bio21 institute

THE UNIVERSITY OF MELBOURNE

Department of Biochemistry and Molecular Biology
Honours/Masters 2017

Photos in this handbook were take by Peter Casamento or Alan Ching

GENERAL OVERVIEW

WHAT IS HONOURS?

Honours is a one-year course of advanced study. It involves an intensive research-based project where you learn to solve problems, analyse data, think critically and communicate your research.

WHY SHOULD I DO HONOURS?

Honours provides high level research training. It enables you to develop skills that include problem solving, critical thinking, project management and oral communication. These are skills that are highly sought after by employers in biological, medical and industrial areas. Completion of Honours is the major route of entry into the PhD program.

ARE THERE SCHOLARSHIPS AND PRIZES AVAILABLE?

Yes! All students that achieve > 83 weighted average for their third year subjects will receive a Biochemistry and Molecular Biology scholarship worth $5000. The student that achieves the highest mark for their Honours year will be awarded with the Grimwade Honours Prize of $2500.

WHEN DOES HONOURS START AND FINISH?

Projects start at the beginning of Semester One and finish in November. Mid-year entry is also possible, where projects start in Semester Two and finish in June the following year.

DO I NEED TO DO COURSE-WORK?

Yes. You will choose two course-work modules from: Cell Biology, Structural Biology or Genomics and Bioinformatics. You will be exposed to major scientific advances and learn essential skills in experimental research. In addition, all students will undertake a module in Research Design and Statistics.

HOW IS HONOURS ASSESSED?

The course-work modules are worth 25 points and are assessed by essays, assignments and/or oral presentations. The Research project is worth 75 points and is assessed by a written thesis and a seminar presentation.

WHAT ARE THE ENTRY REQUIREMENTS FOR HONOURS?

Entry usually requires three years of a B-Sci or B-BMed degree. The Faculty of Medicine, Dentistry and Health Sciences requires a weighted average of 65 or better. However, nearly all students entering honours in Biochemistry and Molecular Biology obtain >73 weighted average, so meeting the minimum faculty level is not a guarantee of admission. For more information refer to: **http://sc.mdhs.unimelb.edu.au/entry-requirements**

HOW DO I FIND A PROJECT?

You can find out about specific Research projects on offer in the Honours handbook, the Department of Biochemistry and Molecular Biology website and the Honours Application and Tracking System (HATS). Make sure you discuss Research projects with prospective supervisors before completing an application form. To meet prospective supervisors, attend the Honours Information Sessions and/or contact them by email.

WHERE WILL MY PROJECT BE LOCATED?

Honours projects in Biochemistry can be conducted in different locations all of which are affiliated with The University of Melbourne. These are Bio21 Molecular Science and Biotechnology Institute, Cell Signalling Research Laboratories, The Centre for Systems Genomics, The Florey Institute of Neuroscience and Mental Health, Peter Doherty Institute for Infection and Immunity and St Vincent's Institute. The location of the research projects are listed under the names of the supervisors involved.

HOW TO APPLY

HOW TO APPLY FOR HONOURS IN THE DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY.

1. Identify a preferred Honours Research project.
2. Discuss Research project and intentions with prospective supervisor.

Option A. Select One Research project.

This means a guaranteed place in the laboratory to undertake the nominated project (providing you meet the entry requirements).

1. Complete Supervisor Provisional Offer form available from the "How to Apply" section of the Honours website: **http://biomedicalsciences.unimelb.edu.au/departments/biochemistry/study/department-student-resources/honour-And-masters#two**
2. Fill in and get supervisor to sign.
3. Email to Dr J. Mintern, Research Training Coordinator, jmintern@unimelb.edu.au.
4. In addition to completing this form, you must also apply via HATS. **http://sc.mdhs.unimelb.edu.au/how-apply**
5. You will receive an official offer of a Research project placement from the University.

Option B. Select up to 3 preferred Research projects.

This option enables you to apply to several projects and laboratories. Based on your preferences, students will be selected by the nominated supervisors.

1. Apply online via the Honours Application and Tracking System (HATS): **http://sc.mdhs.unimelb.edu.au/how-apply**
2. Upon acceptance into the program, you will receive an official offer of a Research project placement from the University.

Key Dates for this process are listed here:
https://hats.mdhs.unimelb.edu.au/

THE MASTER OF BIOMEDICAL SCIENCE

The Master of Biomedical Science offers graduates a pathway into a research or other science-based career, or PhD studies. In this program (200 credit points), students will undertake a major research project (125 credit points) and discipline-specific coursework subjects (50 credit points including the compulsory discipline subject, **Introduction to Biomedical Research (BIOM40001)**, delivered as a 2-week intensive during the last two weeks of February prior to semester commencement). In addition, professional business and communication subjects (25 credit points) complement and enhance career opportunities and the research project.

To be considered for entry, applicants must have completed an undergraduate degree with a major in a relevant discipline with a weighted average mark of at least H3 (65%), or equivalent. Meeting this requirement does not guarantee selection; in ranking applications, the Selection Committee will consider prior academic performance. Applicants are required to satisfy the university's English language requirements for postgraduate courses. For those applicants seeking to meet these requirements by one of the standard tests approved by the Academic Board, performance **band 6.5** is required.

Students entering this course in the Department of Biochemistry and Molecular Biology must organise an academic supervisor and select a research project as part of the application process. The theme and scope of the research project must be negotiated between the student and supervisor at the time of application, and a specific supervisor identified in the application.

