include CXCL12, CSF1, IL-34, IL-1, or others of your choice, chosen in consultation. Experimental approaches include use of blocking antibodies, gene knockdown, migration assays, some simple bioinformatics, T cell activation assays, and T and B cell survival assays.

Project 2: What drives CAF development? Using similar approaches and techniques as above, this cell biology project will explore the fundamental question of what drives a healthy fibroblast to become a CAF, and how might we block this process.
RESEARCH BACKGROUND
Viruses and pathogens are part of day-to-day encounters that the immune system needs to deal with. How the immune system “sees”, recognises and eliminates viral infection is not fully understood. Indeed, viruses are able to mutate in order to escape the immune system surveillance. If we were to develop better vaccine and drugs, or even vaccine against viruses like HIV, it is essential to understand the mechanism of viral recognition and viral escape prior to this.

Project 1 (Influenza virus) Influenza virus is a big health issue. The virus is able of generating a high number of mutation and so more likely to escape the immune system surveillance. We use X-ray crystallography to make 3D structures of viral peptides bound to immune system proteins (HLA molecule) in complex with T cell receptor (TCR). X-ray Synchrotron radiation at the Australian Synchrotron. The atomic structure allows us to observed the details of the interaction between the peptide and the TCR. We discover new Influenza epitope and assess their impact on the immune response to determine if they could be good target for therapeutics development.

Project 2 (HIV virus) HIV has of very high mutation rates and despite the dramatic life improvement provided by current anti-retroviral therapy, HIV and AIDS are still health burdens. To tackle these issues, our work focus on a subset of individuals– named controllers – known to control HIV infection and/or delay disease progression. We use X-ray crystallography to make 3D structures of HIV epitopes bound to immune system proteins (HLA molecule) in complex with T cell receptor isolated from HIV controllers (TCR). The atomic structure allows us to observed the details of the interaction between the peptide and the TCR, and link those observations with the function of the T cell carrying this particular TCR. This help us understand the recognition mechanism of the T cell for the viral particle.

Project 3 (cellular immunology) we have discover new epitope for an unconventional HLA molecule and we will be dissecting the magnitude, functional capacity and molecular characteristics of T cell responses towards novel influenza virus epitopes using a range of techniques including cell culture, flow cytometry and single-cell sorting and multiplex PCR.

Research PROJECT
1./ Structural investigation into T cell response to Influenza
2./ Structural investigation into T cell response to HIV
3./ Role of unconventional HLA molecule in the immune response

First TCR-HIV-MHC-II complex
Galperin, Science Immunology, 2018
Cancer Immunotherapy Laboratory
Professor Nicholas Huntington
0467628854
huntington@wehi.edu.au
https://research.monash.edu/en/persons/Nicholas.huntington

RESEARCH BACKGROUND
Therapies targeting the immune system (immunotherapy) are revolutionising cancer treatment. Natural killer (NK) cells possess an innate ability to detect and kill cancer cells. How NK cells actively detect tumour cells and how cancers evolve to evade immune control is not clear. By generating the first specific NK cell-deficient mouse, our lab has revealed unique role of NK cells in clearing spontaneous metastases and recruiting and priming other immune cells in solid tumours. Furthermore, increasing the activity of NK cells in vivo by deletion of NK cell checkpoints dramatically reduces tumour burden and with industry partners, we are developing drugs against these checkpoints for clinical use. Cancer remains a major disease burden as cancer cells can evolve mechanism to evade immune control. Using genome-wide CRISPR screens, cutting-edge mouse models, human genetic variants, primary human tumours and proteomics/transcriptomics, we have uncovered growth factors and ligands that dictate NK cell ability to detect and kill cancer cells. Mechanistic understanding and therapeutic targeting of these pathways is our research goal.

HONOURS PROJECTS

1. NK cell immunosuppressive pathways (with Dr Fernando Guimaraes). Our laboratory recently identified the opposing roles of the SMAD and STAT transcription factor families in NK cell anti-tumour activity. The intrinsic regulation of these pathways in NK cells and the role of the tumour microenvironment (TME) to trigger these pathways (via IL-15, IL-12, TGF-β and Activin) will be assessed using transgenic mouse models, cell signalling, novel therapeutic strategies and patient samples. We aim to identify the molecular basis of NK cell suppression in the TME to rationally design immunotherapy strategies to overcome them in metastatic cancer.

2. Tumour immune evasion (with Dr Jai Rautela). Cancer cells are detected and eradicated by multiple immune mechanisms including NK cells. Cancer cells evolve resistance to intrinsic immune control and immunotherapy drugs by mutating their genome. This project will exploit our unique mouse strains with varying degrees of NK cell function, NK cell-dependent tumour models, validated CRISPR-Cas9 screens, bioinformatics and computational biology to dissect the essential role of NK cells, the activating receptor NKp46 and tumour ligands in cancer metastasis. NK cell null mice and single cell RNAseq will be used to evaluate the role of NK cells in tumour inflammation including Dendritic Cell and T cell recruitment and activation.

3: Integrated NK cell transcriptomics and proteomics (with Dr Fernando Guimaraes). This project aims to elucidate dominant pathways for NK cell anti-tumour immunity using transcriptomics, proteomics and computation biology. Datasets have been generated from NK cell with a large spectrum of tumour surveillance capacities and will be used to uncover novel and dominant regulatory pathways in NK cells in order to rationalise novel targets for immunotherapy development. Pathways will be confirmed in human NK cells and prioritized for next generation chimeric antigen receptor (CAR) NK cells evaluation.
Human health is dependent on the ability of the immune system to clear the multitude of different foreign pathogens encountered throughout life.

Our research studies the ability of the immune system to clear pathogens and form immunity through production of antibody and B cell memory. Understanding the molecular regulators that underpin this is core to finding new treatments for B cell-mediated disease and progressive vaccine design.

**HONOURS PROJECTS**

**Project 1: Epigenetic regulation of B cell immune responses**

Vaccines exploit the ability of the immune system to provide heightened, tailored responses to pathogens if the host has been infected prior – this is termed immune memory. Antibody-based vaccines are vital for population health, yet very little is known about the factors that are required for antibody memory. This project will identify new regulators of immune memory by investigating histone modifications that allow antibody formation and memory persistence during a secondary response.

**Project 2: Immune memory dysfunction during chronic infection**

Chronic infectious diseases have a devastating effect on global health. HIV and Plasmodium falciparum both cause chronic disease and have evaded effective vaccine design. Production and function of immune memory is altered in chronic infectious diseases, leading to ‘atypical’ memory B cells that may be an impediment to fighting infection. This project will investigate the origin and function of these cells, and how their formation is regulated at the molecular level.
Proteostasis & Disease
Nuclear Signalling

Dr Nadinath B. Nillegoda
Australian Regenerative Medicine Institute

Prof David A. Jans
Dept. Biochem. & Mol. Biol., BDI

Tel: 99053636; 99029341; david.jans@monash.edu
nadinath.nillegoda@monash.edu;
https://research.monash.edu/en/persons/nadinath-nillegoda
https://research.monash.edu/en/persons/david-jans

RESEARCH BACKGROUND

The collective research focus of our laboratories is to dissect critical nodes of the cellular protein homeostasis (proteostasis) network that are linked to disease and develop new strategies to specifically target these nodes for therapeutic gain. We are investigating several disease models including neurodegenerative diseases and viral infection. We focus on how the proteostasis network responds to proteotoxic stresses and help clear potentially cytotoxic protein aggregates in Alzheimer’s disease (AD), Parkinson’s disease (PD) and spinocerebellar ataxia 1 (SCA1). In parallel we study host-pathogen interface, where host-virus interaction is the key to understanding disease and therapeutic approaches to stop it; our key interests are Dengue (DENV), Zika (ZIKV) and respiratory tract infections (RSV). In all of these disease states, efficacious therapies are urgently needed.

HONOURS PROJECTS: NEW THERAPEUTIC APPROACHES

PROJECT 1 NEURODEGENERATIVE DISEASE: Our expertise in proteostasis and nuclear trafficking provides a unique opportunity to examine the potential role of specific components of the Hsp70 chaperone network in SCA1. The SCA1 disease riving polyQ-Ataxin-1 forms striking nuclear bodies (ATX-NBs). We are documenting the contribution of specific components of the Hsp70 chaperone network to ATX-NBs, with the possibility that there could be potential targets to facilitate ATX-NB disaggregation. The project will use proximity-based protein interaction assays to visualise chaperone localisation, with RNA interference (RNAi) and small molecule inhibitors against the key chaperones employed to delineate disaggregation potential.

PROJECT 2 VIRAL INFECTION: Our expertise in proteostasis and virology provides a unique opportunity to examine specific components of the Hsp70 chaperone network manipulated by DENV, ZIKV and RSV for replication. We are investigating effective chaperone-based antiviral targets that could (a) affectively block sudden viral epidemics and (b) combat the emergence of drug-resistant strains. The project will characterize new drug targets with a broad spectrum of action for DENV, ZIKV and RSV therapeutics utilizing several approaches, including RNA interference (RNAi) and small molecule inhibitors against the exploited chaperones.
RESEARCH BACKGROUND

Our Lab is interested in understanding how cancer metastasis occurs in lymph nodes, often the first port of call for metastatic cells from primary tumours. Lymph node fibroblasts have the ability to dampen immune cell responses and prevent differentiation. We have recently identified potential targets to reverse the immunosuppressive effect of fibroblasts and are testing both commercially approved drugs and new therapies in their effectiveness on suppressing metastasis of tumour cells.

We are a group of passionate people that like collaborating in a creative environment. As supervisors we foster an enthusiastic attitude and provide support towards your chosen path.

HONOURS PROJECTS

Our work is looking at the interaction and mechanisms of fibroblasts in both adaptive and innate immunity. We routinely work with both human and mouse samples and use a variety of techniques in our investigations including mouse models, RNA sequencing, flow cytometry, immunofluorescent microscopy, RT-PCR and cell culture.

1. How do lymph node fibroblasts contribute to metastasis in tumour? We have been studying and profiling lymph node fibroblasts in steady state in the human and mouse setting. However, little is known about the behaviour of these cells during metastasis. Using a mouse metastatic tumour model we are looking to characterize the fibroblasts during disease and identify potential drug targets. Experimental approaches include flow cytometry, immunofluorescence, blocking antibodies and T cell activation assays.

