INFORMATION ON RESEARCH PROGRAMS FOR 2018 HONOURS AND POSTGRADUATE STUDENTS

BIOCHEMISTRY AND MOLECULAR BIOLOGY

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RESEARCH BACKGROUND
We work on an immunologically specialised subset of structural cells called fibroblastic reticular cells. We and others have shown that fibroblasts in lymph nodes and organs of immunological relevance are fundamental to healthy immune function, directly maintaining T and B cell survival, and directing their migration and interaction with their environment and dendritic 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) show strong negative correlation with survival and response to immunotherapy (see figure, below), yet our understanding of their function is still in its infancy, due mainly to a lack of effective techniques for their study. We have recently developed effective techniques to purify CAFs from fresh human tumours and we show that human CAFs directly impair T cell activation.

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 (co-supervised with Dr Konstantin Knoblich)

Project 1: Effective isolation of cancer-associated fibroblasts. We have developed new methods to isolate living CAFs from fresh tumour tissue in large numbers, allowing in-depth profiling of different CAF populations for the first time. We are looking for a highly capable student to help us define the CAF populations we isolate, using state-of-the-art flow cytometry, culture, imaging and molecular techniques. If the results warrant, the student will help co-author a manuscript describing the novel technique.

Project 2: How do CAFs interfere with anti-tumour immunity? As the first lab to directly profile freshly isolated, uncultured CAF subsets, 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 using primary CAF cultures and human leukocytes isolated from blood, validated using relevant mouse models of cancer. Candidate genes include CXCL12, CSF1, IL-34, IL-1, or others of your choice, chosen in consultation with your supervisors. Experimental approaches include use of neutralising/blocking antibodies, gene knockdown, migration assays, T cell activation assays, and T and B cell survival assays. The project is flexible but systematic in its approach, to maximise the chance of the student generating interesting, publishable outcomes.

The response of patients to immunotherapy in clinical trials correlates with the proportion of CAFs showing an activated phenotype (unpublished data).
RESEARCH BACKGROUND

We work on an immunologically specialised subset of structural cells called fibroblastic reticular cells. This is a high impact, growing area of immunology. We and others have shown that fibroblasts in lymph nodes are fundamental to healthy immune function, through interactions with T cells, B cells, dendritic cells and macrophages, directly supporting cell survival, function and migration. However, most data has been obtained in animal models, and our initial studies of human fibroblasts isolated from tonsils has shown that these cells can utilise entirely different molecular mechanisms to mouse cells (manuscript submitted). Our current focus is therefore heavily human, and the results we have obtained so far have been surprising, exciting, relevant and novel.

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 and prioritise student inclusion on manuscripts where the data warrant.

HONOURS PROJECTS (co-supervised with Dr Anne Fletcher)

Project 1: In-depth profiling of human tonsils. Our field uses mouse lymph nodes as models for human secondary lymphoid organs, but surprisingly little is known about their human counterparts. We have exciting preliminary data that suggests that the microenvironment of human tonsils looks very different to how our field expects them to, based on mouse studies. We are looking for a creative, driven student to comprehensively image human donor tonsils and profile the cell populations within by imaging, flow cytometry, and transcriptomics, compared with mouse lymph nodes draining similar areas.

Shown: Human tonsil tissue with fibroblasts in red, T cells in green, proliferating cells in blue and nuclei in grey. There are many things about these images that we are surprised about – too many to describe here – please come and discuss if you are interested!

Project 2: Identification of factors influencing innate immune cells. We have evidence that supports an undescribed relationship between fibroblasts and innate immune cells such as monocytes and NK cells. In particular our data suggests that survival and education of the immune cells is highly dependent on the stromal cell network. We would love to work with a talented student to investigate the factors influencing these signals through ex vivo manipulation.

The student would use flow cytometry, mass spectrometry, transcriptomics and imaging to identify the targets and analyse their potential in migration assays. These targets could be evaluated by conditional knockdown assays and other molecular techniques.
RESEARCH BACKGROUND
Our group aims to discover and understand new signalling pathways and processes connecting nutrient supply and demand in the context of heath 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 pathway in the liver metabolic-hormonal axis that connects diabetes and sarcopenia. This project is almost complete and 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 (Maida et al. J Clin Invest 126(9), 2016). 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

A. Proteases in immune defence. Cytotoxic lymphocytes kill infected or cancer cells by releasing proteases (granzymes) which enter the target cell via the pore-forming protein, perforin (Fig). Granzyme B kills cells due to its ability to activate caspases, and is one of the most cytotoxic proteases known. Other granzymes, and related proteases such as cathepsin G, activate cytokine signalling.

B. Regulation of proteases by serpins. Serpins trap and inactivate proteases. Some intracellular serpins protect cells against their own proteases e.g. Serpinb9 protects cytotoxic lymphocytes against granzyme B. Serpin deficiency or misfolding results in blood clots, immune dysfunction, lung and liver disease, cancer or dementia. SerpinA1 misfolding leads to liver and lung disease. We have shown that Serpinb6 deficiency causes inner ear degeneration and hearing loss.

C. Perforin-like molecules in immunity. MPEG1 is an ancient protein related to perforin, and it is found in phagocytes of organisms ranging from sponges to humans. Its molecular role is entirely unknown, but it is suggested to perforate phagocytosed microbes.

HONOURS PROJECTS

To study the above proteins in health and disease we use advanced techniques in molecular cell biology. These include recombinant protein production and analysis, gene manipulation, RNA interference, proteomics, bioinformatics, cell culture and confocal imaging, and the analysis of model organisms such as "knockout" mice and zebrafish. Projects are available in the following areas:

**Cathepsin G.** Inhibitors or neutralizing antibodies to this protease may be useful as anti-inflammatory drugs. We will produce recombinant cathepsin G in yeast or E.coli, for analysis using molecular approaches. New substrates will be identified using proteomics. Recently generated monoclonal antibodies will be tested for neutralizing ability.

**Serpins and cell death.** 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. SerpinA1 misfolding leads to liver cell death, resulting in liver and lung disease. We have made transgenic zebrafish expressing human SerpinA1, and will use them to study the liver disease process, and test inhibitors that could be candidates for human therapeutic development. Zebrafish projects are co-supervised by Dr. R. Bryson-Richardson (School of Biological Sciences).

**MPEG1.** We have made knockout mice lacking MPEG1. We are using these mice to study the expression and role of MPEG1 in the developing and mature immune systems. Using cultured cells we are also investigating its biosynthesis and subcellular localization in phagocytes.
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 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.

2018 HONOURS/PhD PROJECTS:

1. Glucocorticoid-regulated pathways in the pre-term 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 development 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).
Perturbations in cellular signalling play a fundamental role in human cancer and provide the rationale for many targeted therapies. The goal of the Signalling Network Laboratory is to characterize at the molecular level how signalling is altered in cancer, and thereby identify novel therapeutic strategies for particular poor prognosis human cancers, as well as biomarkers that aid classification of patients towards optimal treatments. Ultimately this work will lead to improved treatments for cancer patients with resulting reductions in morbidity and mortality.

The Signalling Network Laboratory utilises a variety of molecular, cellular and biochemical techniques, including mass spectrometry (MS)-based phosphoproteomics and kinomics, siRNA library screens, cellular imaging and protein-protein interaction analysis. In addition, bioinformatic approaches are used to analyse our datasets and integrate these with publically-available data from cancer genome studies and functional genomic screens.

See laboratory website for more information: http://www.med.monash.edu.au/biochem/labs/daly/

PROJECT 1: Novel oncogenes in breast cancer
Our laboratory has a major interest in breast cancer, particularly the triple negative/basal breast cancer subtype, which is associated with poor prognosis and lacks targeted therapies. We have used a variety of approaches, including phosphoproteomic, kinomic and functional screens, to identify novel oncogenes in triple negative breast cancer, which include several poorly-characterized protein kinases as well as the pseudokinase SgK269/PEAK1. This project will use a variety of techniques, including si/shRNA knockdown, CrispR/Cas9 gene editing, use of small molecule drugs, cell signalling and biological assays, to characterize the functional roles of these novel oncogenes and evaluate them as potential therapeutic targets.