For further information, including fees and scholarships, entry requirements, and more on the degree structure, see: **http://mdhs-study.unimelb.edu.au/degrees/master-of-biomedical-science/overview**

PROJECTS

Dr David Ascher
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TREATING THE PERSON NOT THE DISEASE

Dr David Ascher

We have developed a range of computational tools to deconvolute the molecular mechanisms of a mutation giving rise to different phenotypes. In collaboration with clinical partners we have shown that even though patients may present the same disease, they may arise from many different mutations that alter a patient's outcome or how they may respond to a particular treatment. By analysing these mutations and predicting their effects on protein structure and function we are trying to revolutionise treatment strategies, an important step towards personalised medicine. We are currently working on a range of diseases including genetic diseases (Alkaptonuria, Urea cycle disorders, VHL), cancer (renal carcinomas), and drug/vaccine resistance (TB, cancer, malaria, HIV, influenza). These projects combine both computational (bioinformatics) and experimental (protein expression, biophysics, structural biology) approaches to unravel the molecular mechanisms driving these mutations and derive novel predictive methods. This information is then used to help identify and guide the development of novel therapies to treat these conditions. One of the ultimate goals of these projects will be the development of webservers enabling the rapid analysis of mutations to help guide clinical decisions.

Honours/Masters/PhD

FINDING CLARITY WITHIN A BLIZZARD- GUIDING THE SOLUTION OF CRYO-EM STRUCTURES

Dr David Ascher

The recent explosion in the power of cryo-electron microscopy has revolutionised the structural biology field, especially the characterisation of large protein complexes. This is helping us tackle very important biological problems in a way that they could never before. There are, however, inherent limitations that not only pose difficulties to the structure solving stage, i.e., properly positioning a protein chain within an electron density map, but also potentially introducing errors that might be propagated to the refined structure. This is especially relevant for medium resolution structures (4-8 Å). The problem is analogous to looking through blurry (or drunk) glasses and, without good points of reference, not being able to orientate yourself. To improve this procedure we can leverage the power of our existing structural and evolutionary knowledge accumulated over decades and deposited in structural databases in order to help guide the proposal of a more effective methods for this molecule placement. This project will use structural bioinformatics and machine learning to develop novel computational tools to aid cryo-EM and low resolution crystal structure solving, analysing protein residue environments, protein interaction interfaces, and protein functional sites. These methods will be brought together into an integrated platform for the evaluation and validation of medium resolution protein structures.

Honours/Masters/PhD

SMALL MOLECULES FOR BIG TARGETS- TARGETING PROTEIN-PROTEIN INTERACTIONS WITH FRAGMENTS

Dr David Ascher

Most proteins work within a network of interactions with other proteins, and the ability to selectively target specific interactions, modulating protein function and providing the opportunity to develop more selective and effective drugs. But while drugs are usually around 100 Å2, proteins interact tightly using way larger protein-protein interfaces, ranging from 1000-6000 Å2. This raises the challenge of how we can use a small molecule to affect an interface many times larger, which until recently was considered to be flat and undruggable. We and others have had success using fragment-based drug discovery to identify novel protein-protein interaction modulators. This allows us to take advantage of hot-spots within the protein interfaces that mediate a large proportion of the binding energy, growing the molecule to improve binding affinity and drug like properties. The crystal structures of many protein interface modulators with their targets have been solved, which opens up the possibility for us to ask: what are the major components of binding affinity? and can we use this information to predict fragments likely to bind to a given interface? Using structural bioinformatics and machine learning, these questions will be answered, leading to the development of novel programmes. The students will then also have the opportunity to test these experiments in the lab, using biophysical and structural approaches to test fragment binding.

Honours/Masters/PhD

COMPUTATIONAL DRUG SCREENING

Dr David Ascher

Identifying and developing new drugs is a time consuming and expensive process. Using graph-based signatures we have been developing approaches that enable rapid screening of biological activities, along with their pharmacokinetic, pharmacodynamic and toxicity profiles. This can help identify in early stages molecules of interest to focus development efforts upon, reducing failure risks, costs, time and animal usage. These projects will explore the chemical signatures of molecules that could be used to treat different cancers and microbial pathogens (including TB, malaria and the most dangerous hospital-acquired, antibiotic-resistant infections: Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA), as well as the common causes of fungal infections Cryptococcus neoformans and Candida albicans). By mapping the chemical signatures of active and inactive molecules, machine learning will be used to identify patterns that will be used as evidence to generate novel predictive algorithms capable of identifying potential new antibiotics and chemotherapeutics. In collaboration with our partners, these predicted molecules will be tested in models of the diseases.

Honours/Masters/PhD

Prof Ross Bathgate
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TARGETING PEPTIDE G-PROTEIN COUPLED RECEPTORS (GPCRS) FOR NOVEL DRUG DEVELOPMENT

Prof Ross Bathgate, Dr Daniel Scott, A/Prof Paul Gooley

The largest single class of drug targets is the G Protein-Coupled Receptor (GPCR) family, which were targets for 13 of the top 50 prescription drugs sold in the USA in 2010 (26%). However current drugs only target a small proportion of the GPCR family and peptide GPCRs, although showing great potential as targets for treating many diseases (for eg. see neuropeptide project), are poorly targeted with drugs. Modern GPCR drug development is encumbered by a lack of information about the molecular structure underlying GPCR function and the reliance on cell-based assays that are prone to false positives in drug screening. While the past 10 years have seen advances in our knowledge of GPCR structures peptide GPCRs, especially those with large structured ectodomains (ECDs), remain poorly understood. This is mainly because the flexibility of linkers joining the ECDs to the transmembrane domains (TMDs) impedes crystallization. Hence the study of complex peptide receptors requires different approaches. Our laboratory targets peptide GPCRs for drug development utilizing state-of-the-art molecular pharmacology, biochemical and Nuclear magnetic resonance (NMR) techniques. These techniques enable us to map the native peptide binding sites of these receptors and determine the mechanisms of receptor activation as well their cell signalling characteristics. A complete understanding of the mechanism of ligand binding and activation is required to design drugs targeting these receptors. Furthermore we are utilizing novel techniques to study the receptor structures (see project below) and are also studying ligand interactions with receptor domains using soluble receptor domains and NMR (See projects A/Prof Gooley). These studies are complemented by peptide drug development projects and small molecule screening projects with collaborators. Additionally, we are working with pharmaceutical industry partners (eg. Takeda and Novartis) to facilitate drug development efforts. Projects are available on multiple GPCR targets with training in various techniques as outlined above.

Honours/Masters/PhD

How does doing Honours help your future career?