2. Can we inhibit metastasis by reversing immune cell immunosuppression? We want to interrogate the interaction of immune cells (both T cells and NK cells) with fibroblasts. Using a variety of drugs and antibodies followed by gene knockdown approaches we will test the immunosuppressive dampening. These results will inform our selection of most promising targets for in vivo testing in mice.
RESEARCH BACKGROUND

Gastric cancer is the 3rd most fatal and 5th most common cancer in humans. The molecular pathogenesis of gastric cancer remains poorly understood. It is well established that infection by Helicobacter pylori increases the risk of gastric ulcer and gastric cancer. Our team uses state-of-the-art molecular biology and cell biology techniques to understand how the virulence proteins of H. pylori ‘hijack’ host cell signalling pathways to promote carcinogenesis. Our long-term goal is to apply the knowledge gained to the discovery of novel anti-gastric cancer therapeutics and diagnostic markers.

HONOURS PROJECTS

PROJECT 1: Understanding how the bacterium Helicobacter pylori stimulates oncogenic signalling via interaction with the human integrin family of receptors
Aim: The integrin family of eukaryotic transmembrane receptors play fundamental roles in cell adhesion, cell migration, proliferation, angiogenesis and cancer development. Activation of MAP kinases and Src kinase are some of the well-characterised signalling events triggered by integrins which play key roles in cancer development. Interestingly, virulence strains of H. pylori can potently influence these signalling pathways. In collaboration with other cancer research centres, the aim of this project is to understand the molecular mechanisms by which the virulence proteins of H. pylori, CagA and CagL, activate human integrin receptors and how this promotes gastric cancer. Techniques to be used: Gene knockdown by RNAi, tissue culture, transfection, live cell imaging, immunofluorescence microscopy, Western blotting, cloning and animal models.

PROJECT 2: How do H. pylori virulence proteins activate angiogenesis?
Aim: Interesting recent findings indicate that certain amino acid sequence variations of some H. pylori virulence factors are linked to increased gastric cancer risk in infected patients. Angiogenesis (the formation of new blood vessels) plays an essential role in tumour development and wound healing. Our preliminary data indicates that certain H. pylori virulence factors can activate angiogenic responses in human endothelial cells, cells that make up blood vessels, implying that these bacterial factors may play a key role in tumour development. The aims of this project are to characterise the biochemical properties of these virulent proteins and understand how they contribute to the activation of angiogenic responses and increased cancer risk. Techniques to be used: Protein chemistry, molecular cloning, protein purification, ELISA and angiogenesis assays.
**CD8+ T cell activation and function**

Prof Nicole La Gruta  
Phone: 9902 9182  
Email: nicole.la.gruta@monash.edu  
Lab webpage: http://med.monash.edu/biochem/labs/lagruta/

**RESEARCH BACKGROUND**

The primary role of CD8+ T lymphocytes (CTLs) is to recognize and remove pathogen-infected cells and cancers. Indeed, CTLs play a critical role in mediating protection from infections and cancers in humans and many strategies exploiting CTL immunity are already in clinical use. Since T cell based vaccines and immunotherapies rely on the robust activation of potent antigen-specific T cells, it is critical to understand the mechanisms driving effective T cell activation after antigen encounter. The La Gruta laboratory uses a wide range of state-of-the-art molecular and cellular techniques to elucidate the key drivers of effective CTL immunity, and the molecular basis of age-related T cell dysfunction, such that these may be manipulated to elicit optimal CTL immune responses.

**HONOURS PROJECTS**

**Project 1. Understanding the mechanisms underpinning effective TCR recognition of pMHCI.**

T cell receptor (TCR) recognition of peptide + MHC class I complexes (pMHCI) is critical for CD8+ T cell function. Despite the extraordinary diversity of the TCR, it consistently recognises pMHCI in a highly conserved orientation. Why the TCR, with all its diversity, must dock on MHC with this highly reproducible polarity in order to signal successfully remains unknown and intensely debated.

We have identified the first endogenously-derived TCR that docks on its cognate viral peptide+MHC in a reversed orientation. We have shown that the altered positioning of the TCR over the pMHCI negatively impacts the T cell’s ability to be activated and recruited into an antiviral immune response, however the mechanism for this is unclear.

My laboratory aims to use these unique reversed TCR ‘tools’ to understand the mechanism by which TCR docking polarity influences TCR signaling using state-of-the-art approaches, including retrogenic mice, FRET imaging analyses and in vitro T cell stimulation. This work will fundamentally advance our understanding of effective T cell activation. [Co-supervised by Dr Pirooz Zareie; pirooz.zareie@monash.edu]

**Project 2. Cellular and Molecular Analysis of Ageing in CD8+ T cells**

Like many developed countries, the population of Australia is ageing; with 13.8% of the population currently over the age of 65 and predictions this will reach 19.9% by 2031, this increase is predicted to have a considerable effect on the cost to public health services. Aged individuals exhibit increased susceptibility to and severity of a variety of infections, alongside waning vaccine efficacy rates, reflecting, in part, diminished primary CD8+ T cell responses. Studies in mice and humans have demonstrated that intrinsic defects in CTL immunity contribute substantially to overall immune dysfunction in aged individuals. My laboratory aims to elucidate both age related CTL deficiencies and the mechanisms underlying them, addressing key aspects including TCR signalling, metabolism and epigenetics. This work will rigorously define basic immunological, epigenetic, and metabolic mechanisms restricting intrinsic CD8+ T cell immunity in aged individuals.
RESEARCH BACKGROUND

Our research focus is understanding how the sentinels of the immune system, the dendritic cells (DC), sense and respond to “danger” in their environment, and to use this knowledge for improving vaccines and immunotherapies. DC have an array of receptors designed to detect pathogen-associated and damage-associated molecular patterns. These receptors enable DC to sense invading pathogens or other danger (e.g. damaged or dead cells) and to direct the type of protective immune response required. Importantly, there are multiple DC subsets which are tailored for different functions. DC subsets can recognise different pathogens and damage signals, and respond accordingly. Our focus is to determine the receptors that enable the DC to sense and respond to such signals, and their role in inducing immune responses.

HONOURS PROJECTS

Project 1: The dendritic cell receptor Clec9A: dead cell recognition and immune modulation.

DC monitor the environment for potential “danger signals” that signify pathogen invasion, including non-homeostatic cell death caused by viruses. We identified a DC-specific receptor, Clec9A, which plays an important role in the recognition and processing of antigens (Ag) acquired from such dead cells, to initiate effective immune responses. Furthermore, Clec9A is a particularly effective target for the delivery of Ag directly to DC subsets for immune modulation.

We recently identified that Clec9A recognises actin filaments revealed by dead and damaged cells. This project will elucidate the molecular interactions of Clec9A, and determine the role of these interactions in regulating Clec9A function, DC biology and the modulation of immune responses.

Project 2: Molecular Mechanisms that underpin dendritic cell cross-presentation.

DC take up, process and present antigen (Ag) to T cells to initiate immune responses. There are multiple DC subsets that are tailored for different functions. While all DC can take up, process and present Ag on MHC II to induce CD4 T cell responses, only particular DC subsets can take up dead cells and other exogenous Ag and cross-present these on MHC I to induce the CD8 T cell responses essential for killing infected cells and tumours. Using microarray comparisons of DC subsets and DC stages of development, we have identified a panel of genes that are selectively expressed by cross-presenting DC subsets in mouse. In this project, we aim to investigate the expression and function of these genes, and determine their role in Ag presentation and DC function.
RESEARCH BACKGROUND

While most studies in adaptive immunity have focused on peptide-mediated immunity, my research aims to explore the uncharted territory of lipid- and metabolite-mediated immunity. This aspect of immunity represents a new frontier in immunity. Indeed, there is a number of pressing fundamental questions that need to be addressed: (i) What is the extent of the chemical diversity of immunogenic non-peptidic antigens (Ag)? Are there more atypical Ags to be discovered in mammalian and non-mammalian species? (ii) How are these lipid and metabolite Ags presented and recognized? (iii) What are the molecular mechanisms that underpin the recognition event and the signalling outcomes? (iv) How did non-classical MHC molecules evolve to fulfill their molecular functions within a specific species? By applying a multi-disciplinary and highly innovative approaches that include comparative immunology, chemistry, structural biology, cell immunology, advanced atomic and molecular imaging, my research program aims to provide comprehensive and fundamental insights into molecular recognition of non-peptidic Ags, and gain an evolutionary perspective on the structure and function of MHC-like Ag-presenting molecules.

HONOURS PROJECTS

1. **To investigate the MR1 family and metabolites mediated immunity.**

Studies in T cell mediated immunity have focused on understanding the presentation of peptides by the Major Histocompatibility Complex (MHC), and their recognition by αβ T cell receptors (TCRs). However, αβ T cells can respond to other classes of antigen (Ag) associated with both protective and aberrant immunity. Mucosal-associated invariant T (MAIT) cells play a central role in immunity by mediating the recognition of vitamin B metabolites that are presented by the MHC class I-related molecule, MR1. MAIT cells are emerging as key players in antimicrobial immunity, autoimmunity, cancer and metabolic diseases. However, many aspects of MR1-mediated immunity, such as the extent of the MR1 restricted T cell repertoire, and the potential for recognising other MR1-bound Ags, are unknown. Here, we will address one pressing question in MR1-mediated immunity, namely: What are the molecular mechanisms that underpin the recognition of MR1-Ag by these ‘atypical’ T cell subsets? The project will involve a number of biochemistry- and biophysical-based techniques including the recombinant expression, purification, crystallization and 3D structure determination of immune molecules (TCRs and MR1).

2. **To explore the field of comparative immunology (Structure and function of non-classical MHC molecules (MHC-like) in evolutionary distinct species, e.g. Marsupials, frogs, chickens and bats).**

In the past decade, the development of technologies has opened new exciting frontiers and novel opportunities to explore the diversity of immunity in mammalian and non-mammalian species. There is indeed tremendous value and excitement to discover how the immune system in different organisms (non human, non mouse) work, and more importantly to understand how distant species adapted to their immediate environment in order to survive exposure to pathogens throughout evolution. Addressing these fundamental questions may have significant impact in relation to the origin and function of the immune system. These projects aim to investigate the biological function of MHC-like molecules in evolutionary distinct species to humans and will be focusing on the functional and structural studies of families of MHC-like from a wide range of vertebrate species spanning more than 360 millions years of evolution including frogs, marsupials, guinea pigs, bats, rabbits etc...