PROJECT 2: The role of the oncogene Src in human cancer
The tyrosine kinase Src was the first proto-oncogene to be identified, and it is now known that Src plays an important role in several human cancers, including those of the breast, colon and pancreas. Our laboratory has utilized a novel mass spectrometry-based approach to identify protein kinases that respond to Src activation, and then functional screens to determine which ones are critical for cancer cell growth. This project will utilize several approaches, including siRNA knockdowns and assays for cell proliferation, migration and morphology, to determine in detail how these protein kinases contribute to Src-regulated oncogenesis.

PROJECT 3: Characterization of cancer-stroma interactions in prostate cancer
It is now well-established that the cancer stroma, including cancer-associated fibroblasts, plays a major role in development and progression of this malignancy. However, the detailed mechanisms remain unclear. We have used mass spectrometry-based proteomics to characterize how the proteome and phosphoproteome of cancer-associated fibroblasts (CAFs) differs from normal prostate fibroblasts. This project will use a variety of techniques including antibody arrays, cell signalling and biological assays, and knockdown approaches, to further characterize intercellular communication between CAFs and cancer cells, with the potential to identify new therapeutic strategies.
Research Background

Our principal interest is cell surface proteins that promote tumour growth and invasion, and may be targeted by novel therapeutic antibodies. Our focus is on Eph receptor tyrosine kinases, due to their critical roles in tumour tissue patterning and stem cell maintenance, and on ADAM metalloproteases, which regulate signalling by various cell surface receptors implicated in cancer, including the Eph, erbB and Notch families. We employ various biochemical, structure/function and imaging techniques using tumour models in vitro and in vivo. We are developing antibodies against Ephs and ADAMs to inhibit tumour development, with an EphA3 antibody currently in clinical trials.

Honours/PhD Projects

1. Investigation and antibody-targeting of ADAM metalloproteases in cancer. The metalloprotease ADAM10 sheds various proteins from the cell surface, including receptors and/or ligands of the Notch, Eph and erbB families, and thereby activates key oncogenic signalling pathways. We developed a monoclonal antibody (mAb) specific for an active form of ADAM10 which preferentially targets tumour cells, and inhibits ADAM10-dependent Notch signalling. We now wish to investigate the role of ADAM10 in broader tumour signalling through ‘omics’-based approaches, using ADAM10 deletion or antibody-mediated inhibition. We will investigate ADAM10 protein interactions, substrate shedding, and activation of signalling using proteomics and gene expression analyses. This can be applied to tumour models in vitro and in vivo.

2. Eph receptor signalling and cross-talk. Eph receptors constitute the largest family of receptor tyrosine kinases, and bind to their ligands on adjacent cells, to control cell-cell adhesion/de-adhesion during cell guidance and tissue patterning. While active during development, they are generally down-regulated in adult tissues, but reappear in cancers, where they are thought to promote vascularisation, metastasis and tumour maintenance, and resistance to therapy. Despite this they have also been identified as tumour suppressors in some contexts, reflecting their dichotomous role in cell-cell interactions. Ephs are known to co-cluster both with other Ephs and with EGFR/erbB receptors, although consequences for signalling outcomes are not well understood. We will investigate this using tagged receptors which reconstitute a fluorescent protein when dimerised, that can be visualised by microscopy and specifically recovered for analysis of interacting proteins by proteomics (BICAP - Bimolecular complementation affinity purification).
Mitchell lab
Prof Christina Mitchell

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RESEARCH BACKGROUND
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. In addition, deregulation of the PI3K pathway occurs in many other human diseases including diabetes and muscular dystrophy, as well as in developmental disorders.

HONOURS PROJECTS
Role of PI3K regulatory proteins in cancer
Contact: Christina.Mitchell@monash.edu, Lisa.Ooms@monash.edu, Jennifer.Dyson@monash.edu
PI3K signalling is deregulated in many human cancers including breast cancer. We have shown that loss of PI3K pathway regulatory proteins affects tumour initiation, growth and metastasis in mouse cancer models such as breast cancer. These projects will utilise mouse knockout and transgenic oncogene models and gene knockdown in cell culture to investigate the role of PI3K regulatory proteins in the development and metastasis of cancer. Overall these projects aim to identify and characterise novel therapeutic targets for PI3K-driven cancers.

Role of PI3K regulatory enzymes in angiogenesis
Contact: Christina.Mitchell@monash.edu, Michele.Davies@monash.edu
Abnormal angiogenesis results in developmental defects and contributes to human diseases such as cancer and retinopathies. We have identified a PI3K-regulatory enzyme that is essential for angiogenesis during embryonic development and for tumour formation. This project will investigate the mechanisms underlying the role this enzyme plays in angiogenesis during development and in human disease using gene knockout mouse models, with an aim to identify novel angiogenic therapeutic candidates.

Role of PI3K regulatory enzymes in development and ciliopathy syndromes
Contact: Christina.Mitchell@monash.edu, Jennifer.Dyson@monash.edu
Ciliopathy syndromes termed “ciliopathies” are characterised by kidney disease, blindness, obesity, mental retardation, skeletal defects and premature death, associated with abnormal function of primary cilia. Mutations in a PI3K regulator cause ciliopathies by unknown mechanisms. Recently we identified PI3K signalling occurs at primary cilia, but its role in cilia function is only just emerging. Using mouse models that recapitulate ciliopathy phenotypes and state of the art imaging approaches, this project will investigate how PI3K signalling contributes to ciliopathy syndromes, which may lead to the development of novel therapies for human disease.

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

Cells in our bodies respond to extracellular cues utilising not just isolated proteins, but their highly ordered responses result from coordinated actions of networks of proteins. This is analogous to an orchestra symphony which produce beautiful music from multiple instruments. Such understanding together with the availability of a vast amount of large-scale -omics data brought about by advances in 21st century's measurement technologies, has instigated a new and exciting paradigm of biological research termed "systems biology". In a nutshell, "systems biology" aims to obtain a holistic, systems-level view of biological processes, where the system is more than the sum of its parts. The Nguyen Lab deploys systems biology approaches integrating cutting-edge experimental techniques and powerful computational modelling to tackle key issues in cancer research. As cancer is by nature a systems disease and resistance to anti-cancer drugs is inherently a systems problem, quantitative systems approaches have been and will be instrumental in our quest to understand cancer and conquer drug resistance. The ultimate goal of these lines of research is to obtain better network-level understanding of signalling networks in normal and disease states, based on which novel therapeutic strategies can be derived. Prospect candidates will benefit from a highly interdisciplinary and stimulating environment in the Lab by interacting on a daily basis with researchers with both ‘dry’ and ‘wet’ expertise.

HONOURS PROJECTS

PROJECT 1: Move, proliferate or die: how does Raf-1 orchestrate distinct cell fates? Understanding cell fate determination is a fundamental task in cell and developmental biology, but it is challenged by increasing observations that most signalling proteins display multi-factorial functions. A recurring theme that has emerged is that signalling proteins can associate with different effector proteins (i.e. binding partners) to elicit distinct cellular outcomes through different, even opposing signalling pathways. How do multi-functional proteins then orchestrate their divergent functions to specify distinct cell fates in different contexts? This is a fundamentally important question with widespread implications for cellular and organismal homeostasis, as it applies to many cellular proteins. The answer to this question however does not lie in studying individual pathways in isolation, but is only possible through systematic and quantitative analysis of integrated networks that link functionally related pathways. In this project, we address the above question by focusing on a well-known member of the Raf kinase family, Raf-1, which represents a prime example of multi-functional signalling proteins. Our main goals are to understand how Raf-1 orchestrates distinct cellular outcomes (proliferation, apoptosis and migration) through its multi-factorial functions; and to decipher the molecular mechanism that underlies this process.