It's a great way to get a foot in the door for a PhD. Also getting lab skills, doing course work, having to do an oral presentation, all of it coalesces into a great scientific experience. It's incredibly useful for finding a career in science.

Alex Stojilovic

VIRAL-MEDIATED MODULATION OF NEUROPEPTIDE GPCR FUNCTION IN BRAIN

Prof Ross Bathgate, A/Prof Andrew Gundlach

Mental illness is a large and increasing health and economic burden in Australia and worldwide and more research is urgently required to identify new and innovative therapies. In this regard, neuropeptide GPCRs may be better therapeutic targets than receptors for the 'primary' transmitters (amino acids and monoamines), as they offer reduced side-effects, due to their modulatory actions. However assessing the therapeutic potential of neuropeptides is complicated by the difficulties of delivering peptides to the brain and hence alternative approaches are needed. We utilize viral gene transfer by adeno-associated viral (AAV) or lentiviral particles to transduce specific neuronal populations allowing the chronic modulation of neuropeptide or neuropeptide GPCR function. We target specific neuropeptide systems by either gene silencing or by overexpression of peptide agonists or antagonists in adult animals thus avoiding potential compensation that can occur in knockout animals. Such viral targeting allows assessment of long-term modulation of neuropeptide systems on complex behaviours which are assessed in state-of-the-art rodent behavioural facilities at the Florey Institute. We are also utilizing viruses to express excitatory and inhibitory 'designer receptors exclusively activated by designer drugs' (DREADDs), GPCRs activated only by an artificial ligand which allows a pharmacogenetics approach to selectively and reversibly activate or inhibit specific neuropeptide expressing neurons and assess effects on physiology and behaviour in freely-moving animals. This complementary approach assesses the role of the neuropeptide expressing neural circuits in the modulation of behaviour and can be extended to studies in genetic models of social, cognitive and other deficits seen in psychiatric illnesses. We have successfully employed these strategies to demonstrate that the neuropeptide relaxin-3 has roles in regulating behaviours which are perturbed in mental illnesses including anxiety, depression, sleep disorders and memory deficits. Students will receive training in molecular cloning, viral production, stereotaxic surgery; behavioural assays and analysis; mRNA/peptide/protein analysis; and light/confocal microscopy.

Honours/Masters/PhD

Why did you do Honours in Biochemistry?

Most of my lecturers from third year were part of the Biochemistry department so I was really interested in the research they were doing. I really liked the projects they had on offer and their enthusiasm.

Robyn McConville

Dr Katrina Binger
Prof Malcolm McConville
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IMMUNOMETABOLOMICS – CONNECTING OUR DIET WITH OUR IMMUNE SYSTEM

Dr Katrina Binger, Prof Malcolm McConville

Our research is focused on understanding how the consumption of the "Western" diet, comprised of processed foods high in sugar, salt (sodium chloride) and fat, increases the risk of developing chronic non-communicable diseases such as diabetes and cardiovascular disease. It has previously been shown that malfunctioning immune cells, particularly macrophages, are important in initiating the low-grade inflammation that drives the onset of these diseases. Our recent work has identified a putative mechanism where too much salt was found to affect macrophages in diverse ways. Pro-inflammatory 'M1' macrophages, important in clearing pathogens such as *Leishmania*, respond positively to high salt (Jantsch et al Cell Metab 2015). Conversely, anti-inflammatory 'M2' macrophages, which play critical roles in combating inflammation, were inhibited by high salt (Binger et al JCI 2015). The overall result of these seemingly contradictory studies is that high salt pushes the immune system out of balance by boosting pro-inflammatory cells and weakening cells which stop inflammation. This project will focus on understanding the molecular mechanisms by which high salt differentially affects M1 and M2 macrophage activation and metabolism. This project will use a range of approaches, including metabolite profiling and ^{13}C-isotope labeling experiments (mass spectrometry), live cell respiration and glycolysis (Seahorse metabolic analyzer), and analysis of macrophage activation (flow cytometry and molecular biology assays) of wildtype and mutant macrophages exposed to isotonic and high salt conditions. The findings from this project will be used to identify potential targets to ameliorate the adverse effects of dietary salt on the immune system, and thus to treat or prevent chronic diseases.

Honours

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KEEPING JNK IN ITS PLACE

A/Prof Marie Bogoyevitch

The nucleus, housing the genomic information store of DNA in the protein-complexed form called chromatin, is of central importance in the compartmentalised eukaryotic cell. c-Jun N-terminal Kinase (JNK) signaling plays key roles in the signaling events activated by stress and during fundamental processes of development. Our recent studies analysing JNK1 nuclear trafficking challenge the paradigm of uni-directional signaling: live imaging reveals continued nucleus-cytoplasmic shuttling of JNK1 with its major determinant for nuclear entry mapped to its C-terminal sequence and not correlated with its activation. Furthermore, unanticipated links between JNK and the Nuclear Factor-Y (NF-Y) transcription factor may provide additional levels of control, whereby chromatin-associated acetylases modify JNK in an unappreciated regulatory platform for sending nucleus-initiated signals back to the cytoplasm, i.e. nucleo-radiant signaling, representing a novel mechanism that integrates nuclear actions by creating a signal transduction circuit triggered by environmental or genetic factors. These observations underpin a series of projects that aim to define the critical features of JNK that dictate its nuclear trafficking, to explore the impact of JNK post-translational modifications on its trafficking and kinase activity and to define JNK's contribution to the NF-Y regulatory platform and nucleo-radiant signalling. Key methodologies used in these projects will include high resolution and live imaging, together with fundamental biochemical approaches to the creation and deployment of novel recombinant forms of modified JNKs. The studies are underpinned by a national and international collaborative network including Prof David Jans (Monash), Prof Attila Remenyi (Hungary) & Prof Roberto Mantovani (Italy) and supported by an Australian Research Council Discovery Grant.