These projects will involve a number of biochemistry- and biophysical-based techniques including the recombinant expression, purification, crystallization and 3D structure determination of immune molecules.
RESEARCH BACKGROUND

Human Cytomegalovirus (HCMV) is a β-herpesvirus that infects over 60% of the adult population. HCMV is a significant cause of morbidity and mortality in immuno-compromised individuals such as organ transplant recipients. However, the largest burden of disease occurs from intrauterine HCMV transmission during pregnancy, occurring in at least 1% of pregnancies worldwide. This can cause permanent hearing loss, vision impairment, and mental retardation. No vaccine exists, and discovery of new antivirals is urgently required.

Research in our laboratory is at the interface between cell biology, virology and quantitative proteomics, and we have proprietary virus libraries and reagents to make unique discoveries. All projects have an opportunity to learn standard (tissue culture, cloning, western blotting, immuno-precipitation, confocal microscopy, RNAi, CRISPR) and advanced (liquid chromatography, mass spectrometry, bioinformatics, electron microscopy) laboratory techniques and skills.

HONOURS PROJECTS

PROJECT 1: Understanding assembly and egress of HCMV virions
The HCMV virion comprises a nucleocapsid that houses the DNA genome, and is surrounded by a proteinaceous tegument layer, and glycoprotein-containing membrane. Precisely how the virion is assembled and released (egress) remains unknown. However, infection causes extensive organelle remodelling in infected cells, and produces a structure known as the viral assembly complex (vAC) to package HCMV virions (see image above). Projects in the lab use a library of mutant viruses to identify the viral proteins that are required for vAC biogenesis, as well as proteins involved in later stages of virion maturation and release.

PROJECT 2: Investigating hijacking of host exosome pathways by HCMV
It is known that maturing nucleocapsids bud into host membrane-derived structures to acquire the outer virion envelope. However, the origin of the membrane is unknown, and the precise molecular mechanisms remain elusive. Proteomics in our lab identified HCMV virions were significantly enriched with host exosome proteins, and deletion of these host proteins in cells severely compromised production of virus. Therefore, these host proteins represent novel antiviral candidates, and projects in the lab explore their ability to block viral processes including membrane envelopment, cellular trafficking, or vesicle fusion.
RESEARCH BACKGROUND
The Clinical immunology Group examines key interactions between the TCR/MHC/peptide complex that are not only imperative for pathogenic control and clearance but also are pertinent to many human disorders (i.e. autoimmunity, allergy, cancer) and therapies for end-stage disease (i.e. transplantation). The clinical impact of this research has the potential to be far-reaching including translatable outcomes in aiding clinicians to tailor individual patient clinical management (personalised medicine) and influence development and design of immunotherapeutics.

HONOURS PROJECT
Understanding the role of T cell cross-reactivity in human disease
Throughout life exposure to a vast array pathogens shapes our immune system to establish a repertoire of specific memory T cells that can be rapidly recruited to combat secondary challenges from previously encountered pathogens. Whilst T cells provide essential immunosurveillance to combat and eliminate pathogenic assault, these heroes can turn into villains by mediating unwanted immune responses against self via T cell receptor (TCR) cross-reactivity. This cross-reactivity trigger is important in a number of human disorders (i.e. autoimmunity, allergy, cancer) and therapies for end-stage disease (i.e. transplantation). This project explores how the virus-specific T cell repertoire is able to recognise different human leukocyte antigens (HLA; human MHC) allotypes via TCR cross-reactivity. We have identified several cross-reactive virus-specific T cells that recognize different HLA allotypes that require further characterisation to elucidate molecular interactions between the TCR/peptide/HLA complex (1,2). This mechanism is considered to be potential driver associated with transplant rejection (3,4) and autoimmune diseases. You will be embedded in a start-of-the-art laboratory with access to the latest technology associated with the phenotypic and functional dissection of human T cell responses (various T cell assays, flow cytometry, RNASeq, TCRSeq), and interrogation of antigen processing and presentation (proteomics and mass spectrometry, gene editing technologies including CRISPR/Cas9).

RESEARCH BACKGROUND

Superbugs are not only resistant to current antibiotics but they are also highly effective in evading immunity. This leads to several human diseases that are increasingly difficult to treat. Thus, there is an urgent need to develop alternative approaches to antibiotic therapy. Rather than killing the bacteria, targeting host-factors that promote pathogen survival has emerged as a promising strategy in infectious diseases. To develop this further we need a better understanding about how superbugs evade immunity on the molecular and cellular levels.

To identify new host-pathogen interactions we follow infections by live-cell imaging. This enables the identification of host cell responses on the single cell level in a high-temporal resolution. In addition, we employ super-resolution imaging to uncover how pathogens target host factors in immune cells. Finally, by screening host genome libraries we identify the host factors that enable superbugs to survive immune attack. This has led to new therapeutic approaches by re-purposing existing drugs to kill infected cells (Speir et al, Nature Micro, 2016).

HONOURS PROJECTS

1. **Targeting host factors to prevent MRSA infections**
   Methicillin resistant *S. aureus* (MRSA) utilizes secreted toxins to kill innate immune cells and to cause disease. The project will identify host factors that are activated by these toxins. For this, a whole genome CRISPR library will be screened to identify mutant immune cells that resist toxin mediated killing. Identified genes will be further validated in infections that depend on transgenic stem-cell derived human immune cells. This will utilize live-cell imaging, immunological and biochemical assays.

2. **Cell death signalling in infections**
   The superbug *Neisseria gonorrhoeae* that causes sexually transmitted infections evades immunity, but the mechanism behind this remain unclear. We have identified bacterial secreted vesicles as novel delivery system employed by these bugs to manipulate cell death signalling to evade immunity. The project will utilize proteomics and transcriptomics to characterize bacterial vesicles. In addition, super-resolution imaging will identify how vesicles deliver bacterial proteins into immune cells to hijack host signalling pathways. This will identify host-pathogen interactions that promote infectious diseases.
Dendritic cells link innate and adaptive arms of the immune system

RESEARCH BACKGROUND

Dendritic cells are sentinels of the immune system that produce cytokines and interferons upon sensing danger. They are also professional antigen presenting cells, thereby connecting the innate and adaptive immune systems.

Our laboratory investigates how pathogens and their products or danger signals of ‘self’, activate dendritic cells. We aim to decipher how this activation influences the function of dendritic cells. We aim to understand the role of dendritic cells in interferon-mediated diseases, including autoimmune diseases such as Lupus, in cancer immunotherapies and in antibiotic-resistant bacterial infections.

HONOURS PROJECTS

1. The function of checkpoint inhibitors on dendritic cells
The anti-tumour effects of immunotherapies targeting checkpoint inhibitors is currently attributed to the ‘re-awakening’ of T cells. However, we have now discovered that DC can express high surface levels of checkpoint inhibitors. We find that the functions of dendritic cells are themselves directly regulated by checkpoint inhibitor expression. This project will examine the biological consequences of, and molecular pathways leading to, checkpoint inhibitor regulation of dendritic cell function.

2. The effects of interferon-lambda on dendritic cells
Interferon-lambda is an anti-viral cytokine that is highly expressed by dendritic cells and attributed with major regulatory roles in the immune system. This project will investigate the effects of interferon-lambda on dendritic cell function. It will particularly examine the nature of the interferon-lambda receptor on dendritic cells.
RESEARCH BACKGROUND

The Purcell laboratory specialises in identifying novel targets of the immune response using a combination of functional immune profiling, proteomics, transcriptomics and structural biology. The laboratory has an outstanding track record in delivering high end outcomes including recent publications in highly regarded peer reviewed journals including Nature (5), Nature Immunol (7), Nat Methods, Nat Struc Mol Biol (2), PNAS (13), J Exp Med (8), Immunity (8), J Clin Invest, Mol Cell Proteomics (5), Proteomics (4) & J Proteomics Res (3). The Purcell lab works closely with other groups at Monash, nationally and internationally to study all aspects of immune recognition. We work closely with industry and pharma to translate our findings into the clinic with major collaborations with local, European (Belgium, Denmark, UK) and US based pharma and startups.

HONOURS PROJECTS

Honours projects are available in a variety of areas (see lab web site for details) and include the following example projects

Project Title: Novel peptide antigens in infectious disease, autoimmunity and cancer
Project Description: Recent studies have highlighted a prominent role for a novel classes of peptides generated by various post-translational modifications including proteasomal splicing of different polypeptide chains (1-3). These peptides represent an untapped resource for vaccination and immunotherapy (4-7). Our lab has developed new tools to identify and study these peptides. Several projects of relevance to influenza infection, psoriasis, rheumatoid arthritis, diabetes, melanoma, lung and bowel cancer are available. The projects will involve biochemistry, mass spectrometry and/or immunology focussed techniques. Our lab has access to cutting edge instrumentation, collaborates through a world wide network of scientists and industry to deliver research and health outcomes.

References:
Human Leukocyte Antigens (HLA) molecules of the major histocompatibility complex (MHC) regulate the adaptive immune response. HLA molecules present peptides derived from self and non-self proteins to T cells as a means to detect and destroy invading pathogens. The capacity to distinguish between peptides derived from self and non-self proteins is thus a crucial feature of the immune system. However, failure of this self/non-self discrimination can result in T cell reactivity against self-peptides and, consequently, autoimmunity. Indeed, in addition to the role of HLA in protective immunity, they are also important genetic determinants in autoimmunity. We are interested in how post-translational modifications such as citrullination, deamidation and hybrid peptide generation leads to the conversion of self and innocuous environmental derived peptides (e.g. gluten peptides in celiac disease) into antigenic triggers for a deleterious immune response. We investigate how these neo-antigens are presented by MHC Class II (MHC-II) molecules and how these complexes are recognised by the T cell receptor (TCR) on CD4+ T cells that initiate these disease processes. The other major area of research in the laboratory focuses on understanding differences between T cell antigen recognition by regulatory and effector T cells in autoimmune disease. The projects described below will employ biochemical, biophysical, structural and cell based approaches to investigate the cellular immune response to self and modified self antigens in autoimmune and inflammatory diseases.