PROJECT 2: Elucidating mTOR signalling: a network-centred approach. The mTOR signalling network plays a central role in cell growth and proliferation, and is highly intricate with multiple upstream regulators and downstream functions. Its frequent aberration in cancers makes it an important drug target. However, the effort in inhibiting mTORC1 and/or mTORC2 has been slow due to emerging drug resistance. This project will employ integrated approaches that combines computational modelling and cutting-edge experimentation (biochemistry assays, mass-spectrometry based proteomics, etc.) to understand the dynamic properties of the mTOR network and utilize this knowledge to design new therapeutic strategies capable of overcoming resistance.
The PI3K-Akt-mTOR cascade is a key intracellular signalling pathway that mediates several biological processes including cell growth, proliferation, metabolism and migration. As such it is not surprising that mutations in key regulators of this pathway are frequently associated with cancer. Activating mutations in the **PIK3CA** gene (the gene encoding the catalytic subunit of the PI3-Kinase) are observed in up to 40% in ER+ and/or HER2+ breast tumors; whilst a negative regulator of this pathway, **PTEN** (phosphatase and tensin homology on chromosome 10) is found frequently inactivated or silenced in a range of human cancer and cancer-syndromes.

It was shown that mice expressing an activating PI3K mutation, Pik3caH1047R specifically in the breast, develop ER+ breast cancer, demonstrating that constitutive activation of PI3K signalling is oncogenic in the mammary gland. In addition, we have recently shown that mice with loss of Pten function (Pten knock-in, KI mice) also develop breast cancer, however with distinct features from the PI3K mice and with a more aggressive nature, suggesting that PTEN and PI3K may have non redundant functions.

**Honours Project:** The proposed project aims to define the role of PTEN in suppression of breast tumorigenesis driven by mutant PIK3CA. To this end, we will establish new mouse models of breast cancer by taking advantage of previously generated mouse models with compound PI3K activation and loss of Pten function (PI3K and Pten knock-in mice).

**Experimental plan:** 1- *In vivo* studies will be focused on the characterization of the breast tumour phenotype developing in newly established cohorts of mice in terms of tumor onset and penetrance. Tumor growth will be monitored at specific time points and in aged mice in order to establish overall survival rate. Histopathological analyses will be performed to characterize different histological features developing in tumour samples and to identify presence of metastasis. 2- *Ex vivo* studies will be performed on primary mammary epithelial cells derived from mouse tumour. Levels of activation of different signaling cascades will be monitored by western blot analyses in order to determine the contributions of the PI3K-Akt-mTOR pathway and other PTEN targets to breast tumorigenesis.
RESEARCH BACKGROUND

Mutations leading to deregulation of β-catenin activity are frequent in colon and other cancers. However, despite large scale efforts by academia and pharma we currently lack approaches to inhibit β-catenin driven cancers. As an alternative approach for targeting these cancers my group will use state of the art functional genomic approaches including pooled CRISPR mediated loss of function screens, ORF gain of function screens and drug resistance screens to map and understand the landscape of genetic vulnerabilities in β-catenin driven cancers.

In addition to developing new approaches for cancer therapy research in the lab will focus on developing new genomic technologies such as high throughput RNA sequencing and genetic approaches for rational combination therapy. The long term goal of the lab is to identify new targets for drug development that will enable treatment options for “un-treatable” cancers.

HONOURS PROJECTS

Project 1: Regulation of YAP1 in β-catenin driven cancers.
My previous work identified the transcription regulator YAP1 to be essential for survival of β-catenin driven colon cancers (Rosenbluh et al. Cell, 2012). However, we lack mechanistic understanding of how YAP1 regulates these cancers. Using a combination of proteomic profiling and genetic interaction mapping I recently identified that YAP1 could regulate the SWI/SNF chromatin remodelling complex in β-catenin driven cancers (Rosenbluh et al. Cell Systems, 2016). The aim of this project will be to gain mechanistic understanding of how YAP1 regulates the SWI/SNF complex and how this is related to β-catenin activity.

Project 2: Genetic approaches for rational combination cancer therapy.
Combination therapy aims at simultaneous inhibition of multiple targets. However, our current technologies are not suitable for systematic studies aimed at identifying effective combination therapies. To address this need we will use state of the art molecular biology techniques to construct a combinatorial gene suppression library that will enable simultaneous knock-out of genes with known inhibitors.

Project 3: SRP19 a new novel target of β-catenin driven cancers.
SRP19 is a component of the signal recognition particle and is essential for survival of all cells. Due to its close physical proximity to the tumor suppressor APC one allele of SRP19 is lost in ~20% of colon cancers creating a unique vulnerability. The aim of this project is to explore this vulnerability as an approach to treat this population of colon cancer patients.
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:

**PROJECT 1 - CNS CONTROL OF BEIGE ADIPOCYTE THERMOGENESIS:** The identification of classical brown and beige adipocytes in white fat depots in adults humans, has heralded a new era in adipose tissue biology with a focus on energy homeostasis. The capacity of brown/beige adipocytes to utilise lipids and glucose as a fuel source, and to expend the energy as heat, accompanied by their decreased abundance in older and overweight individuals, has garnered interest in promoting brown and beige fat thermogenesis to combat the obesity epidemic. Studies from our lab have shown that brown and beige fat thermogenesis is controlled by POMC and AgRP neurons in the brain. Projects are available to determine the molecular mechanism by which the brain controls brown and beige adipocyte thermogenesis and body weight.

**PROJECT 2 - OBESITY & LIVER DISEASE/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.

**PROJECT 3 - OXIDATIVE STRESS & INSULIN RESISTANCE:** Obesity or increased visceral adiposity are causally linked to the development of insulin resistance, a state of diminished insulin responsiveness, which is the major etiologic factor of the metabolic syndrome and precedes the development of frank type 2 diabetes. The molecular processes underlying the development of insulin resistance remain unclear. Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the antioxidant capacity of the cell. Oxidative stress is thought to drive insulin resistance and type 2 diabetes. Projects are available to determine the mechanisms by which ROS contribute to the development of insulin resistance.
RESEARCH BACKGROUND

The research conducted by the Biochemistry Education Research Team focuses on student. The group uses quantitative and qualitative methods to examine approaches to enhance student learning. The data from these studies can be used to maximise curriculum design and approaches to learning. Of particular interest is how to engage students with their learning and improve academic outcomes. The educational scholarship of the group emanates from our strong belief that the teaching and learning methods we introduce to our students should be thoroughly researched to ensure that we are truly developing and implementing the highest possible quality in teaching and learning.

HONOURS PROJECTS

Student networks (Janet Macaulay and Caroline Speed)
The focus of this project is to look at how students form and use peer networks. Instruments with which to best collate and analyse data will be designed and will include surveys and focus groups and include questions such as: What type of networks are used? Face to face, online (what type)? How are the networks established? What do they use them for? i.e. social, learning, assisting with assignments?

Designing and evaluating active learning strategies (Janet Macaulay and Caroline Speed)
Research has shown that traditional, didactic teaching can disengage students from their studies and encourage passive rather than active learning. The use of active learning in the large class environment is being adopted to motivate and engage students in their learning. This allows class time to be used to contextualise and apply knowledge through exercises, problem solving, the application of the concepts and discussions to ensure that knowledge is assimilated and deeper understanding developed. However, students and teachers are often resistant to the implementation of active learning. This project will encompass the development and evaluation of active learning from the perspective of both students and teachers.

Learning Biochemistry: effectiveness of partnerships as a strategy in the Faculty (Nirma Samarawickrema)
Engaging students and academics as partners in learning and teaching is critical in the current higher education context. These partnerships may take many forms. The proposed study will involve a scan of all the Biochemistry units offered in the Faculty to: (1) Identify all the learning activities and assessments that draw on partnerships with students and academics; (2) Classify the knowledge built, skills and Monash graduate attributes developed and assessed within each learning activity/assessment and (3) Evaluate effectiveness of these.

Other projects:
A range of other projects related to learning and teaching and the student experience are also available. Please contact Janet Macaulay for details
RNA Systems Biology
Dr Traude Beilharz
Phone: 99029183
Email: traude.beilharz@monash.edu
Lab webpage:
http://rnasystems.erc.monash.edu/

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

From their birth till their destruction, mRNAs are always associated with proteins. Deciphering the language of how mRNAs interact with specific RNA-binding proteins is a fundamentally important concept in modern molecular cell biology. The cytoplasm of eukaryotic cells typically contain 250,000-500,000 mRNAs. Strikingly, although mRNAs are the template for protein production, recent genome scale analysis indicates that the correlation between mRNA levels and protein abundance is not particularly strong, indicating that most mRNAs must undergo specific post-transcriptional gene regulation. Our laboratory is focused on understanding the mechanisms of post-transcriptional gene regulation by using a diverse set of experimental approaches, including cell biology, genetics, biochemistry and genomics. We use the small non-parasitic nematode C. elegans as our model eukaryote, as it provides an exceptional tool for in vivo and in vitro studies.