Honours/Masters/PhD

DISCOVERY OF POLYQ ATAXIN-1 REGULATION AND REGULATORS

A/Prof Marie Bogoyevitch

The worldwide impact of the broad spectrum of neurodegenerative diseases is staggering: >35 million people experience debilitating dementia and cognitive decline, most commonly in the progression of Alzheimer's disease, but millions more suffer the loss of coordinated movement and memory in neurodegenerative conditions such as Parkinson's disease, Huntington's disease, and the Spinocerebellar Ataxias (SCAs). Polyglutamine (PolyQ) expansions in ataxin-1, an RNA-binding protein with poorly understood physiological and pathophysiological roles, are the cause of neurodegeneration in Spinocerebellar Ataxia 1 (SCA1). A robust feature of polyQ-ataxin-1 is its formation of prominent nuclear bodies comprised of polyQ-expanded ataxin-1 together with other, mostly uncharacterized, partners, but how ataxin-1 influences cellular functions has remained poorly understood. We recently discovered that several nuclear transport proteins are proximal partners of polyQ-ataxin-1, and that expression of polyQ ataxin-1 is sufficient to disturb the cytosolic milieu and negatively impact cell survival. Furthermore, we observed a stress-induced remodelling of ataxin-1 intranuclear bodies typical of a transition to a hydrogel-state, a hallmark of neurotoxicity. Our studies open exciting new avenues to probe the mechanisms of ataxin-1-induced toxicity by defining the impact of ataxin-1 on nucleocytoplasmic transport and characterizing stress-dependent remodelling of ataxin-1 nuclear bodies. Key methodologies used in these projects will include high resolution and live imaging, as well as proteomics approaches to define the ataxin-1 interactome. The studies are underpinned by a national and international collaborative network including Dr Liz Hinde (UNSW), Prof David Jans (Monash), A/Prof Danny Hatters (Bio21) and Prof Annalisa Pastore (London).

Honours/Masters/PhD

PROBING MICROTUBULE ORGANISATION

A/Prof Marie Bogoyevitch

Microtubules, cytoskeletal polymers of tubulin, are critical contributors to normal brain development and maintenance. Across a spectrum of neurodegenerative conditions including Parkinson's and Alzheimer's disease, microtubule functions are dysregulated, and mutant microtubule and/or their regulatory microtubule-associated proteins result in severe brain developmental defects, intellectual disability and epilepsy. Despite this clear significance, large gaps remain in our understanding of how MAPs are regulated and how disease-causing mutations impact on their actions. In this project, our focus is Doublecortin X-linked (DCX); the importance of DCX is highlighted by clinical studies showing multiple different DCX gene mutations as the cause of a disease spectrum typified by epilepsy, seizures, and intellectual disability arising from abnormal neuron migration during development and the disruption of the layering of the brain cortex. At a biochemical level, DCX interacts with microtubules via its structured DC domains. Although clustering of pathogenic mutations in the DC domain has strengthens a hypothesis that defects in DCX-microtubule interactions underlie brain developmental defects, many pathogenic DCX mutants continue to associate with microtubules, highlighting the need to explore additional regulatory defects. Indeed, some pathogenic mutations lie outside the DC domains. Our studies exploring the impact of truncated forms of DCX, pathogenic DCX mutations lying outside the DC domains and a novel N-terminal phosphorylation site have reinforced the importance of regions outside the DC domains. We are now evaluating how DCX actions can be controlled by its N- and C-termini to direct appropriate spatial and temporal control of microtubule organization. Key methodologies used in these projects range from high resolution and live imaging in cells to focused structural and biochemical studies. The studies are enhanced by a national and international collaborative network including Dr Dominic Ng (University of Queensland), A/Prof Paul Gooley (Bio21) Prof Staffan Persson (Biosciences) and Prof Carolyn Moores (London).

Honours/Masters/PhD

Dr Kristin Brown
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FUELING CHEMOTHERAPY RESISTANCE IN TRIPLE-NEGATIVE BREAST CANCER

Dr Kristin Brown

Triple-negative breast cancer (TNBC) is a molecularly heterogeneous group of diseases defined by the lack of estrogen receptor (ER), progesterone receptor (PR) and absence of human epidermal growth factor receptor-2 (HER2) amplification. Consequently, TNBCs are impervious to therapies commonly used in other breast cancer subtypes and treatment options are largely limited to conventional genotoxic chemotherapy agents. Approximately 30% of TNBC patients respond to chemotherapy. The long-term prognosis for the majority of patients with residual disease after chemotherapy is poor. Identification of novel and actionable strategies to sensitize cancer cells to chemotherapy would represent a major advance for the management of TNBC.

Cancer cells exhibit dramatic alterations in cellular metabolism, which support cell growth, proliferation and survival. Indeed, metabolic reprogramming is a recognized hallmark of cancer induced by numerous genetic or epigenetic alterations. Targeting the existing metabolic perturbations that occur in cancer cells has emerged as a promising strategy for cancer therapy. Recent studies suggest that reprogramming of cellular metabolism is also a component of the highly coordinated response to genotoxic stress. However, the metabolic response to clinically relevant genotoxic chemotherapy agents is poorly understood. The aims of this project will be to 1) identify adaptive metabolic reprograming events triggered upon chemotherapy exposure, and 2) identify novel therapeutic approaches to exploit adaptive metabolic reprogramming events and circumvent chemotherapy resistance. This research will lead to the identification of critical mechanisms driving chemotherapy resistance in TNBC and establish combination therapy strategies with potential to have a major impact on patient survival. Students will gain experience in mammalian cell culture, metabolomics (mass spectrometry) and stable-isotope labeling techniques.