RESEARCH BACKGROUND

Human Leukocyte Antigens (HLA) molecules of the major histocompatibility complex (MHC) regulate the adaptive immune response. HLA molecules present peptides derived from self and non-self proteins to T cells as a means to detect and destroy invading pathogens. The capacity to distinguish between peptides derived from self and non-self proteins is thus a crucial feature of the immune system. However, failure of this self/non-self discrimination can result in T cell reactivity against self-peptides and, consequently, autoimmunity. Indeed, in addition to the role of HLA in protective immunity, they are also important genetic determinants in autoimmunity. We are interested in how post-translational modifications such as citrullination, deamidation and hybrid peptide generation leads to the conversion of self and innocuous environmental derived peptides (e.g. gluten peptides in celiac disease) into antigenic triggers for a deleterious immune response. We investigate how these neo-antigens are presented by MHC Class II (MHC-II) molecules and how these complexes are recognised by the T cell receptor (TCR) on CD4+ T cells that initiate these disease processes. The other major area of research in the laboratory focuses on understanding differences between T cell antigen recognition by regulatory and effector T cells in autoimmune disease. The projects described below will employ biochemical, biophysical, structural and cell based approaches to investigate the cellular immune response to self and modified self antigens in autoimmune and inflammatory diseases.

HONOURS PROJECTS

Project 1: T regulatory vs T effector T cell receptor recognition of self peptide-MHC Class II

Regulatory T cells (Treg) play a critical role in the control of the adaptive immune response. This includes controlling the effector T cell (Teff) response once an infection has been cleared as well as preventing the Teff response from eliciting autoimmune attack. Disturbances to the Treg control of Teff responses are observed in autoimmune diseases. This project aims to understand some of the discerning features of Treg and Teff responses by examining the structural basis for Treg and Teff T cell receptor recognition of self peptide-MHC Class II complexes.

Project 2: Defining key determinants of HLA mediated T cell tolerance and autoimmunity

Some HLA alleles predispose individuals to autoimmunity whereas others provide dominant protection against disease. We have recently provided a mechanism for dominant protection by demonstrating in Goodpasture’s Disease (GP), a kidney autoimmune disease, that the HLA molecules encoded by the susceptibility and protective alleles both present the dominant autoantigenic peptide but stimulate Teff (disease causing) and Treg (protective) cells, respectively. This project will use validated structure-function systems to determine the phenotype and function of GP peptide specific Treg cells, how different HLA presentations of the same peptide affect T cell repertoire and phenotype, and to define how peptide-HLA/TCR interactions determine phenotype and function.
Immune Recognition Laboratory

PROF JAMIE ROSSJOHN  FAA FLSW FAHMS FMedSci
ARC Australian Laureate Fellow
Phone: (03) 9902 9236
Email: Jamie.Rossjohn@monash.edu
Lab webpage: https://rossjohnlab.com/

RESEARCH BACKGROUND

The academic research program within this laboratory is focused on defining the key molecular interactions underlying receptor recognition events that are the primary determinants of immunity. The laboratory’s research has provided an understanding of the basis of peptide, metabolite and lipid presentation – events that underpin protective immunity and deleterious immune reactivity. **The team’s research on anti-viral immunity** has provided an understanding of the factors that shape MHC-restriction (e.g. Nature Immunology 2015; Immunity 2016; Nature Rev Immunol 2018), while also demonstrating how the pre-TCR, a receptor crucial for T-cell development, functions by autonomous dimerization (Nature 2010). **In relation to aberrant T-cell reactivity**, our team has provided insight into alloreactivity (Immunity 2009), Tregs and autoimmunity (Nature, 2017), Celiac Disease (Immunity 2012, NSMB 2014, Cell 2019), rheumatoid arthritis (JEM 2013) and HLA-linked drug hypersensitivities (Nature 2012). **Regarding innate and innate-like recognition**, the team has shed light into how Natural Killer cell receptors interact with their cognate ligands and viral immunoevasins (Nature 2011; JEM 2016; NSMB 2017; Cell 2017; PNAS 2018). **Further, we have provided fundamental insight into TCR recognition of lipid-based antigens in protective and aberrant immunity** (e.g. Nature 2007; Nature Immunology 2016; Nature Communications 2016; Nature Immunology 2018). Most recently, our team identified the long sought after ligand for MAIT cells, namely showing that MAIT cells are activated by metabolites of vitamin B and can also respond to commonly prescribed therapeutics (Nature 2012, 2014; Nature Immunology 2016, 2017).

**Our research program uses numerous biochemical and biophysical techniques including protein expression and purification, surface plasmon resonance and three-dimensional structure determination with the use of the Australian Synchrotron.**

Further, cellular immunology techniques are taught within the laboratories of collaborators of the Rossjohn laboratory.

The laboratory is funded by the National Health & Medical Research Council (NHMRC), the Australian Research Council (ARC), the ARC Centre of Excellence in Advanced Molecular Imaging, Cancer Council Victoria, National Institutes of Health, Worldwide Cancer Research and Wellcome Trust. A large number of students and ECRs from this laboratory have been awarded various fellowships/honours including the Premier’s award, NHMRC Dora Lush Postgraduate research scholarships, CJ Martin Fellowships, Peter Doherty Fellowships and CDA fellowships, ARC QEII fellowship and Future fellowship, EMBO fellowship and Victoria Fellowships. Honours scholarships are available.

HONOURS PROJECTS

1) Investigating lipid-based immunity in the context of Mycobacterium tuberculosis infection.
2) Investigating the role of lipids in skin-based allergies (e.g. contact hypersensitivities).
3) A chemical/biochemical study into vitamin B metabolite recognition.
4) Investigating T cell mediated autoimmunity (e.g. Celiac Disease).
RESEARCH BACKGROUND

*Helicobacter pylori* is a causative agent of gastric and duodenal ulcers, mucosa-associated B-cell lymphoma and gastric adenocarcinoma. Although it is a definitive carcinogen, there is no effective vaccine against this bacterium. Standard *H. pylori* eradication therapy now fails in up to 30%-40% of patients, mainly due to an increase in clarithromycin resistance. There is a clear demand for new strategies to fight *H. pylori* infections, strategies that involve new or unconventional targets for drug design. A key to success with this lies in strong basic knowledge of the molecular basis of bacterial virulence and survival. Our laboratory focuses on the mechanisms of acid acclimation, damage to gastric epithelial cells and motility and chemotaxis. We use *in vitro* molecular biophysics and crystallography techniques to investigate structure and dynamics of biomolecules and formulate hypotheses about molecular mechanisms which we then test *in vivo* using genetics, enzymology and cell biology methods.

HONOURS PROJECTS

Dissecting architecture of high torque bacterial motor

The bacterial flagellar motor is a remarkable nanoscale molecular engine. *H. pylori* evolved to be highly motile in the very viscous mucus layer of the stomach, and its flagellar motor is specialised for locomotion in viscous liquids – it produces a significantly higher torque (turning force) than, for example, enteric bacteria. Preliminary cryo-electron tomography reconstruction of this motor revealed a unique protein cage that supports a wider power-generating ring allowing it to sustain the larger torque. Our aim is to unravel the make-up of this cage and the structural basis for its ability to recruit more force-generating units.

How does *H. pylori* sense environmental cues?

Many bacteria are motile. Chemotaxis, mediated by chemoreceptors, plays an important role in bacterial survival and virulence. In this project, we shall investigate what ligands such receptors recognize and why some molecules are attractants and some repellents, how binding to the receptor leads to signalling, how mutations in the sensor domain affect ligand specificity and, building on this, how bacterial chemoreceptors can be redesigned to recognize and respond to non-native ligands for innovative applications in biotechnology and bioengineering.

Applications are welcome from students with a strong interest in structural biology, X-ray crystallography, the biology of *H. pylori*, or protein biochemistry.
RESEARCH BACKGROUND

Inflammation is the body’s response to injury or infection. A key feature of inflamed tissues is the accumulation of leukocytes (white blood cells), which carry out a variety of defence and tissue repair functions. However, excessive or inappropriate leukocyte recruitment can give rise to the chronic pain and tissue damage observed in inflammatory diseases. Leukocyte recruitment in inflammation is regulated by chemokines, which are secreted at the site of injury or infection and then activate chemokine receptors, G protein-coupled receptors (GPCRs) expressed on the target leukocytes. Research in our lab aims to understand how the interactions of chemokines with their receptors control leukocyte recruitment and to develop novel ways of suppressing these mechanisms.

HONOURS/PhD PROJECTS

SIGNALLING MECHANISMS CONTROLLING RECRUITMENT OF WHITE BLOOD CELLS IN INFLAMMATION

In this project, we are studying the molecular details of how chemokines induce transmembrane signalling by their receptors and the intracellular signalling pathways that are activated by chemokine-receptor interactions. These studies will provide a solid mechanistic foundation for future development of selective therapeutic interventions.

NATURAL ANTI-INFLAMMATORY PROTEINS: CHEMOKINE INHIBITION BY TICK EVASINS

Ticks, which live on mammalian hosts, produce proteins called evasins, which interact with host chemokines and thereby prevent inflammatory responses, allowing the ticks to live longer on their hosts. We have discovered a large number of new evasin proteins and shown that they bind and inhibit human chemokines, suggesting they have enormous potential as chemokine-targeted anti-inflammatory agents. This research project focuses on characterising the interactions of evasins with chemokines. Our results will enable the development of evasin-like proteins that target specific groups of chemokines in inflammatory disease therapy.

TRAINING OPPORTUNITIES

We are seeking talented PhD, Honours and undergraduate research students. Students involved in these projects will develop high-level critical thinking, project planning and communication skills as well as a variety of technical skills in biochemistry, molecular biology, pharmacology and bioinformatics.
RESEARCH BACKGROUND

Microbial pathogens kills millions of people every year. We are in a critical situation, with raising drug resistance leading to fewer and fewer options for the treatment of serious infectious diseases. It is predicted that, if the current trends continue, by 2050 as many as 10 million people might die every year from drug-resistant infections.

The go-to strategy for overcoming antimicrobial drug resistance has been to develop new drugs. However, this approach has not worked so far, and microbes can rapidly develop resistance to any new drug. Therefore, new thinking is needed.

The Traven lab focuses on understanding how metabolic processes drive host-pathogen interactions and virulence of the major human fungal pathogen, Candida albicans. Candida sp are responsible for a large number of infections, including around 400,000 invasive, life-threatening disease that have high mortality.

The has a record of publications in top journals including Cell Metabolism, Cell Reports, PNAS, Molecular Cell, mBio, PLoS Genetics, and a recent review on metabolism in infection for EMBO Reports (2019). We collaborate broadly with groups in Australia and internationally to use comprehensive approaches to understand mechanisms of virulence and define new strategies to combat deadly human infections.

To work on these questions, we use a range of techniques, from molecular cell biology to live cell imaging and animal infection models. Our Honours students will receive highly relevant training and transferrable skills that can be broadly applied in biomedical science.

HONOURS PROJECTS

- Understanding how microbial pathogens reprogram their metabolism to conquer immune cells. Live cell imaging and molecular cell biology will be used to decipher how metabolic switches enable C. albicans to resist immune attack and drive infections.