HONOURS/PHD PROJECT 1: Functions of small RNAs in development

Small RNAs play a major role in controlling gene expression and genome organisation, but do not encode for any proteins. We have identified an RNA-binding protein that function in two distinct small RNA pathways: the miRNA pathway in somatic cells, and the siRNA pathway in germ cells that is required for maintaining chromatin organisation, suppression of transposons and chromosome segregation. This project will use cell biology, genetics, biochemistry and genomics approaches to elucidate how this RNA-binding protein functions in these two small RNA pathways.

HONOURS/PHD PROJECT 2: Post-transcriptional gene regulation during oogenesis

Post-transcriptional gene regulation during oogenesis: One of the most remarkable examples of posttranscriptional regulation occurs during formation of oocytes (oogenesis) in sexually reproducing animals. Thousands of mRNAs are transcribed prior to the characteristic shutdown of transcription late in oogenesis, and a large proportion of these mRNAs are maintained in a translationally silent state until specific stimuli initiate their translation, facilitating the completion of oocyte development, fertilisation and early embryonic development. This project will investigate how a conserved protein complex is required for translational repression of many mRNAs and localisation of specific RNA-binding proteins to key sites of post-transcriptional gene regulation in germ cells.

HONOURS/PHD PROJECT 3: Poly(A) tail dynamics

At the 3’ end of mRNA molecules is the poly(A) tail. The longer the ploy(A) tail the more stable an mRNA and the more efficiently it will be translated. Interestingly, for some mRNAs the length of the poly(A) tail is dynamic and this is thought to “fine tune” gene expression. The use of poly(A)-length change to regulate gene expression is common in the brain, germline, circadian rhythm and is mis-regulated in disease. However, the mechanism(s) of mRNA target selection is often unclear, as is the functional specificity of the enzymes involved. This project we will use C. elegans as a model to examine how poly(A)-length change is regulated and the key factors involved. This project is a Collaboration with Dr Traude Beilharz.
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 - Understanding normal and abnormal kidney development.
Nephron number is highly variable in human populations but the developmental basis for this difference is poorly understood. This project will utilise our imaging expertise and an array of different models to explore how perturbations to fetal environment like alcohol intake and maternal diabetes impacts on genetic pathways central to progenitor cell maintenance and nephron formation. Doing so will help us to understand how kidney disease develops and what might underlie the enormous variation in nephron number which is an important predictor of kidney failure and hypertension.


Project 2 - Dissecting the molecular basis of congenital kidney diseases.
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 and Aurka. Our group is also involved in an Australia-wide program which aims to identify novel genes in patients with kidney disease. 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.

RESEARCH BACKGROUND

Emerging and re-emerging 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, hepadnaviruses such as hepatitis B 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. The present research proposed will shed important new light on RLR signalling, and how it is regulated/exploited by host and viral proteins. Importantly, this will open up the exciting possibility to target RLR signaling to develop broad-spectrum anti-viral agents.

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

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 for their own advantage.

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- How do giant human viruses assemble their infectious particles?
2- Cryo-electron microscopy of viral particles
3- Development of MicroCubes, a novel vaccination platform

HONOURS PROJECTS

How do giant human viruses assemble their infectious particles?

Background: Smallpox is an ancient and dreadful disease that enormously influenced human history causing over 300 millions of death in the 20th century alone. Smallpox had mortality rates as high as 30% with no available cure and debilitating injuries for survivors. Fortunately, a worldwide vaccination campaign succeeded in eradicating this scourge almost 30 years ago. However, in recent years, human cases of monkeypox virus in the US and Africa, and the potential use of smallpox as a bioterrorism weapon have called for an urgent improvement in Australia’s preparedness against poxviruses. Poxviruses are the largest viruses infecting humans and among the most complex members of the virus world. Their incredibly complex assembly involves the initial formation of lipid-containing precursors called viral crescents, subsequently forming poxvirus immature particles. These structures have fostered a controversy with conflicting reports that suggest either de novo synthesis or a cellular origin for the membranes of viral crescents.

Project: the project will focus on structure-function analysis of the viral protein complex that is responsible for the assembly of viral crescents using X-ray crystallography and cryo-EM. This complex is the target of a rare example of a natural antibiotic that also has a specific antiviral activity - albeit with a very different target. The overall aim of the research will be to understand the role of key proteins and their lipidic ligands in the mechanism of membrane assembly, a process that is most likely in part conserved in many giant DNA viruses.

Fields of research: molecular virology; structural biology

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

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

Due to the unprecedented success rate of several cancer immunotherapies, humankind has never been more hopeful or interested in immunology than now. While the success stories are truly inspiring, responses to immunotherapies remain variable and some patients get no benefit. Nobody understands why this is the case. Immunotherapies have enormous potential to transform lives, but advances still rely on information from basic immunology that is as close to the truth as possible.

Our research focuses on the immune response to self-antigens. How do immune cells recognise and tolerate the ~200 different cell types present in a healthy human body? This response begins in the thymus, the birthplace of T cells. The thymus selects just a few T cells out of the many available, and dispatches them to a precise organ, where continual recognition of the same self-antigen tells the immune system that there is no need for inflammation. We hypothesise that genetically determined variation in this process underlies variable responses to cancer immunotherapies and susceptibility to autoimmune diseases observed in the clinic.

HONOURS PROJECT

TITLE: Defining the B-cell-dependent T cell repertoire

PROJECT BACKGROUND: During immune responses T cells interact with B cells, the antibody producers. These interactions are thought to determine whether the B cells make antibodies that are protective (anti-microbial) or pathogenic (anti-self “autoantibodies”). We hypothesise that T cell recognition of self-antigens tells the immune system that the threat from infection has passed and that the immune response can be terminated. The self-antigens that B cells display to T cells are unknown.

HYPOTHESIS: The absence of B cells removes a subset of self-antigens from the body, leaving a “hole” in the self-antigen repertoire recognised by T cells.

AIMS:
1. Deep-sequence the T cell repertoires of B-cell-sufficient and B-cell-deficient mice.
2. Test whether autoantibodies are associated with a defect in self-antigen recognition by T cells.

OUTCOMES:
• Apply cellular and molecular immunology and mouse genetics to a cutting-edge research topic.
• Access to dedicated supervisors in a small lab setting.

METHODS/SKILLS:
• Mouse handling and dissection
• Flow cytometry
• Sample preparation for deep-sequencing (RNA isolation, cDNA synthesis and PCR amplification)
• T cell repertoire interrogation using established software, called “VDJTOOLS”
RESEARCH BACKGROUND

The Nuclear Factor of kappa B (NF-κB) signal transduction pathway plays a key role in the immune system and features prominently in disease associated pathology. Our group focuses on the use of genetically modified mice to understand how NF-κB regulates normal development, differentiation and cellular functions particularly in the immune system, as well as its roles in autoimmune disease and cancer. The long-term goal of our research is to identify ways of manipulating NF-κB transcription factor activities for therapeutic purposes.

HONOURS PROJECTS

Honours/PhD project 1: NF-κB, inflammation and aging.
Despite a strong causal association between inflammation and organismal aging (inflammaging), the underlying mechanistic links are poorly defined. Mice lacking the NF-κB1 transcription factor age prematurely. This is in part due to increased systemic inflammation arising from an over-production of pro-inflammatory cytokines resulting from biased M1 polarization of monocyte/macrophage lineage cells. This project will examine how NF-κB1 controls the development and function of M1 macrophages.

Honours/PhD project 2: NF-κB1 and cellular senescence.
Senescence, which is an important mechanism for preventing cell division in response cellular stress increases with age. The NF-κB pathway is a key regulator of age-dependent gene expression, with NF-κB1 serving a key role in preventing cellular senescence. This project will examine the cell intrinsic mechanisms by which NF-κB1 prevents cellular senescence.