Honours/Masters/PhD

ELUCIDATING THE SERUM AND GLUCOCORTICOID KINASE 1 (SGK1) KINOME AND INTERACTOME

Dr Kristin Brown

The phosphoinositide 3-kinase (PI3K) pathway has emerged as a master regulator of numerous cellular phenotypes associated with cancer including cell survival, proliferation, growth, altered metabolism and malignant transformation. Deregulation of the PI3K pathway is implicated in virtually all human cancers and the pathway has been aggressively targeted for cancer therapy. Although most work has focused on the Akt kinase family as major downstream effectors of PI3K, the closely related serum and glucocorticoid regulated kinase (SGK) family of serine/threonine kinases has by comparison received little attention. The SGK1 isoform was initially discovered as a gene transcriptionally responsive to serum and glucocorticoids in mammary tumour cells. More recently, SGK1 has been shown to play a critical role in driving the expansion of tumour cells and promoting resistance to conventional chemotherapy and targeted therapy agents. However, the molecular mechanisms that enable SGK1 to elicit such oncogenic activities are unknown. This is largely because information regarding the substrates and interaction partners of SGK1 is extremely limited. In this project, students will identify SGK1 substrates and interacting proteins using the proximity-dependent biotin identification (BioID) method. Students will gain experience in mammalian cell culture and proteomics (mass spectrometry) techniques. Targets identified in the BioID screen will be validated using a variety of biochemical and molecular biology techniques.

Honours/Masters/PhD

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BIOCHEMICAL BASIS OF THE TUMOUR SUPPRESSIVE MECHANISM OF CSK-HOMOLOGOUS KINASE (CHK)

A/Prof Heung-Chin Cheng, Dr Michael Griffin (Bio21 Institute), Dr Heather Verkade, Dr Hong-Jian Zhu (Surgery), Dr Nick Williamson (Bio21 Institute) and Ching-Seng Ang (Bio21 Institute)

Src-family kinases (SFKs) are protein products of the Src-family of oncogenes and proto-oncogenes. Constitutive activation of SFKs contributes to many forms of cancer. In normal cells, SFKs are kept in the inactive form by phosphorylation of the C-terminal regulatory tyrosyl residue (referred to as YT). CSK-homologous kinase (CHK) is a major tumour suppressor enzyme constraining SFK activity in normal cells. Furthermore, we and others recently discovered that CHK is down-regulated by epigenetic silencing and inactivating missense mutations in colorectal cancer, suggesting that lack of expression of functional CHK permits over-activation of SFKs in cancer cells. This project focuses on deciphering (i) the structural basis of two inhibitory mechanisms employed by CHK to constrain SFK activity, (ii) how CHK activity and expression are regulated in cancer cells and (iii) how expression of CHK induces growth arrest of the cancer cells using quantitative proteomic approaches.

Honours/Masters/PhD

DECIPHERING THE ROLE OF SRC-FAMILY KINASE C-SRC IN EXCITOTOXIC NEURONAL DEATH DURING ISCHEMIC STROKE

A/Prof Heung-Chin Cheng, Dr Carli Roulston (St. Vincent's Hospital), Dr Nick Williamson (Bio21 Institute) and Dr Ching-Seng Ang (Bio21 Institute)

An ischemic stroke occurs when the one of the blood vessels (e.g. the middle cerebral artery) supplying the brain is blocked, leading to the damage of the neurons nearby. The damaged neurons induce a second round of neuronal death by secreting an excessive quantity of the neurotransmitter glutamate. Over-stimulation of glutamate receptors in the neighbouring healthy neurons causes cell death – a process known as excitotoxicity. Exactly how over-stimulation of glutamate receptor induces neuronal death is poorly understood. Results of the studies by us and others indicate that inhibitors of c-Src kinase are protectants, indicating that aberrant activation and/or subcellular localisation of c-Src contribute to excitotoxic neuronal death. We are using cultured mouse primary cortical neurons and rat model of ischemic stroke to decipher how c-Src is aberrantly activated in neurons undergoing excitotoxic neuronal death and how the activated c-Src induces premature death of neurons in cultured and in rats. Students participating in this project will be co-supervised by Dr. Carli Roulston of St. Vincent's Hospital (refer to publications for further information).

Honours/Masters/PhD

DEFINING THE SIGNALLING MECHANISM GOVERNING EXCITOTOXIC NEURONAL DEATH BY PROTEOMICS AND MOLECULAR APPROACHES

A/Prof Heung-Chin Cheng, Dr Joe Ciccotosto (Pathology), Ching-Seng Ang and Nick Williamson (Bio21 Institute)

Excitotoxicity, a neuronal death process caused by over-stimulation of glutamate receptor, governs neuronal loss in acute and chronic neurological conditions such as cerebral stroke and neurodegenerative diseases. The exact mechanism of excitotoxic neuronal death remains unclear. Previous studies by us and other researchers indicate that aberrant regulation of proteases, protein kinases and phosphatases at the early stage of excitotoxicity directs neurons to undergo cell death. In this project, we aim to employ quantitative protease proteomics and phosphoproteomics approaches to define the targets of the aberrantly activated proteases, protein kinases and phosphatases in neurons in excitotoxicity. The first stage of our study has identified multiple neuronal proteins that are modified by these dysregulated enzymes. In the second stage of our study, we aim to use molecular and proteomics approaches to define the roles of these neuronal proteins and investigate how they interplay to direct neuronal death.

Honours/Masters/PhD

PROTEIN KINASES INVOLVED IN THE PATHOGENESIS OF PARKINSON'S DISEASE

A/Prof Heung-Chin Cheng, Dr Vijaya Kenche (Pathology and Melbourne Brain Center)

Parkinson's disease (PD) is a neurodegenerative disorder of movement known to result from the progressive, preferential loss of dopaminergic neurons from the *substantia nigra* of the mid-brain. However, the cause of such neuronal death in PD is not known. Genes encoding PTEN-induced kinase 1 (PINK1) and leucine repeat-rich kinase 2 (LRRK2) were identified as PD-causative genes. The aim of this project is to identify the physiological substrates and define the biochemical and structural basis of regulation of PINK1 and LRRK2 (refer to publications for more information).

Honours/Masters/PhD

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FISHING FOR METABOLIC CLUES: THE HIPPO/YAP PATHWAY REPROGRAMS METABOLISM TO FUEL TISSUE GROWTH.