- Deciphering the impact of short-chain fatty acids made by microbiota on virulence and innate immune interactions of C. albicans. C. albicans is a gut commensal and opportunistic human pathogen, but how microbiota-derived metabolites controls its virulence is poorly defined. This project uses molecular cell biology approaches to understand this question.
RESEARCH BACKGROUND

The projects centre broadly on the role of Natural Killer Cell receptors in disease progression. The projects will focus on the role of KIR receptors in the control of HIV replication and progression of haematological malignancies. It is well established that KIR receptors are grouped into activating and inhibitory sub-types that together dictate the cellular immune response. Yet, how these two receptor sub-types differ in terms of ligand recognition and how this plays out with regard to sensing and controlling HIV infection and other diseases is unknown. We are interested in characterising these receptors at the cellular, molecular and atomic level. See reference Vivian et al., Nature (2011) 479:401-5

Techniques and the Lab:

The projects will involve challenging and multi-disciplinary approaches. They will provide an opportunity to learn bacterial, insect and mammalian techniques for protein expression. Biophysical techniques for protein characterisation including small-angle X-ray scattering, analytical ultracentrifugation, surface-plasmon resonance and atomic resolution protein structure determination by X-ray crystallography. Also, immunological techniques including cell culture and flow cytometry. The laboratory is exceptionally well funded and well equipped and is host to a large number of helpful researchers with expertise in a diverse set of disciplines. In all, it provides an excellent research environment for students.

HONOURS PROJECTS

Project 1: The enigmatic receptor KIR2DL5.

KIR2DL5 is the least understood member of the KIR family. By sequence, KIR2DL5 is a hybrid of KIR3DL1 (see Vivian et al., Nature (2011) 479:401-5) and KIR2DL4 (see Moradi and Vivian J Biol Chem. (2015) 290(16): 10460–10471). Yet, KIR2LD5 has the properties of neither. This project will involve biophysical and functional characterisation of KIR2DL5. This is a an opportunity for the candidate to learn protein Chemistry X-ray crystallography and SAXS. The function of KIR2DL5 will be probed by cellular assays. This project is a collaboration with Prof. John Trowsdale, Cambridge Uni. UK.

Project 2: KIR3DL1 in acute myeloid leukaemia.

This project centres on KIR3DL1 polymorphism and how it translates to improved outcomes in hematopoietic stem cell transplantation for the treatment of haematological malignancy (Vivian et al. J. Exp. Med (2016)) Combining functional and clinical data, the aim is to understand KIR in donor selection for HSCT treatment of AML.

Project 3: The activating receptor KIR2DS5

KIR2DS5 is an activating KIR that was long thought to be a receptor without a ligand. We have recently identified the ligand for KIR2DL5 (unpublished). This project is an opportunity for the student to learn the structural/functional techniques listed above to validate these preliminary results and establish the framework for the field to understand KIR2DS5. This project is a collaboration with Prof. Peter Parham, Stanford Uni. USA and Prof. Andrew Brooks, Melb Uni.
RESEARCH BACKGROUND

The overarching goal of research in the Zaph lab is to define the cellular and molecular mechanisms that control immunity and inflammation at mucosal sites such as the intestine and the lung. The various subsets of immune and non-immune cells at mucosal sites are present in a tightly controlled equilibrium that when perturbed by infection, chemicals or genetic predisposition, results in dysregulated inflammation and diseases including asthma and allergy, inflammatory bowel diseases (IBDs), food allergies and cancer. Understanding the molecular and cellular principles underlying mucosal inflammation represents a potential target for identifying novel therapeutics for the treatment of these diseases.

HONOURS PROJECTS

**Project #1. Epigenetic regulation of mucosal immunity and inflammation.**
We have been at the forefront of defining the epigenetic mechanisms that control T cell differentiation and function (Lehnertz (2010) J. Exp. Med.; Antignano, (2014) J. Clin. Invest.), focusing on lysine methyltransferases (KMTs), enzymes that methylate histones to repress or activate gene expression. This project involves the characterization of the epigenomic regulators of T cell physiology and will focus on linking genome-wide histone modifications (via ChIP-Seq) to functional assays in vivo.

**Project #2. Retinoic acid, Hic1 and intestinal immune homeostasis.**
Micronutrients such as Vitamin A (and its derivative retinoic acid (RA)) play a critical role in intestinal immune homeostasis. However, the molecular mechanisms that link RA signaling to immune cell function in the gut are unclear. We have recently identified a role for the transcriptional repressor Hic1 as an RA-responsive gene that controls intestinal immune cell homeostasis and function. This project will use novel mouse models to define the role of Hic1 in immune cells during the steady state and following infection and inflammation.

**Project #3. Methylation is the new phosphorylation: Dynamic regulation of signal transduction by methylation.**
We have recently identified a novel role for the methyltransferase SETD7 in regulation of the Hippo/YAP signalling pathway (Oudhoff (2013) Dev. Cell; barsyte-lovejoy (2014) Proc. Natl. Acad. Sci. USA). We have now extended these findings to show that SETD7/YAP interactions control activation of the Wnt/β-Catenin pathway, and regulate intestinal regeneration and tumourigenesis. This project will define how SETD7-dependent methylation controls Wnt-dependent processes in the intestine.
RESEARCH BACKGROUND:
With the advent of personal genomic medicine a detailed understanding of gene function has never been more important. In to the future, our health may be monitored by regular “omics” measurements overlaid on our individual genomes. Each of us carries numerous “disease” mutations and countless further genetic variation, with mostly unknown consequences. My lab studies RNA metabolism: the birth, life and death of RNA molecules. A growing list of RNA-metabolic enzymes and binding proteins are implicated in intellectual disability, neuronal disorders and other diseases. I am motivated by a conviction that through the combined use of next generation technologies and evolutionary conservation in model organisms we can significantly accelerate discovery of basic gene function and the network-effect of loss-of-function mutations.

My laboratory makes extensive use RNA-seq, traditional wet-lab methodologies and computational approaches. Each of the project areas outlined below can be customised to a mix, that best suits interested applicants. Students with computational interests are especially encouraged to make contact, since this is one of the major growth area of the future.

HONOURS PROJECTS

PROJECT 1: Investigating the role of the natural compound Cordycepin in RNA expression: The addition of a poly(A)-tail is an essential step in mRNA biogenesis and protein translation. However, the common use of alternative polyadenylation sites is revealed by deep-sequencing. The purpose of such additional noncoding RNA is hotly debated and the mechanisms of its synthesis and general metabolism remain largely unknown. Cordycepin a natural compound with a long history in traditional medicine influences mRNA 3’-end dynamics. In this project, RNA dynamics will be analysed in yeast strains with mutations in genes implicated in various aspects of RNA metabolism. The level and position of adenylation in the transcriptome will be used to probe functions in RNA biogenesis, translation, and/or recycling.

PROJECT 2: Investigating the switch from silence to activation of translation: The poly(A)-tail that terminates mRNA can be specifically altered in the cytoplasm to regulate protein translation. Poly(A)-extension activates translation whereas poly(A)-trimming is associated with silencing. Tuning of gene expression by poly(A)-length change is common in the brain and germline, and is misregulated in disease. Here, the functional consequence of changes to adenylation state will be probed in neuronal lineages.

PROJECT 3: An Investigation into the host-pathogen synapse: An infection by microorganisms induces changes in both the host and the pathogen. Both sides reprogram gene expression in an attempt to over come the other’s defences. In the host, this means an activation of the immune response; in the pathogen, it means immune evasion. Here we will investigate how changes in RNA metabolism can influence the balance.
RESEARCH BACKGROUND

In germ cells a diverse array of small RNAs provide an important layer of complexity in gene regulation and genome organisation. These small RNAs can be genomically encoded and endogenously produced (endo-siRNAs). Recently it has been discovered that transgenerational inheritance of small RNAs is required for establishing epigenetic memory that identifies regions of the genome that should or should not be express. As outlined in the diagram below, this process is based on small RNAs binding to Argonout proteins (CSR-1 (green) or HRDE-1 (red)) that are subsequently imported into the nucleus where they recruit factors that either support or inhibit transcription (effector stage). Understanding how these process works is one of the most important questions in biology today.

<table>
<thead>
<tr>
<th>Initiation</th>
<th>Amplification</th>
<th>Effector</th>
<th>Transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>• pIRNAs</td>
<td>• RNA-dependent RNA polymerase</td>
<td>CSR-1</td>
<td>HMTase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRDE-1</td>
<td>HMTase</td>
</tr>
<tr>
<td>• exogenous RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HONOURS PROJECTS

Transgenerational Epigenetic inheritance

C. elegans is at the forefront of understanding small RNA biology and has been the platform upon which many paradigm-shifting concepts have emerged. Using this system, we are investigating proteins that are required for normal functions of specific small RNA pathways required for maintaining the epigenetic landscape of the genome. We have Honours projects that will investigate RNA-binding proteins required for multiple arms of the small RNA pathways. The projects will use a combination of biochemical, cell biology, genetic and genomic strategies to identify how this new player in the small RNA biology can impact so many different pathways.
RESEARCH BACKGROUND

Our group studies how the embryo develops with a view to understanding the basis for congenital diseases and those caused by a compromised fetal environment. In particular we are interested in understanding the developmental mechanism known as "branching morphogenesis", which is employed by a large number of organs to establish the tissue architecture required to facilitate exchange of nutrients, gases or waste in the adult organ. Understanding this process will provide insights into the developmental origins of congenital diseases and how the "normal" variations observed in the structure of organs are influenced by their experiences and exposures as an embryo.

HONOURS PROJECTS

Project 1: Can we cure renal cysts by inhibiting Aurora kinase A?

Abnormal kidney development is one of the most common birth defects. We are pursuing a number of projects aimed at understanding their mechanistic and biochemical basis with a particular focus on renal cyst development and vesicoureteral reflux (VUR). One project in this area will focus on examining cyst development in the kidney and explore the interactions between the primary cilia-associated proteins INPP5E, PKD1 and AURKA. You will use mouse and cell based models of genetic deletion and pharmacological inhibition to study how polycystic kidney disease arises and the role these proteins play in its initiation and progression.