Hons/PhD project 3: NF-κB and epithelial cells.
The importance of the NF-κB pathway in the gastrointestinal system is underscored by the prominence of deregulated NF-κB signalling in gastrointestinal inflammatory diseases and cancers. This project will involve understanding the roles different NF-κB transcription factors serve in the development, homeostasis and function of gastrointestinal epithelial cells.

Hons/PhD project 4: NF-κB and T cell subsets.
The roles particular NF-κB transcription factors serve in various T cell populations is largely unknown. Projects within this theme will investigate how the c-Rel, RelA and NF-κB1 transcription factors each control the differentiation and function of different CD4+ and CD8+ T cell subsets. A major focus of this work will be understanding how modification of the T cell epigenetic landscape by these transcription factors determines specific outcomes for T cell subsets.
RESEARCH BACKGROUND

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.
RESEARCH BACKGROUND
Nuclear transport is central to eukaryotic cell processes such as signal transduction and differentiation, where changes in transcription within the nucleus are mediated by transcription factors which gain access to the nucleus through cellular transporters called importins (IMPs). IMPs are also critical in oncogenesis, whereby their misregulation can cause mislocalisation of transcription factors. Finally, nuclear transport is critical in viral infection, where viruses utilise or disrupt mechanisms of the infected host cell to ensure the precise subcellular localisation of critical viral proteins, as well as to mislocalise host proteins, resulting in increased virus replication and inhibition of host anti-viral responses.

We focus on viruses of medical significance (Dengue, HIV etc.) and cancer as model systems to study the importance of nuclear transport during infection and tumorigenesis. The results will identify new targets for therapeutic intervention and help develop novel urgently required anti-viral/anti-cancer agents.

HONOURS PROJECTS

PROJECT 1: The NS5 gene product from the causative agent of dengue fever, dengue virus (DENV), localises predominantly in the nucleus, even though its function in virus replication is in the cytoplasm. We have shown that NS5’s trafficking into and out of the nucleus is essential to virus replication, and that inhibitors of NS5 nuclear accumulation can block DENV infection. Project 1 examines interactions of NS5 with host-cell proteins within the nucleus and their importance to infection, as well as developing small molecule inhibitors of NS5 nuclear import to stop DENV infection.

PROJECT 2: Respiratory syncytial virus (RSV) accounts for more human deaths than influenza each year. RSV matrix (M) protein plays an important role in pathogenesis through its ability to enter the host cell nucleus to inhibit transcription, thus shutting down the host cell immune response. Using various approaches, we have identified a series of M-protein interaction partners that may aid M in its nuclear and cytoplasmic function(s). Project 2 focusses on determining the mechanism of inhibition of transcription by M, validating nuclear and cytoplasmic targets of M and the effect of knocking down these targets on RSV infection. We will also develop small molecule inhibitors of RSV infection.

PROJECT 3: We have found that IMP levels can be markedly increased in transformed cells, resulting in increased nuclear transport efficiency, and that this is in part regulated by microRNAs (mIRs). Project 3 will examine the extent to which misregulation of IMPS may play a role in cancer cell migration, proliferation and resistance/sensitivity to anti-cancer drugs in a novel breast cancer progression model, and the extent to which this may involve specific mIRs.
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.
RESEARCH BACKGROUND

Aged individuals exhibit increased susceptibility to, and severity of, a variety of infections and cancers, alongside waning vaccine efficacy rates, which reflects dysfunction in adaptive responses to newly encountered antigens. While ageing likely compromises a number of arms of the immune system, studies in mice and humans have demonstrated deficits that are intrinsic to naïve CD8 T cells. The projects described here aim to define the molecular mechanisms underpinning age-related CD8+ T cell dysfunction and to test pharmacological interventions to rescue CD8+ T cell function.

HONOURS PROJECTS

Project 1. To characterize the metabolism of CD8+ T cell subsets in young and aged mice

Project Description: T cells display distinct metabolic profiles depending on their activation state. Naïve T cells have low energy demands and typically utilize oxidative phosphorylation as their major source of energy. Upon activation, effector CD8+ T cells undergo a metabolic switch to predominantly use glycolysis over OXPHOS. Finally, memory cells revert to the use of OXPHOS (fueled by fatty acid oxidation) but now have greater mitochondrial mass and elevated spare respiratory capacity, thought to reflect a metabolically ‘primed’ state for a rapid response upon reinfection. This project will investigate the metabolic processes in aged, compared to young, T cell populations, including true naïve T cells (Tn), memory T cells (Tm) and a population of T cells that are antigenically naïve, but express some markers of activation, termed virtual memory T cells (Tvm) that accumulate significantly with age. This project will determine whether there is a metabolic phenotype underpinning age-related T cell dysfunction.

Techniques: Mouse handling and dissection, Flow cytometry, In vitro cell culture, T cell stimulation assays, Seahorse XFe96 bioanalyser assays.

Project 2. To characterize and selectively eliminate senescent cells from the aged CD8+ T cell population

Project Description: We have transcriptional evidence that a subset of aged CD8+ T cells (‘virtual memory’ cells; Tvm) are senescent, a specific state that allows cells to persist in vivo but they are unable to respond to further activation. These cells may also limit the function of surrounding cells. To determine if aged Tvm cells negatively impact on the function of true naïve (Tn) cells, we will coculture these cells and assess Tn cell proliferation in a variety of assays. We then aim to selectively eliminate aged Tvm cells in vitro, using newly described “senolytic” drugs that target senescent cells, while leaving the more functional Tn intact. In this way, we will determine the extent to which senescent CD8+ T cells compromise global naïve CD8+ T cell function.

Techniques: Mouse handling and dissection, Flow cytometry, In vitro cell culture and functional proliferation assays.
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 (eg. 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

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. This occurs in at least 1% of pregnancies worldwide, and can cause permanent hearing loss, vision impairment, and mental retardation. There is no vaccine currently available, and discovery of new antivirals is urgently required. Importantly, the process by which infectious virus is packaged and released is not well understood, and this presents a novel molecular loci to develop antiviral therapeutics.

Research in our laboratory uses systems biology to better understand host defense mechanisms, and identify cellular pathways hijacked by viruses. We utilize cutting-edge proteomics and mass spectrometry approaches to better understand viral pathogenesis and immune-evasion. We work at the interface between cell biology, virology and instrumentation, and have proprietary virus libraries and reagents to make unique discoveries. All projects involve a multidisciplinary approach, and the opportunity to learn a wide range of laboratory techniques and skills.

HONOURS PROJECTS

1) **Dissecting the viral assembly complex**
   
   HCMV causes profound remodelling of host organelles. Using a library of mutant viruses, we investigate which viral proteins are essential for vAC formation. Skills used include tissue culture, confocal microscopy, and quantitative proteomics.

2) **Hijacking of host secretory pathways**
   
   HCMV co-opts host machinery (exosomes) to assembly and release infectious virions. We identify host proteins that are engaged for virion maturation, and identify critical host-viral interactions. Approaches used include CRISPR, viral assays, electron microscopy.

3) **Exploring the mitochondrial function of SIRT4**
   
   We discovered SIRT4 as the first cellular lipoamidase (Mathias et al. Cell 2014). We now characterize its enzymatic activity to substrates containing lipoyl and biotin modifications, to reveal how it controls cellular metabolism. Enzyme kinetics, mass spectrometry, and bioinformatics are employed.
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.
**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 and/or self nucleic acids activate dendritic cells. We aim to decipher how this activation influences the function of dendritic cells. We investigate how this process may differ in different body locations, at different ages and in different disease settings. Major aims are to understand the role of dendritic cells in bone marrow malignancies, in autoimmune diseases such as Lupus and in infectious disease.

**HONOURS PROJECTS**

1. **What role do checkpoint inhibitors play in dendritic cell function?**

   Immunotherapy with reagents that block checkpoint inhibitors such as PD-1 and CTLA4 have shown great promise in the clinic against tumours, including melanoma. T cells are traditionally considered the main cells to express these molecules but we have recently found that dendritic cells also upregulate the expression of numerous checkpoint inhibitors. This project will investigate the functional consequences of checkpoint inhibitor expression by dendritic cells.