Dr Andrew Cox

One of the fundamental questions in biology is to understand how organ size is regulated at the cellular and organismal level. The Hippo/Yap pathway has recently emerged as an evolutionarily conserved cascade that plays a fundamental role in governing organ size control, stem cell homeostasis and cancer. The Hippo/Yap pathway is regulated by a range of environmental cues including nutrient status. Although many of the inputs into the Hippo pathway have been identified, less is known about the Yap target genes responsible for the remarkable effects on tissue growth. We have made efforts to develop zebrafish as a model system to study the role of the Hippo/Yap pathway in regulating tissue growth during liver development, regeneration and cancer. Using a combination of metabolomic and transcriptomic approaches, we have recently discovered that Yap reprograms glutamine metabolism *in vivo* to stimulate nucleotide biosynthesis and fuel premalignant liver growth. Building on this initial investigation, we currently have research projects that aim to 1) Examine how Yap coordinates nutrient sensing to metabolic output in the liver. 2) Elucidate the mechanisms by which Yap reprograms metabolism to fuel liver growth in the context of regeneration and cancer. The students will use a combination of innovative biochemical, genetic and imaging approaches in zebrafish to identify the metabolic dependencies of tissue growth during regeneration and cancer. Specifically, students will gain expertise in stable isotope labeling, mass spectrometric profiling and metabolic flux analysis as well as zebrafish molecular genetics and confocal microscopy.

Honours/Masters/PhD

Prof Paul Gleeson
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INTRACELLULAR TRAFFICKING IN NEURONS AND ALZHEIMER'S DISEASE

Prof Paul Gleeson, Dr Wei Hong Toh

Alzheimer's disease is characterized by the accumulation of amyloid plaques in the brain consisting of an aggregated form of ß-amyloid peptide (Aß) derived from sequential amyloidogenic processing of the Amyloid Precursor Protein (APP) by membrane-bound proteases BACE1 and ß-secretase. The initial processing of APP by BACE1 is regulated by intracellular sorting events of the enzyme, which is a prime target for therapeutic intervention. We are interested in defining the intracellular trafficking pathways of APP and BACE1 and the sorting signals of these membrane proteins that define their itineraries. We have mapped the itineraries of these cargos in cultured human cell lines and our findings show that the distinct trafficking pathways of APP and BACE1 provides the capacity to finely regulate their co-localization and thereby regulating APP processing and Aß production. There is considerable evidence that dysregulation of membrane trafficking events is associated with an increased risk of Alzheimer's disease. It is now important to define the itineraries of APP and BACE1 in a more relevant cell type, namely primary neurons. The project will map the post-Golgi anterograde transport pathways of APP and BACE1 in neurons, determine the selective trafficking routes to axons and dendrites, and assess the impact of neuronal signaling on these trafficking pathways. A wide range of biochemical and cell biological approaches will be employed including super-resolution light microscopy to determine the extent of co-localization of these cargoes, live cell imaging and photoactivatable fluorescent probes to monitor transport itineraries coupled with silencing of transport machinery.

Honours/Masters/PhD

WHY IS THE GOLGI APPARATUS A RIBBON STRUCTURE IN VERTEBRATE CELLS?

Prof Paul Gleeson, Dr Prajakta Gosavi

Vertebrates have evolved mechanisms for joining the individual Golgi stacks into a ribbon, typically found in a juxtanuclear location in interphase cells. The organisation of the Golgi apparatus is highly dynamic and the Golgi ribbon can dissociate and re-organise under a variety of conditions, for example, during mitosis and to reposition the Golgi to accommodate a number of processes, including directed secretion and pathogen invasion. Surprisingly, and despite our knowledge of Golgi dynamics, the fundamental biological relevance of the "ribbon" structure of the Golgi in vertebrates remains a mystery. We have discovered that a modest overexpression of one of the TGN membrane tethers (golgins) (GCC88) results in disruption of the Golgi ribbon and dispersal of intact Golgi mini-stacks throughout the cytoplasm. On the other hand, silencing this particular TGN golgin results in an enhanced Golgi ribbon structure. Our finding that the dose of GCC88 regulates the balance between Golgi mini-stacks and Golgi ribbon provides the first opportunity to explore the function of the Golgi ribbon in stable cell lines. This project will investigate the role of the ribbon structure of the Golgi on the higher order functions of metabolism and autophagy in cells in culture, especially primary cells of the immune system. We are using a wide range of technologies in this project including viral transduction, high resolution light microscopy, electron microscopy, flow cytometry, quantitative immunoblotting, proteomics and metabolic analysis.

Honours/Masters/PhD

EXTENDING THE SERUM HALF-LIFE OF NOVEL THERAPEUTIC PROTEINS

Prof Paul Gleeson, Dr Jade Louber

The neonatal Fc receptor (FcRn) plays a critical role in regulating the half-live of a range of serum proteins, including IgG and albumin, in the adult individual. The Fc receptor protects these serum proteins from degradation by binding to IgG and albumin in endosomes after internalization by cells and releasing the proteins back into the plasma. There is considerable interest in exploiting this protective pathway to prolong the life time of engineered therapeutic proteins by attaching the FcRn ligand binding motif to recombinant therapeutic proteins. In collaboration with CSL, this project will define the membrane recycling pathway of the FcRn, and the itinerary of albumin-based ligands, information which is critical for the optimizing the life span of therapeutic proteins. The project will analyse the role of FcRn in specific cell types including macrophages, dendritic cells and hepatocytes which are considered to be the major sites for recycling in the body. Both cultured and primary cells, derived from FcRn engineered mice, will be employed. A wide variety of biochemical and cell biological methods will be used including transfection cell systems, trafficking assays, coupled with RNAi silencing of transport machinery, to dissect the pathway of ligand internalization and recycling, the kinetics of recycling using quantitative biochemical assays, as well as mass spec analysis of the recycled ligands to determine if the itinerary of recycling has resulted in post-translational modifications which may impact on function.