Project 2: Characterising novel genes which cause congenital kidney disease

Our group is involved in an Australia-wide program which aims to identify novel genes in patients with kidney disease. Individuals with genetic kidney disease who do not have mutations in known genes will These individuals will have their genomes sequenced and we will then use CRISPR/Cas9 genome engineering approaches to model disease causing mutations in mice. Using these models, honours students will have a unique opportunity to establish how novel disease genes function in the kidney, how their protein products regulate cell biology and how their mutation leads to congenital renal malformations.
Mitophagy & mitochondrial quality control in disease

Dr Michael Lazarou
Email: michael.lazarou@monash.edu
Web: www.med.monash.edu.au/biochem/labs/lazarou-lab.html

RESEARCH BACKGROUND

Parkinson’s disease (PD) is one of the most common of the neurodegenerative disorders, affecting 1-2% of the population worldwide. Multiple lines of evidence place mitochondrial dysfunction as a central player in the pathogenesis of PD. Two proteins commonly mutated in familial PD, PINK1 and Parkin, play a key role in maintaining mitochondrial health by identifying damaged mitochondria and degrading them through a selective form of autophagy termed mitophagy (Lazarou et al., (2015) Nature). Our lab investigates the molecular mechanisms of PINK1/Parkin mitophagy. We are interested in how PINK1 and Parkin drive the sequestration of damaged mitochondria within double membrane structures called autophagosomes, before delivering them to lysosomes for degradation. The PINK1/Parkin mitophagy projects on offer provide experience with a variety of biochemical and cell biological techniques including state-of-the-art confocal microscopy, the latest generation of genome engineering technology (CRISPR/Cas9), tissue culture, western blotting, stable protein expression using retrovirus, molecular biology and mass spectrometry. These techniques enable students to gain experience in a range of scientific approaches and provide students with a strong scientific foundation to build on.

HONOURS PROJECTS

Project 1: Characterisation of novel PINK1/Parkin mitophagy factors Using quantitative proteomics of autophagy defective cell lines, we have identified a number of novel PINK1/Parkin mitophagy candidate proteins. Some of these proteins have predicted functions in vesicle trafficking and membrane fusion, while others have no known function. This project will utilise CRISPR/Cas9 gene editing to generate knockout cell lines of the putative novel mitophagy factors in order to determine whether the factors are required for PINK1/Parkin mitophagy. Furthermore, how the factors regulate the molecular signals that govern the clearance of defective mitochondria will be investigated. For example; are they required for autophagosome formation, or for the recognition of damaged mitochondria?

Project 2: Can bacterial infections predispose you to Parkinson’s Disease? There is a growing body of evidence linking inflammation to neurodegeneration in Parkinson Disease. Some of the latest research in the field has revealed that PINK1/Parkin mitophagy plays a key role in preventing inflammation (Sliter et al., (2018) Nature). PINK1 and Parkin clear damaged mitochondria before they rupture and release their mitochondrial DNA, which is the source of inflammation. This project will investigate whether pathogenic bacteria which damage mitochondria can cause excessive inflammation when mitophagy is defective. Bone derived macrophages from WT and Parkin KO mice will be infected with different bacteria (e.g. Salmonella or Legionella) to assess whether Parkin can play a protective role in mitigating mtDNA derived inflammation during infection. The role of other mitochondrial quality control systems will also be investigated with the aim to find new pathways that we can target to help prevent Parkinson’s Disease.
Organelle Biology/Advanced Cellular Imaging Lab
Dr Georg Ramm
Location: Building 75 ground floor and 13D level 2
e-mail: Georg.ramm@monash.edu
phone: 9905 1280
www.monash.edu/researchinfrastructure/cryo-em

Background: Our lab is focused on high-resolution imaging of cellular architecture and intracellular trafficking. We are the first in Australia to use cryo-tomography (on Titan Krios) in combination with cryo-focused ion beam milling (cryo-FIB on cryo-Helios) to reveal cellular structures at the highest resolution. We focus on fundamental cell biological problems that are relevant to human diseases. This includes the intracellular degradation of organelles by autophagy and mitophagy, mitochondrial ultrastructure and dynamics in healthy and stressed cells, and cellular structural changes during cell death.

Honours/PhD Project 1: High-resolution imaging of mitochondria under stress. Cryo-electron tomography (CET) allows for the highest resolution cellular imaging achievable at the moment. To get access to all areas of the cell we use CET in combination with cryo-focused ion beam milling to cut windows into thicker parts of the cell. In collaboration with the Kile lab, we were the first to show herniation of the inner mitochondrial membrane (green) through an apoptotic pore in the outer membrane (red) in apoptotic cells. We will use high resolution imaging to investigate how macromolecules such as ATP synthases (yellow) in the crista membrane (blue) are undergoing changes during apoptosis.

Honours/PhD Project 2: Our lab is applying and developing new imaging tools such as cryo-correlative light and electron microscopy to combine state of the art optical and electron microscopy techniques. The project will apply these high-resolution techniques to study the spatio-temporal regulation of intracellular organelle traffic in autophagy.

Honours/PhD Project 3: Molecular Imaging of key metabolic signalling nodes. We use cryo-EM to determine molecular structures of key molecules involved in the metabolic regulation of cells. While structural cryo-EM is traditionally being used to solve molecules larger than 100kDa, we will apply recent advances that allow for high resolution imaging of smaller molecules.
Mitochondrial biology & disease
Prof Mike Ryan
Phone: 99024909
E-mail: michael.ryan@monash.edu
Lab page: www.ryanlab.org

RESEARCH BACKGROUND
Yes, mitochondria are the powerhouses of our cells, but they are also important in other processes including apoptosis, innate immunity and in neurological diseases including Parkinson’s. Defective mitochondria cause degenerative diseases and often lead to infant death. We investigate the molecular and cellular processes related to mitochondrial function, dysfunction and disease, and dynamics.

Each student will perform a range of techniques, attend weekly lab meetings, and gain expertise for future scientific and non-scientific careers. Students will be mentored in the lab by one of our friendly postdocs.

HONOURS PROJECTS

Understanding the function of novel human proteins
Complex I of the mitochondrial respiratory chain is a huge machine containing 44 different subunits. Following up from our study, (Stroud et al., Nature 2016) we are identifying and characterising proteins involved in complex I assembly and how their mutations cause disease. This involves using CRISPR/Cas9 to make specific gene knockouts for study plus other approaches. We also wish to determine how specific mutations in complex I subunits and assembly factors cause defects leading to disease by re-expressing these mutants in knockout cell lines. The study will provide important new insights into an essential process.

Determining the function of proteins that cause mitochondrial disease
With next-gen sequencing approaches, pathogenic gene mutations have been identified but what is often lagging is understanding what the protein encoded by the gene actually does. This project will involve characterising one such protein whose mutation causes mitochondrial disease to determine how it functions. The work will provide important new insights into pathogenic mechanisms of mitochondrial disease.

Investigating the importance of novel mitochondrial dynamics proteins
Mitochondria form dynamic networks that rely on fission, fusion and trafficking along the cytoskeleton. A number of proteins involved to specifically function in mitochondrial dynamics have been identified but they have not been properly characterised. This project will investigate the importance of three proteins in these activities. We will establish their function and the interactions they make to determine new insights.

Techniques may include: CRISPR/Cas9, cloning, tissue culture, fluorescence microscopy, growth assays, pull downs and proteomic analysis, SDS-PAGE, blue native PAGE & western blot analysis.

For more info on our great team and where our former members have gone, browse www.ryanlab.org/lab-team
Mass spectrometry laboratory
Proteomics, Metabolomics and Lipidomics
Dr Ralf Schittenhelm
Phone:  990 54324
Email:  ralf.schittenhelm@monash.edu
https://www.monash.edu/researchinfrastructure/mpmf

RESEARCH BACKGROUND

Mass spectrometry has emerged as the leading technology to comprehensively identify and quantify proteins and other biomolecules in virtually every biological sample.

Our lab (the Monash Proteomics & Metabolomics Facility) – equipped with the latest mass spectrometric instrumentation – focuses on utilizing and improving mass spectrometry for biochemical and clinical research.

HONOURS PROJECTS

All honours (and PhD) projects are highly technology-driven. They are about establishing, applying or developing state-of-the-art techniques or software scripts to improve, automate and simplify mass spectrometric analyses.

While a background in mass spectrometry and/or bioinformatics is beneficial, it is not mandatory and we do consider every student’s interest and skill set to find a suitable project.

The following projects are just a few examples, which highlight the diversity of the available projects:

1) Establishment of non-canonical phosphoproteomics
   Phosphorylation events on amino acids other than serine, threonine and tyrosine are believed to play an important role in cancer cell biology. We would like to establish a robust methodology to study this ‘dark phosphoproteome’ and analyze various cancer cell lines.

2) Development of new features to improve MZMine
   MZMine is an open-source software package, which is heavily used by the metabolomics and lipidomics community. We would like to contribute new features and improve existing functions to enhance the functionality of MZMine.

3) In-depth comparison of various mass spectrometric quantification methods across multiple instruments
   Several methodologies have been developed in the last years to accurately quantify peptides and proteins in complex biological samples such as DIA/SWATH or DDA. We would like to thoroughly compare the performance of these methodologies on multiple mass spectrometers (for example Orbitrap vs TOF) to understand their limitations and benefits.
RESEARCH BACKGROUND
Our group aims to discover and understand new signalling pathways and processes connecting nutrient supply and demand in the context of health and diseases such as diabetes and cancer. We conduct genetic and recombinant virus gain- and loss-of-function experiments in mouse models to uncover novel aspects of metabolic control.

HONOURS PROJECTS

Projects:

a. **Systemic metabolic crosstalk connecting sarcopenia and obesity.** The comorbidities of sarcopenia (i.e. muscle shrinkage and weakness) and diabetes are often associated in obese people but the underlying mechanisms are not understood at all. Here we have serendipitously discovered a liver metabolic-hormonal axis that connects diabetes and sarcopenia. This project will require implementation of mouse metabolic phenotyping using genetic and adeno-associated virus mediated loss- and gain-of-function enzyme(s) controlling metabolism.

b. **Novel nutrient signalling pathways connecting dietary protein supply and metabolism.** The dietary protein to carbohydrate ratio is a powerful environmental variable that determines longevity and disease risk including diabetes and cancer. Here we will conduct proteomic and metabolomic screening of mouse samples already collected in order to identify novel liver signalling nodes in metabolic control.

c. **Novel metabolic signalling pathways downstream of amino acid restriction.** We have previously shown that the dietary protein restriction can retard the risk of metabolic disease via the hormone FGF21. Here we aim to identify the precise amino acids responsible for this as well as the intermediary signalling molecules involved in linking metabolism and transcription.
RESEARCH BACKGROUND
Our group focuses on peptide-based drug design and biomembrane nanotechnology. We are developing novel compounds that allow us to exploit the potential of peptides as drugs. We are currently applying our technology to the development of new compounds for treatments of cardiovascular disease and new bio- and nano-materials for tissue engineering and drug delivery. Our membrane nanotechnology projects involve studying the mechanism of antimicrobial peptide resistance, apoptosis and angiotensin receptor function.