2. **How does the nucleoporin Nup98 regulate plasmacytoid dendritic cell function?**

   The nucleoporin Nup98 is highly expressed in plasmacytoid dendritic cells. It is also an interferon-regulated gene and shown to be required for optimal anti-viral responses. In the NHD13 model of Myelodysplasia, in which plasmacytoid dendritic cells express a mutant form of Nup98, the ability of plasmacytoid dendritic cells to produce large amounts of interferon-alpha is abrogated. This project will investigate how Nup98 regulates plasmacytoid dendritic cell function.

3. **How is the expression of the interferon-lambda receptor regulated?**

   Interferon-lambda is made at high levels by plasmacytoid dendritic cells and human CD141+ and mouse CD8+ dendritic cells. Dendritic cells and neutrophils express the interferon-lambda receptor, it is upregulated on dendritic cells with activation, but it is unclear how its expression is regulated. Moreover, a truncated soluble form of the receptor has been reported but it is unclear which cell types express this potentially immunomodulatory molecule. This project will investigate human and mouse interferon-lambda receptors and address how the expression of these receptors, including the soluble forms, are regulated.
RESEARCH BACKGROUND


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: The molecular immunology of adverse reactions to anti-epileptic drugs

Project Description: Adverse drug reactions (ADRs) result in an estimated $660 million in hospital related costs annually in Australia, a subset of these is mediated by T cells. Moreover, many of these reactions are strongly associated with different alleles of the Human Leukocyte Antigens (HLA). One of the strongest associations is between a severe cutaneous ADR to carbamazepine and HLA-B*15:02 in Asians\(^1\). This association does not extend to European populations, where HLA-A*31:01 has been associated with ADR\(^2\).

The aim of this project is to use an array of cellular and molecular assays to determine the phenotype, function and signature of T cells that respond to the anti-epileptics. Primary investigations will utilise flow cytometry to quantitate and phenotype drug-specific T cells combined with a single-cell PCR technique to sequence drug reactive TCRs. These studies will be combined with biochemical analysis of the HLA and its antigenic cargo using mass spectrometry, structural biology and biophysical methods. These investigations will contribute to the understanding of immunological mechanisms associated with drug hypersensitivity as recently reported by the host laboratory for the anti-viral drug abacavir\(^3\).

References:


Project Title: The link between bacterial infection and autoimmunity

Project Description: Several HLA allotypes are associated with the development of Ankylosing spondylitis (AS) a debilitating arthritis of the spine. This project will investigate recently reported links between HLA B40 subtypes\(^4\) and AS, and contribute to a large database of HLA-bound peptides known to bind to other disease associated alleles from the HLA B27 and B14 family. These studies will involve the biochemical analysis of the HLA and its antigenic cargo using mass spectrometry, structural biology and biophysical methods. These investigations will contribute to the understanding of immunological mechanisms associated with AS development and the association of prior bacterial infection as a trigger for this disease.

References:

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), 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), 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 (Nature, 2011, JEM 2016; NSMB 2017; Cell 2017) interact with their cognate ligands and viral immunoevasins. 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 Comms 2016). 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 the collaborators of the Rossjohn laboratory. The industrial research program of the laboratory includes a close collaboration with Janssen, for the development of new therapies to treat psoriasis and rheumatoid arthritis.

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. My 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

**New targets for the old drugs: exploring the antimicrobial potential of carbonic anhydrase inhibitors.**

*H. pylori* has a unique ability to withstand high acidity of the stomach by buffering its periplasm at pH 6, through the action of urease and carbonic anhydrase (CA). In this project, we shall evaluate the potential of *H. pylori* CA as a novel target for treatment against *Helicobacter*. We have determined the first crystal structure of this enzyme in complex with inhibitors that have been used clinically for a different purpose, *i.e.* as antiglaucoma or antiulcer drugs. The project will include analysis of structure-activity relationships, isolation of mutants with spontaneous resistance and genomic investigation of the resistance mechanisms.

**The mechanism of assembly and activation of the *H. pylori* flagellar motor.**

The bacterial flagellar motor is a highly efficient rotary nanodevice made of proteins and powered by the electrochemical gradient across the cytoplasmic membrane. In this project, we shall identify protein components that are essential for the assembly and activation of the force-generating component of the flagellar motor and elucidate the structural basis of their function.

**How do bacteria 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 recognise and respond to non-native ligands for innovative applications in biotechnology and bioengineering.
RESEARCH BACKGROUND

Inflammation is the response of a tissue and its microvascular system to injury or infection. A hallmark of inflammation is the accumulation of leukocytes (white blood cells), which remove pathogens and necrotic tissue by phagocytosis and proteolytic degradation. However, excessive leukocyte recruitment or activity leads to the release of toxic substances and degradation of healthy tissue, i.e. inflammatory disease.

Leukocyte recruitment in inflammation is controlled by the expression and secretion of small proteins called chemokines at the site of inflammation and by the subsequent interaction of those chemokines with chemokine receptors located on the surfaces of circulating leukocytes. A detailed understanding of chemokine-receptor interactions is required in order to rationally develop novel therapeutic agents against inflammatory diseases.

Our group is investigating several important aspects of chemokine and chemokine receptor biochemistry with the overall goals of better understanding and ultimately controlling their biological functions.

HONOURS/PhD PROJECTS

HONOURS/PHD PROJECT 1: BIASED RECEPTOR AGONISM BY CHEMOKINES

We have recently discovered that a chemokine receptor expressed on macrophages can be activated by two different chemokines giving different arrays of downstream, cellular signals. In this project we aim to understand the mechanism of this fascinating phenomenon, its consequences for the cellular phenotype, and its relevance to macrophage-mediated inflammatory diseases such as atherosclerosis.

HONOURS/PHD PROJECT 2: STRUCTURAL BASIS OF CHEMOKINE RECOGNITION

A critical factor that regulates chemokine-receptor interactions is the post-translational sulfation of tyrosine residues in the chemokine receptors. We have shown that chemokine receptor sulfation influences chemokine binding affinity, selectivity and receptor activation. To understand the structural basis of these effects, we are using biophysical methods (such as NMR) to determine the structures of chemokines bound to sulfated receptor fragments.

HONOURS/PHD PROJECT 3: Tick Evasins – Natural Chemokine Antagonists

Ticks naturally produce proteins called evasins, which bind to chemokines on the host, thereby suppressing host inflammatory responses and allowing the ticks to feed on the host blood for extended periods. Evasins have the potential to be developed into useful chemokine-targeted anti-inflammatory agents. In this project we aim to: (a) discover and characterise new members of the evasin family; (b) determine the structural basis of how evasins recognise chemokines; and (c) engineer modified evasins to target specific chemokines.
Host-pathogen interactions
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RESEARCH BACKGROUND

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

The go-to strategy for overcoming drug resistant infections has been to develop new drugs. However, this approach has not worked so far. A major reason is that microbes can rapidly develop resistance to any new drug that we design. Therefore, new thinking is needed.

In my laboratory, we focus on this problem from two aspects:

(i) We are looking at new ways to treat infections by nutritional and metabolic approaches. We have exciting evidence that microbes compete with immune cells for essential nutrients, and thereby cause a state akin to immune paralysis. We are studying how we could overcome this through mechanisms that target both host and pathogen pathways, to boost immune responses and empower the host to clear infection.

(ii) We are taking the approach of drug repurposing, focusing on compounds that are being developed to treat non-infectious human conditions. As our major focus are pathogenic fungi, which cause devastating human infections with mortality as high as 40%, we are exploiting the conservation of human and fungal cell biology to find innovative ways to treat infections. We have evidence of antifungal activity for a small molecule inhibitor, and are now exploring the mechanism of action.

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

HONOURS PROJECTS

1. Understanding how microbes evade innate immune responses.
2. Defining of a novel mechanism that prevents the transition of a normally benign inhabitant of the human microbiome to a highly deadly pathogenic form.
RESEARCH BACKGROUND
Breast cancer remains one of the leading causes of death in Australia, with current treatments often causing debilitating unwanted toxic side effects. By determining the underlying cellular differences between cancer and normal cells, we are able to understand the causes of these changes and to develop new drugs and delivery agents to target them specifically.
Similarly, infectious diseases such as those caused by viruses and cellular stress conditions often rely upon or generate changes in the subcellular targeting of various proteins, particularly those involved in transport between the cytoplasm and the nucleus. We identify these protein interactions and harness them to develop novel anti-viral drugs and to uncover the cellular pathways, which underpin these important conditions.