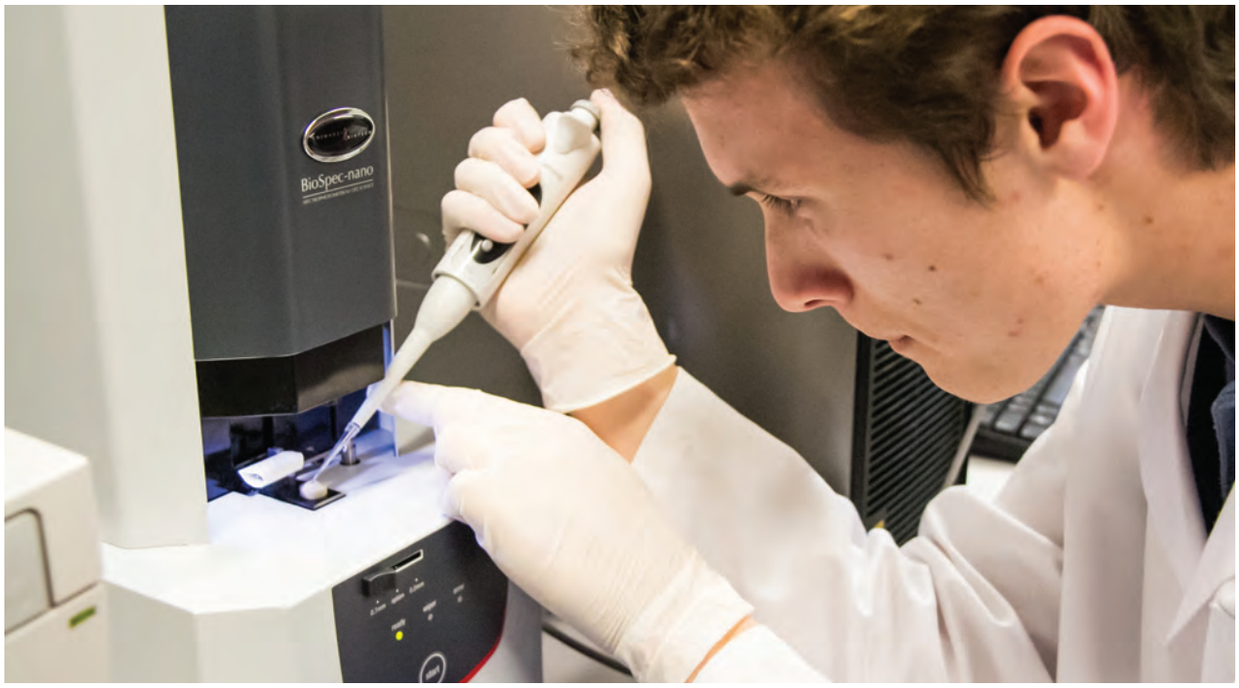
Honours/Masters/PhD

MEMBRANE TRAFFICKING OF THE TOXIN RICIN IN CELLS

Prof Paul Gleeson, Dr Peter Gray and Dr Damien Chong (Defence Science and Technology Group, Department of Defence, Fishermans Bend)

The plant toxin ricin poses a potential threat to humans and animals and the design of effective therapeutics and prophylactics require a greater knowledge of the cell biology of ricin action. Ricin binds to terminal galactose residues on cell surface glycoproteins and glycolipids and is then endocytosed into cells where some of the internalized ricin is trafficked by retrograde transport pathways to the Golgi apparatus then the lumen of the ER. In the ER, ricin is translocated into the cytoplasm where it inhibits protein synthesis. Ricin is a heterodimer with a catalytic A chain and a carbohydrate binding B-chain which is responsible for binding to cell surface glycoproteins and glycolipids. The functional transport pathway to deliver ricin to the ER and the functional receptors for ricin responsible for the retrograde transport to the ER have yet to be identified. In this project we will use a variety of trafficking assays, coupled with RNAi silencing of transport machinery, to dissect the pathway of the carbohydrate-binding ricin B-chain to the Golgi, including light microscopy and flow cytometry. We will also use a novel in vivo labelling strategy (BioID) to identify ricin binding partners by proteomics/mass spectrometry. In addition different cell types will be compared, especially primary lung epithelium as these represent one of the relevant target cells.

Honours/Masters



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DEFINING THE HOST-VIRAL MOLECULAR INTERACTIONS OF RABIES PROTEINS

A/Prof Paul Gooley, Dr Greg Moseley

Symptomatic infection by rabies virus causes an incurable and invariably lethal disease. The virus manipulates the immune response of the host cell to avoid detection during replication. To achieve this viral proteins interact with and manipulate the function of proteins of the host cell. Key to this process is the rabies virus P-protein whose full functions are not understood. P-protein interacts with other viral proteins, including the rabies N-protein, for viral replication, but it also is known to bind the immune-signalling transcription factors STAT1 and STAT2, preventing them from entering the nucleus to activate antiviral in response to interferons. The regions and amino acid residues of P-protein that are involved in these processes are unclear, and the full extent of host protein/P-protein interactions remain to be resolved. This project broadly aims to understand the molecular interactions of P-protein with its multiple targets and includes techniques such as mutagenesis to perturb specific interactions, and characterization of these mutants by cell-based assays. Mutants that lose (or gain) function will be structurally characterized and the impact on their molecular interactions determined using an array of techniques. These studies will lead towards the design of novel anti-virals and vaccines.

Honours/Masters/PhD

THE COMPLEX BINDING MODE OF THE PEPTIDE HORMONE H2 RELAXIN TO ITS RECEPTOR RXFP1

A/Prof Paul Gooley, Dr Daniel Scott, Prof Ross Bathgate

The insulin-like hormone relaxin has received recent clinical interest as a treatment for acute heart failure. The biological processes involving relaxin generally are through the activation of the G-protein coupled receptor, RXFP1. However, the molecular details of how relaxin interacts and activates RXFP1 are unclear. In part this is due to the complex multidomain structure of RXFP1: an N-terminal LDLa module essential for activation, a large leucine rich repeat (LRR) domain that is known to contain a relaxin binding site, and a C-terminal transmembrane domain that contains critical regions for activation. Structurally, we have only characterized the LDLa module. However, we have recently discovered that the 32-residue linker between the LDLa module and the LRR domain contains a second relaxin binding site, and therefore we hypothesize that relaxin binds to both this linker and the LRR domain to induce a conformational change, possibly of the linker, that reorients the LDLa module so it can effectively bind and activate the transmembrane domain. This hypothesis requires proving and opens opportunities in understanding receptor activation and the design of novel agonists and antagonists. There are multiple projects available involving mutagenesis of RXFP1 and relaxin, peptide synthesis and cell-based assays to monitor binding and activation of these mutants/analogues; expression and purification of the domains of RXFP1, structural determination of these domains and characterization of their molecular interactions.