The long-term aim of these studies is to increase our understanding of the molecular basis of peptide and protein function and allow the rational design of peptide and protein based therapeutics.

RESEARCH PROJECTS

HONOURS/PHD PROJECT 1:  
PEPTIDE-BASED NANOMATERIALS
Supramolecular self-assembly represents a powerful approach to the design of functional nanomaterials in biomedicine and engineering applications. Peptide-based materials offer the advantages of biological compatibility, ease of synthesis, low toxicity and functionalisability. This project involves the design and synthesis of novel self-assembled nano-materials for application as novel agents in wound healing and drug delivery.

HONOURS/PHD PROJECT 2:  
MECHANISM OF RESISTANCE TO ANTIMICROBIAL PEPTIDES
Antibiotic resistance continues to emerge and intensify and while antimicrobial peptides (AMPs) are a promising alternative to current antibiotics, bacteria have also evolved a range of resistance mechanisms to AMPs, which include thickening of the cell wall, modification of the phospholipid composition, changing the net surface charge, increasing the membrane fluidity, releasing proteinases to degrade the peptides and discharging amino acids into the environment to reduce hypo-osmotic stress. This project aims to characterise how bacteria transiently modify their lipid content and repel the action of AMPs and how the membrane barrier can be more effectively targeted with agents tailored to lyse compositionally different membranes.

HONOURS/PHD PROJECT 3:  
NEW LIGANDS FOR CARDIOVASCULAR DISEASE
Cardiovascular disease affects one in three adults in Australia and kills one Australian every 11 minutes. Hypertension, as the main risk factor, is managed by either AT1R antagonists or ACE inhibitors which act on the angiotensin 1 receptor (AT1R). The binding of Ang II to AT1R mediates vasoconstriction, cell growth, and remodelling leading to increased blood pressure (BP); cardiac, renal, and vascular hypertrophy; and fibrosis, which is the molecular basis for the clinical application of AT1R antagonism. While this receptor is a major therapeutic target, the therapeutic regimes can be complex and response to drugs is highly variable. This project involves the design and synthesis of novel receptor ligands involved in cardiovascular disease.
RESEARCH BACKGROUND

We are interested in understanding the structure, folding and dynamics of proteins that play a role in human physiology and disease. Our current research focus is to harness this knowledge to design and engineer proteins for therapeutic and diagnostic application. Using a combination of protein engineering, protein crystallography, biophysics and computational techniques, three current research focuses are:

HONOURS PROJECTS

Design and engineering of antibody and non-antibody based therapeutics
We are interested in designing novel proteins based upon the antibody fold as well as the adnectin (FN3) scaffold. The ultimate aim is the development of new biologics for therapeutic and diagnostic use.

Design of new α-1 antitrypsin molecules as therapeutics
We recently designed a novel serine protease inhibitor that possessed the same activity as α-1 antitrypsin (AAT) without the unwanted instability and aggregation properties. We are engineering this molecule further to generate a panel of optimised AAT variants as new pre-clinical candidates for use in human AAT deficiency augmentation therapy.

Novel biosensors
In collaboration with Dr Simon Corrie's Nanosensor Engineering Lab in Dept. Chemical Engineering. Projects focus on designing antigen-binding domains that change their fluorescent properties when bound to antigens, and also incorporating these domains into nanoparticles for biosensing applications.

Techniques Used
General molecular biology (PCR cloning, mutagenesis) and biochemistry, X-ray crystallography & SAXS (Australian Synchrotron), directed evolution (yeast surface display, FACS), biophysics (energetics and kinetics of folding and biomolecular interactions), bioinformatics, molecular modelling and dynamics simulations (CPU/GPU supercomputers). Students will receive training in all these techniques.

See https://www.facebook.com/BuckleLab/
And http://monash.edu/research/explore/en/persons/search.html
RESEARCH BACKGROUND

My group investigates two main topics: the biosynthesis of the glycopeptide antibiotics (GPA) and the development of novel antibiotics to treat serious bacterial pathogens. GPAs include vancomycin and their natural biosynthesis remains the only route to their commercial production: by studying and understanding GPA biosynthesis we aim to identify new, more effective antibiotics and how to produce them. We also develop new antimicrobial therapies against multi-drug resistant *Staphylococcus aureus* that exploit a combined immune/antibiotic approach. Our projects are multi-disciplinary, supported by our expertise in chemical synthesis, X-ray crystallography, enzymatic catalysis & protein engineering.

HONOURS PROJECTS

**Project 1: Combining immune recruitment with antibiotics to kill Golden Staph** – antibiotics have undoubtedly improved life expectancy and underpin modern medicine: however, increasing resistance means that society is badly in need of new approaches to treat antibiotic resistant bacterial infections. In this project, you will explore combinations of a clinical antibiotic and innate immunity peptides that recruit the immune system to target and treat bacterial infections. This will involve generating modified antibiotics, testing their activity against clinically relevant isolates of the superbug *Staphylococcus aureus* (Golden Staph) and assessing the immune recruitment effects of antimicrobial peptides on neutrophils, the immune systems “first responders” involved in fighting bacterial infections.

**Project 2: Structural and biochemical characterisation of an unusual peptide antibiotic producing assembly line** – non-ribosomal peptide synthetases (NRPSs) are amazing peptide assembly lines that produce highly modified and bioactive peptides. Antibiotics are without doubt one of the most important classes of natural product, and many are produced by NRPS assembly lines. Whilst the modular architecture of most NRPSs is conceptually well understood, we known comparatively little about the structure of complete multi-modular assembly lines. In this project you will reconstitute an unusual tripeptide antibiotic producing NRPS machinery, characterise the behaviour of this machinery *in vitro* and structurally characterise the assembly line using cryo-electron microscopy (Cryo-EM).

**Project 3: Understanding the biosynthesis of the glycopeptide antibiotics via ancestral sequence reconstruction** – the glycopeptide antibiotics are produced by the interplay of both a fascinating enzymatic peptide assembly line – a non-ribosomal peptide synthetase (NRPS) – and Cytochrome P450 enzymes. By reconstructing the sequences of ancestral versions of these enzymes, our aim is to explore the substrate tolerance and catalytic properties of these potential biocatalysts. In this project you will produce and characterise ancestral variants of the Cytochrome P450 enzymes responsible for the antibiotic activity of the glycopeptide antibiotics in terms of their structure and function.
How genes are turned off and then maintained repressed throughout countless cell divisions? During embryonic development, large sets of lineage-specific genes become transcriptionally inactive and spatially compacted. The process of maintaining genes in a repressed state requires chromatin modifiers: enzymes that modify DNA and histone proteins at the immediate vicinity of repressed genes.

Long-term repression of developmentally-expressed genes, often referred to as epigenetic repression, is dysregulated in most types of cancer, leading to the expression of oncogenes and the repression of tumour suppressor genes. Chromatin modifiers considered promising anticancer drug targets and there is, therefore, a large interest to understand how they are regulated at the molecular level. The Davidovich lab utilises cutting edge approaches in structural biology, biochemistry and cell biology in order to determine how chromatin modifiers are regulated at the molecular level.

HONOURS PROJECTS

**Project 1:** Reconstitute epigenetic regulation in the test tube: "What I cannot create, I do not understand." (Richard Feynman). For a mechanistic study of epigenetic repression, the entire process is reconstituted in the test tube. DNA of entire genes is reconstituted with nucleosomes and then targeted by selected epigenetic modifiers and transcriptional regulators. Structure-function studies are carried using high-resolution cryo-EM, X-ray crystallography, electron cryotomography, mass spectrometry-based approaches to map protein–protein, protein–DNA and protein–RNA interactions, and next-generation sequencing-based approaches to detect molecular interactions and enzymatic activity.

**Project 2:** Develop a platform for drug screens, to target epigenetic modifiers in cancer: Epigenetic modifier complexes, including multiple protein subunits, DNA, RNA and nucleosomes, will be subjected to in vitro reconstitution combined with binding and enzymatic assays. The project will set the foundation for the development of a platform to screen for highly specific drugs selectively targeting subtypes of epigenetic modifiers.

**Project 3:** Epigenetic regulation of oncogenes and developmentally expressed genes: The molecular mechanism of gene repression and derepression (activation) will be identified through the utilization of genome editing techniques (CRISPR/Cas9), combined with proteomic approaches, next-generation sequencing techniques and genetic screens.
Technology development for in situ structural biology
A/Prof Alex de Marco
Phone: 0399053791
Email: alex.demarco@monash.edu
Lab webpage: www.demarco-lab.com

RESEARCH BACKGROUND

Biological systems are extremely complex and dynamic. Over the past 10 years there has been an explosion of technical advancements in both and Electron Microscopy. Those advancements led to the possibility to obtain structural information about any protein directly while still within their natural environment: the cell.

HONOURS PROJECTS

All projects are conducted in the framework of the ARC centre of Excellence for Molecular Imaging. The first two are adequate for a student with a background in physics and interest in Optics. The third project would be suitable for a student with a background in Chemistry and interested in Organic and Analytical Chemistry.

Development of cryo-correlative FIB milling
Through the use of correlative microscopy it is possible to identify any event in a cell, study its dynamics and resolve the structural conformation. In my lab we are developing an ultra-stable cryo-light microscope that will be able to perform super-resolution imaging on cryopreserved samples. We will use the information retrieved through this imaging technique to drive the isolation of regions of interest through cryoFIB milling. Those regions will be then imaged using cryo-Electron Tomography.
The project will need some basic coding capabilities.

Development of fast super-resolution Light Sheet Microscopy
Super resolution light microscopy is an extremely powerful tool in cell biology, unfortunately it is limited by either its time resolution or the photon dose required, which can be phototoxic. We will combine structured illumination and the use of a light-sheet in order to obtain fast and low-dose super-resolution light microscopy.
The project will involve ray-tracing and design of optical systems.
RESEARCH BACKGROUND

Pore forming toxins (PFTs) are fascinating proteins have the ability to breach cell membranes by forming pores in the lipid bilayer. These pores can be either lytic to the target cell, e.g. by osmotic flux, or the pores can mediate the translocation of toxic proteins into the target cell. They are found in all kingdoms of life, especially pathogenic bacteria. My research looks at the structure and evolution of pore forming toxins such as the MAC, fungal toxins and aerolysin. PFTs are being developed for Third Generation Sequencing, biosensors and pest control in agriculture.