HONOURS PROJECTS
Project 1. Identification of novel tumour biomarkers for advanced triple negative breast cancer.
Triple negative breast cancers remain some of the most aggressive forms of breast cancer and there are no specific treatment options available. This is in large part due to the fact that there are no known specific biomarkers for these cells upon which to base a specific therapeutic strategy. We have developed a series of highly specific tumour targeting agents that recognise these cells only and do not bind to normal breast tissue, even from the same patient. **Project 1** will identify through a range of techniques the biomarkers on the tumour cells to which these agents bind. Techniques will include, pull-down/mass spectrometry, confocal laser scanning microscopy, tissue culture, siRNA and immunofluorescence.

Project 2. The role of nuclear transport in cellular stress, DNA damage and repair.
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. **Project 2** will define some of the characteristics of this pathway and its contribution to DNA damage and repair following cellular stress insult. 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.

Nuclear protein transport is a critical part of many infectious diseases. We have developed a novel screening approach to identify compounds that target the interaction of the viral protein with the host nuclear transport machinery specifically. **Project 3** will utilise a range of tools to develop and characterise anti-viral agents directed at the nuclear transport machinery. Techniques will include protein-protein interaction assays, small molecule inhibtors, confocal microscopy, viral infections/viral techniques and tissue culture.
**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.
Mitophagy & mitochondrial quality control in disease

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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: Investigating the role of ER morphology in PINK1/Parkin mitophagy Autophagosomes are formed in close association with the endoplasmic reticulum (ER). The ER is the cradle of autophagosome formation which is required to encapsulate damaged mitochondria during PINK1/Parkin mitophagy. Atlastin and Reticulon2 are mutated in hereditary spastic paraplegia and function to maintain correct ER morphology. This project will investigate whether changes in ER morphology associated with disease can impact autophagosome formation during PINK1/Parkin mitophagy. Knockout cell lines of Atlastin and Reticulon2 will be generated to disrupt ER morphology and determine whether autophagosome formation is hindered. Disease associated mutations in Atlastin and Reticulon2 will also be investigated to assess whether they affect PINK1/Parkin mitophagy. This research has the potential to reveal novel disease mechanisms.
RESEARCH BACKGROUND

Fluorescent protein technology has revolutionised the way in which we carry out experiments in the life sciences, and few areas of biological research remain untouched by the technology. Fluorescent proteins such as the green fluorescent protein (GFP) cloned from the jellyfish *Aequorea victoria*, have been engineered to produce proteins with different fluorescent properties (for example see picture) useful for sensing a vast range of events in living cells. GFP is just one member of the protein superfamily found in marine organisms. Although each member folds to form the same 11-stranded β-barrel a variety of different chromophores (the light emitting component buried inside the barrel) together with the complex network of interactions between the chromophore and the surrounding amino acid side-chains (the protein matrix) determine the myriad range of optical properties.

Members of this protein family possess a constellation of fascinating and biotechnologically useful features. For example fluorescence emission of different proteins can with light be ‘switched’ from green to red, cyan to green, fluorescent to non-fluorescent, or vice versa, in a process that can be reversible or non-reversible. Such photoswitchable FPs are useful as ‘optical highlighters’ or optical data storage systems. They are the foundation of emerging super-resolution microscopy techniques that allow cellular features 10 times smaller than can be seen with conventional light microscopy to be visualised.

Our aim is twofold: (a) understand the complex and subtle relationship between FP structure and optical properties, and (b) use newly acquired knowledge to design and engineer new FPs for novel biotechnology applications. In particular we are exploring their use in the fields of autophagy research, super-resolution microscopy and optogenetics. In the new and exciting field of optogenetics light-sensitive probes are used together with focussed light to switch processes ‘on’ and ‘off’ in living cells, tissues and intact organisms.

HONOURS PROJECTS

1: Engineering and characterization of FPs with useful optical properties.
2: Developing probes for optogenetics.
3: Developing probes and using FP for super-resolution microscopy.
BACKGROUND: Our lab is focused on the regulation of autophagy, a major intracellular degradation process. In cancer, autophagy plays complex roles and can suppress tumours, but also helps tumour cells survive in other cases. Autophagy delivers cellular and cytoplasmic structures to the lysosome, where they are degraded. This process is tightly linked to the cellular metabolism and is an evolutionary conserved survival mechanism that helps cells to cope with nutrient starvation. We have recently discovered a link between metabolic control and the Serine/Threonine kinase ULK1, a key regulator of autophagy. We aim to get a detailed understanding of how these regulatory networks are causing changes in intracellular membrane trafficking during autophagy.

Honours/PhD Project 1: High-resolution imaging of the mitophagy pathway. Our lab is applying and developing imaging tools such as correlative light and electron microscopy to combine state of the art optical and electron microscopy techniques. The project will apply high-resolution techniques to study the spatio-temporal regulation of intracellular organelle traffic in mitophagy. You will have access to and use the latest imaging technology including in the new cryo-EM facility housing the first Titan Krios cryo-TEM in Australia.

Honours/PhD Project 2: Autophagy and Cancer. You will undertake a project to examine the role of autophagy in cancer progression. Work from our lab has integrated the autophagy kinase ULK1 into a signalling network of kinases that play an important role in cancer. The study will use optical and electron microscopy imaging techniques to visualise the role of autophagy and mitophagy in cancer tissues.

Laboratory techniques that can be learned and applied during the honours project include: live cell microscopy, super-resolution optical microscopy, immunofluorescence microscopy, immuno EM, correlated light and electron microscopy, TEM, SEM, cryo-EM, tomography, cell culture, cell transfection, molecular cloning, in vitro kinase assays, western blotting, and immunoprecipitation.
Mitochondrial biology & disease
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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. Disorders of mitochondrial energy generation cause degenerative diseases and often lead to infant death. Through funding from the NHMRC and ARC, we investigate the molecular and cellular processes related to mitochondrial function, dysfunction and disease, and dynamics. Honours projects are designed to ensure each student encounters a range of techniques and along with weekly lab meetings, will give them expertise for future scientific and non-scientific careers. Students will be mentored in the lab by one of our friendly postdocs.

HONOURS PROJECTS

Systems approaches to understand human mitochondrial protein function
Complex I of the mitochondrial respiratory chain is a huge machine containing 44 different subunits. We are identifying and characterising proteins involved in its assembly and how mutations cause disease. We have used CRISPR/Cas9 to make a series of human cell lines with specific gene knockouts and are identifying the consequences of gene loss to complex I and to the cell (Stroud et al., Nature 2016). Using proteomics, we have identified new proteins that respond to changes in mitochondrial function. The function of these proteins are unknown and require characterisation. 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 by other groups, however 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 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
Our group’s focus is to link genes and complex biological pathways to physiological function and dysfunction by the use of novel genomics, proteomics, metabolomics and other systems biology approaches. The main disease area of interest is in Metabolic Disease using genetically modified models to study Obesity, Diabetes, Lipid Disorders and Immune & Inflammation Function

Honours Projects

In what tissues is the carbohydrate sensing protein (ChREBP) expressed?

The level of expression of the carbohydrate responsive element binding protein (ChREBP) correlates to both glucose and lipid flux. We have generated a mouse line that expresses a reporter gene to help identify whole body, tissue and cellular expression of ChREBP. We aim to identify novel tissues that express ChREBP and are important in the regulation of lipogenesis and glucose sensing. The primary technique that you will be trained in will be immunohistochemistry of mouse paraffin tissue sections.

Key words: Immunohistochemistry, ChREBP, histology

How does liver expression of the carbohydrate sensing protein (ChREBP) modulate glucose flux?