HONOURS PROJECTS

Using immune system hole-punching proteins to fight inflammation and cancer

The Membrane Attack Complex (MAC) is a giant hole-punching complex that targets and kills invading bacteria and parasites. Insufficient MAC leads to susceptibility to bacterial infection. Too much MAC assembly leads to unwanted inflammation. Currently we do not understand how the MAC assembles on the target membrane or how it is inhibited by CD59.

This study of MAC will tell exactly how the MAC changes shape to punch into the membrane and how we can stop this hole-punching action in inflammation or to fight cancer. This will include the use of high resolution techniques including Single Particle-cryo Electron Microscopy and cryo-Electron Tomography.

Pore forming toxins in agriculture and biosecurity

Pore forming toxins are used in Australian cotton and canola crops to convey resistance to specific insect pests and reduce the dependence on chemical pesticides. However there is emerging resistance in insect populations to the actions of these toxins. Nematodes can also be an issue in optimal growth of crops. We are working on several new pore forming toxins that are insecticidal or nematocidal that can be used to enhance the resistance of crops to insects and nematodes.

Pore forming proteins in nanotechnology

Pore forming proteins in the lab will be used to develop a “proof of concept” experiment to test if MACPF/CDC pore forming toxins can be used in electrophysiology sensing. Electrophysiology sensing can be applied to develop biosensors or third generation sequencing (TSG). Collaboration with Oxford Nanopore Technologies (UK).
RESEARCH BACKGROUND
Alterations in the finely tuned balance of signalling pathways underlie the pathogenesis of a host of diseases from cancer to inflammation. Capturing an atomic view of ‘signaling in action’ by determining the structures of key signaling components is central to the development of targeted therapeutics. The laboratory’s research vision is to determine structures of critical multi-component protein complexes formed by tumour-suppressor proteins, and oncogenes. This resolution is enabled by combining the latest advances in single-particle cryoEM and crystallography with advanced single-cell fluorescence techniques. Importantly, the incorporation of proteins into signalling complexes often reveals unique sites that can be therapeutically exploited to both increase specificity of medicines and decrease unwanted ‘on target’ side effects. In parallel, the team has a long-term aim to translate key mechanistic findings on the anti-inflammatory signalling of IL-1 family cytokines to the clinic.

HONOURS PROJECTS
Project 1. IL-1 family receptors in inflammation.
Interleukin-1 (IL-1) family cytokines play key roles in the initiation and regulation of innate immunity and inflammation. In 2015, Dr. Ellisdon (BDI) and Prof. Whisstock (BDI) formed a collaboration with A. Prof. M. Nold and Dr. C. Nold (Hudson Institute) to translate the anti-inflammatory activity of IL-1 family cytokines to the clinic. Publications from the collaboration include studies in Science Immunology (Ellisdon et al, 2017) and Nature Immunology (Nold-Petry et al, 2015). Current projects include harnessing X-ray crystallography to understand the structural basis of IL-1 receptor signalling in inflammation.

Project 2. CryoEM and crystallography of GEF and GAP complexes in cancer.
RhoGTPases are small G protein members of the Ras superfamily that regulate cytoskeletal organization, cell-cycle progression and gene expression, and their dysregulation drives tumourigenesis and metastatic dissemination. Guanine-nucleotide-exchange factors (GEFs) turn on signalling by catalysing the exchange of GDP for GTP on target G-proteins, whereas GTPase-activating proteins (GAPs) terminate signalling by promoting GTP hydrolysis.
Current projects include characterisation of the activation of the metastatic factor and GEF P-Rex1 at the plasma membrane by Gβγ, gaining a mechanistic understanding of the inhibitory complex formed between the tumour-suppressor protein PTEN and the oncogenic GEF P-Rex2, and determining the first architectural insights into the complete tuberous sclerosis complex (TSC).
RESEARCH BACKGROUND

Our lab uses cryogenic electron microscopy (cryo-EM) to elucidate the structure and dynamics of large macromolecules involved in processes of fundamental biological and medical importance. In addition, we develop new computational methods for solving the most challenging problems in cryo-EM image processing and integrative structural biology. Biological topics include cancer biology, transcription regulation and mRNA export. Cryo-EM images are acquired at the newly established Clive and Vera Ramaciotti Centre for Structural Cryo-EM, housing the world-class FEI Titan KRIOS instrument.

HONOURS PROJECTS

1) Cryo-EM of the housekeeping transcription initiation complex
2) Elucidating the role of SAGA in transcription elongation by a combination of biochemical methods and cryo-EM
3) New computational methods for cryo-EM image processing & integrative structural biology

For more information visit our website: http://simplecryoem.com
Fibrinolysis and Wound Healing

Dr Ruby Law  
Phone: +61 3 99039308  
Email: ruby.law@monash.edu  
Lab webpage: https://research.monash.edu/en/persons/ruby-law

RESEARCH BACKGROUND

Plasmin plays a vital role in fibrinolysis, tissue remodeling, wound healing and cell migration. During traumatic injuries and systemic pathogen infections, inhibition of plasmin and fibrinolysis save lives. On the other hand, in the case of ischemic stroke and pulmonary embolism, therapeutically promoting plasmin formation promotes blood clot dissolution and blood flow. Our studies investigate the intermolecular structure and function relationships of the plasminogen activation system.

*Key research techniques:* X-ray crystallography, cryo-electron microscopy, enzyme kinetics, flow cytometry, small angle x-ray scattering, surface plasmon resonance, mass spectrometry and novel monoclonal antibody discovery.

HONOURS PROJECTS

1. *How does Streptococcus pyogenes hijack plasminogen for invasion? (with Dr Adam Quek)*

2. *Mechanism for plasminogen mediated wound healing (With Blake Mazzitelli)*

3. *Improving the outcome of tissue transplantation therapy (collaboration with Prof Whisstock)*

The group A streptococcal plasminogen-binding M protein and plasminogen kringle5 (J Mol Biol. 2019 2836(19):30424)  
Protein conformational change measured by small angle x-ray scattering
RESEARCH BACKGROUND

Professor James Whisstock is an Australian Laureate Fellow, Director of the ARC Centre of Excellence in Advanced Molecular Imaging and Scientific Head of EMBL Australia. The Whisstock laboratory uses X-ray crystallography and cryo-electron microscopy, small angle x-ray scattering, bioinformatics, cell biology, biophysics techniques and monoclonal technology to study inflammation, infection, immunity, blood clotting, cell signaling and developmental biology.

These studies are focused on knowledge-based research as well as the translation of such knowledge in the development of therapeutics and diagnostics for inflammation, immune driven disorders, cancer, neurological disorders and thrombotic diseases.

HONOURS PROJECTS:

1. Improving the outcome of tissue transplantation therapy (in collaboration with Dr Ruby Law)

2. Role of pore forming toxin in natural killer cell mediated immunity

3. Lateral transfer of antibiotic resistance genes in bacteria

4. Structural studies on pathological thrombi by cryo-EM (in collaboration with Dr Alex de Marco)

5. Development of image processing tools for high throughput 3D-electron Microscopy (in collaboration with Dr Alex de Marco)

Component of the bacterial conjugation complex (Nat Comm 9:3732)
RESEARCH BACKGROUND AND PROJECTS

Protein-RNA interactions in antiviral cellular defence and inflammation
Protein-RNA interactions are integral to cellular biology – both in normal cellular function and also in cells subject to the stresses of viral invasion. Proteins are responsible for the detection of viral RNA, and initiation of the innate immune response. Proteins direct post-transcriptional regulation of cytokines produced as a result of cellular stress, and are responsible for preventing their over-expression. In some cases, cellular proteins that normally function in translational control are hijacked by viral RNA as part of the viral mechanism of replication in the cell. Underlying each of these types molecular events are intricate and specialised molecular interactions. Their understanding would greatly advance our knowledge of antiviral cellular defence and potentially lead to new means to combat virus-related disease and inflammatory disorders.

Our lab has specialised in the study of protein-RNA interactions, using biophysical and structural tools to better understand the basis for their affinity, specificity and conformational consequences underlying their mechanism of action. Our objective is to delineate specific protein-RNA systems relevant to antiviral cellular defence.

Figure 1. Protein-RNA interactions underlie many cellular events upon viral invasion. The RIG-I family detect foreign dsRNA and initiate the immune response, ARE-binding RBPs dictate cytokine expression, and viral RNA hijacks PCBP for translation and replication.
HONOURS PROJECTS IN 2020 (cont)

Project 1: Structural characterisation of picornavirus RNA/protein complexes.

*Picornaviridae* family are positive strand RNA viruses with many members including enteroviruses (poliovirus and coxsackieviruses), human rhinoviruses, encephalomyocarditis virus, aphthoviruses and hepatitis A virus. Members of the *Picornaviridae* family cause a range of significant diseases such as paralysis, hand-foot-and-mouth disease, the common cold, myocarditis and hepatitis. Replication of the viral RNA requires the formation of a specific interaction between viral “stem loop IV” and poly-C-binding protein (PCBP). We are currently investigating the structural basis of this interaction that is required for ribosome docking of the viral RNA as a potential new anti-viral target.

![Figure 2. Viral Stem Loop IV interactions with PCBP.](image)

(A) The poliovirus SLIV interacts with PCBP via three C-rich loops. (B) structural studies of individual PCBP domains reveal precise basis for molecular recognition. (C) structural studies using SAXS give overall shape information about complexes.

Project 2: TIA proteins in RNA recognition and stress granule formation.

One of the cell’s primary rapid responses to stress is to sequester specific proteins and RNA into dense clusters known as “stress granules” (SGs). In this way the proteins and RNA are removed from their normal cytoplasmic sites of activity, and held at bay until the stress is relieved. This process is essential for regulating the expression of pro-inflammatory proteins as well as stress-response proteins and oncoproteins. Accordingly, improper SG formation is implicated in many pathologies including inflammation and cancer and neurodegenerative diseases. We are currently investigating the way in which TIA proteins recognise RNA and self-associate to form stress granules.

![Figure 2. TIA protein interactions](image)

(A) The crystal structure of TIA protein individual domains reveal the basis for recognition of target mRNA. (B) stress granule formation in cells can be initiated by TIA proteins.

These (and other projects in the Wilce lab) are currently supported by NHMRC funding. Please come and speak to us to find out more!