The liver expresses the highest level of the carbohydrate responsive element binding protein (ChREBP) and this significantly modulates whole body glucose metabolism. We have generated mouse lines deficient of liver ChREBP, and are studying mechanisms of lipogenesis and glucose sensing. The primary aim of this project will be to conduct glycolytic flux measurements on isolated hepatic cells from ChREBP null mice. Key words: glycolytic flux, Seahorse XF24, ChREBP, liver

How does adipose tissue expression of the carbohydrate sensing protein (ChREBP) modulate whole body insulin sensitivity?

The level of expression of the carbohydrate responsive element binding protein (ChREBP) in adipose tissue is strongly correlated to whole body insulin sensitivity. We have generated mouse line that is deficient in adipose ChREBP expression, to aid in our understanding of this important regulator of lipogenesis and glucose sensing. The primary aim will be to help conduct in-depth phenotypic studies of these specific ChREBP tissues specific null mice. Key words: lipogenesis, ChREBP, adipose

What is the role of the TNFSF14 in the hepatic steatosis and NASH?

Unregulated deposition of fat in the liver ultimately leads to fatty liver but the progression to more severe forms of steatosis are believed to be due to secondary inflammatory insult. The primary aim of this project is to study the role of CD8+ T cell derived TNFSF14 (or LIGHT) in regulation of hepatic lipid uptake. Key words: TNFSF14, NASH, inflammation and obesity
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. Our membrane nanotechnology projects involve the development of new methods for membrane protein purification and analysis with application to Alzheimer’s, G protein-coupled receptor function, apoptosis, antimicrobial peptide function and new biosensor devices.

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.

HONOURS 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.

HONOURS/PHD PROJECT 2: ROLE OF THE MITOCHONDRIAL MEMBRANE IN APOPTOSIS
The Bcl-2 family of proteins is crucial for apoptosis (a form of programmed cell death) regulation and in spite of the recognised importance of the membranes in the function of Bcl-2 family members, this process is still poorly understood. The overall aim of this project therefore is to perform a systematic evaluation of the membrane interactions of the Bcl-2 family of proteins in complementary biophysical and cellular experiments. This project will redefine the mechanism of apoptosis and provide new avenues for the development of compounds to selectively modulate diseases in which apoptosis is dysregulated.

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 adnectins for diagnosis and therapy
My group recently developed the world’s most stable adnectin domain, which we are now investigating as a scaffold for the development of new candidate agents for therapeutic and diagnostic use.

Harnessing protein dynamics in antibody engineering
We are dissecting the molecular mechanisms of antibody stability, paving the way for stable antibody reagents with optimized dynamic properties that will be highly beneficial for applications in basic research, medicine and the biotechnology industry.

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/
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 develop lead compounds and ultimately new, more effective antibiotics. We also develop new approaches for antimicrobial therapy, with our current focus on multi-drug resistant *Staphylococcus aureus*. In this research, we are exploring how combining the ability to inhibit crucial infection pathways with compounds that specifically “target” bacteria can be used to develop novel treatments that we badly require. These multi-disciplinary projects are 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 constraints of the peptide synthesis machinery that generates the glycopeptide antibiotics** – the glycopeptide antibiotics are produced by a fascinating enzymatic peptide assembly line – a non-ribosomal peptide synthetase (NRPS) – which can generate peptides from non-proteinogenic amino acids entirely independently of the ribosome. One major question in the field is the source of the famed selectivity of NRPS systems, and condensation domains – responsible for peptide bond formation – have been implicated in this selectivity. In this project you will chemically synthesise peptide substrates for condensation domains and test their acceptance into altered peptide products, thus testing and identifying the rules for peptide synthesis by these impressive molecular machines.
RESEARCH BACKGROUND

During cancer, repression of tumour suppressor genes and activation of oncogenes is driven or contributed by dysregulation of epigenetic modifiers. The polycomb repressive complexes 1 and 2 (PRC1 and PRC2) are histone modifiers that required for the repression of thousands of genes during development and are dysregulated in cancer. Yet, although PRC1 and PRC2 are considered as promising targets for anticancer therapeutics, the molecular mechanism underlying their regulation is unknown.

HONOURS PROJECTS

1. **Epigenetic repression and derepression of polycomb-target genes**: The molecular mechanism of repression and activation of thousands of polycomb-target genes will be identified through the utilization of genome editing techniques (CRISPR/Cas9), combined with proteomic approaches, next generation sequencing techniques and genetic screens.

2. **Structural basis for the regulation of epigenetic modifiers, using high resolution cryo-EM and X-ray crystallography**: Macromolecular complexes of epigenetic modifiers, including multi-protein subunits, RNA and nucleosomes will be reconstituted and their structure will be determined in the presence and absence of anticancer drugs or disease associated mutations.

3. **RNA-binding specificity by epigenetic modifiers**: We will combine next generation sequencing techniques with classical methods for the detection and perturbation of RNA binding specificity for the development of novel anti-cancer therapeutics and diagnostic tools.
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 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.

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.
RESEARCH BACKGROUND

Electron Microscopy of immune system proteins
Currently we do not understand the structure of the terminal complement pathway Membrane Attack Complex (MAC). The MAC is a giant holepunching complex that targets and kills invading bacteria and parasites. Not enough MAC leads to susceptibility to bacterial infection. Too much MAC assembly leads to autoimmune disease. The study of MAC using Single Particle-cryo Electron Microscopy and cryo-Electron Tomography will tell exactly how the MAC changes shape to punch into the membrane and how we can stop this hole-punching action in autoimmune disease.

Enhancing the activity of antibiotics using the MAC (collaboration with Prof Li, Pharmacy)
The MAC is proposed to deliver other antimicrobial agents such as lysozyme that chews up the peptidoglycan layer leading to bacterial cell death. We want to investigate the use of the MAC to improve the delivery, and therefore efficacy, of antibiotics. This study will use electron microscopy and atomic force microscopy to explore the structure of the MAC on the surface of bacteria. This will be an important step in the fight against multidrug resistant bacteria.

Application of the MACPF/CDC superfamily of pore forming proteins
The fungal toxin, pleurotolysin, 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). This project includes early engagement with TSG sequencing industry.

HONOURS PROJECTS

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 proteins (typically toxins) into the cytoplasm of 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.
RESEARCH BACKGROUND

Our lab uses cryogenic electron microscopy 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.

HONOURS PROJECTS

All projects will be executed within the ARC Centre of Excellence in Advanced Molecular Imaging and in collaboration with Centre associated chief investigators, including Prof James Whisstock, A/Prof Mike Lawrence (WEHI), Dr Max Cryle and Dr Chen Davidovitch. Biological topics include cancer biology, transcription regulation & mRNA export. Cryo-EM images will be acquired at the newly established Clive & Vera Ramaciotti Centre for Structural Cryo-EM, housing the world-class FEI Titan Krios instrument.

Collaborative Cryo-EM Projects

Do you want to be in the forefront of structural biology research? Do you want to add a competitive edge to your set of competences? Join the lab and learn how to master the entire process of cryo-EM structure determination, from data acquisition, to image processing and interpretation and validation of the results.

New Computational Methods for Cryo-EM Image Processing & Integrative Structural Biology

Are you apt with computers? Do you want to contribute to the development of HPC codes for hybrid CPU/GPU architectures and field-programmable gate arrays (FPGAs)? Do you want to develop codes that integrate interactome data obtained by cross-linking and mass-spectrometry MS/MS with X-ray structures and cryo-EM maps to solve the most difficult problems in contemporary structural biology?
The Whisstock Laboratory
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RESEARCH BACKGROUND
Professor James Whisstock is an NHMRC Senior Principal Research 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
Please email James Whisstock (james.whisstock@monash.edu) for further detail of the projects.

1. Structural studies on the pore forming toxin perforin
   Development of small molecules and single chain monoclonal antibody inhibitors to block perforin mediated cell killing in organ transplant rejection
2. Role of host proteases in bacterial infection
   Development of therapeutic for flesh eating disease
3. Role of autoantibody on neurological function
   Focusing on autoantibody to glutamic acid decarboxylase
4. Lateral transfer of antibiotic resistance genes in bacteria
   Structural studies on the bacterial DNA conjugation system
5. Functional role of mammalian pore forming toxin
   Structural studies on macrophage perforin-like pore forming toxin
6. Signaling and cancer
   To investigate the structural basis for the dysfunction of second messenger signaling systems in cancer
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 2018 (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!