Honours/Masters/PhD

PROBING THE CONFORMATIONAL DYNAMICS OF G PROTEIN-COUPLED RECEPTORS WITH NMR

A/Prof Paul Gooley, Dr Daniel Scott

G protein-coupled receptors (GPCRs) are a large family of signalling proteins (>800 gene members) located on the surface of all cells in the body, particularly in the brain. GPCR signalling controls virtually every physiological process in the body, making these receptors the targets of many current drugs treating conditions like pain, hypertension, schizophrenia and asthma. GPCRs exist as an ensemble of conformational states (inactive, intermediate and active) in equilibrium, with agonist binding shifting this equilibrium towards active states to stimulate cell signalling. A deeper understanding of the structural basis underlying GPCR signalling is needed to guide the design of improved therapeutics. The low expression levels and instability of GPCRs makes them difficult proteins to biochemically characterize. We have engineered high expressing, stabilized GPCRs that we can isotopically label for structural analysis with NMR. Using NMR we can observe that the receptors are dynamic and as expected that the conformational equilibrium is influenced by the binding of particular ligands (e.g. agonists vs antagonists). This project is focused on introducing NMR probes into these GPCRs so that we can characterize the structure and dynamics of specific domains within the proteins. Ultimately this will give us generic insight into how ligands engage GPCRs and how GPCRs transmit ligand binding signals into the cell.

Honours/Masters/PhD

THE RELATIONSHIP OF THE GUT MICROBIOTA AND CHRONIC FATIGUE SYNDROME.

A/Prof Paul Gooley

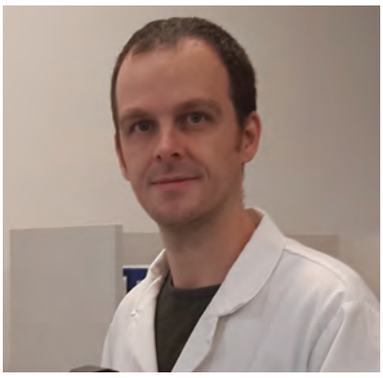
The microbiota of the human gut acts as a "hidden organ" with the ability to modulate host metabolism. There is evidence of a relationship of the microbiota with diseases and syndromes as diverse as type 2 diabetes, irritable bowel syndrome, allergy and autism. The impact of the microbiota on host metabolism can be assessed through metabolic profiling of the microbiota and the host biofluids, for example blood, urine and faecal matter, to determine associations significant of a host-microbe relationship. In this project we are applying metabolomics methodology to Chronic Fatigue Syndrome (CFS). CFS, also known as Myalgic Encephalomyelitis (ME), is a long-term disorder of fatigue that is exacerbated by exercise and not sufficiently relieved by rest. CFS is likely to be a spectrum of health issues and thus is accompanied by a large variety of other symptoms increasing the complexity of diagnosis. CFS may develop following a triggering event, such as an infection or immunity issue, resulting in an altered state of homeostasis. As the gut microbiota is important in the development of immunity it also may have a role in the development of CFS. We have conducted a metabolomics study on a cohort of CFS sufferers and found a relationship between the species of the gut microbiota, the metabolites of the microbiota and how they correlate with metabolites of the blood and urine. This project will continue this research, developing the analytical techniques of Mass Spectrometry and Nuclear Magnetic Resonance on the samples we have, as well as engaging in a longitudinal study of CFS.

Honours/Masters/PhD

What have been the highlights of your Honours year so far?

I've really enjoyed the camaraderie and the sense of community that's between the labs and within the lab. It's a great environment to study and do research. The Friday night social nights and pub nights are fun.

Alex Stojilovic



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APOLIPOPROTEIN A-I AND AMYLOID FIBRIL FORMATION IN DISEASE: HOW DOES IT CHANGE SHAPE AND MISFOLD?

Dr Michael Griffin

Apolipoprotein A-I (apoA-I) binds phospholipid and cholesterol and mediates reverse cholesterol transport; the process by which cholesterol is removed from the body via high density lipoproteins (HDL). High plasma levels of apoA-I provide a number of beneficial effects in the cardiovascular system. However, apoA-I also forms amyloid deposits in atherosclerosis and in hereditary amyloidosis, which leads to failure of vital organs. Amyloid deposition is associated with many debilitating neurological and systemic diseases including Alzheimer's disease, Parkinson's disease, and diabetes type II. Pathological apoA-I amyloid fibrils are generally composed of the N-terminal portion of the protein; however, very little is known about the mechanism of apoA-I amyloid formation in disease.

We have demonstrated that oxidation of the methionine residues of apoA-I leads to amyloid fibril formation by the intact protein, providing a possible mechanism for amyloid deposition in atherosclerosis where oxidative damage to proteins is common. In vivo, the majority of apoA-I is bound to lipid in the form of HDL, which inhibits its propensity to misfold and form amyloid. This project will investigate the structural mechanism by which folded, lipid-bound apoA-I converts to a misfolded form and aggregates into amyloid fibrils. This will be achieved using a variety of biophysical and structural analyses of lipid-bound and lipid free apoA-I, including the use of conformationally-specific antibodies that recognise specific apoA-I structures.
Honours/Masters/PhD

THE STRUCTURE AND FUNCTION OF THE INTERLEUKIN-11 SIGNALLING COMPLEX, A NEW CANCER TARGET

Dr Michael Griffin

Interleukin (IL)-11 is a member of the IL-6 family of cytokines that performs a wide range of biological functions. Recombinant human IL-11 is administered as a standard clinical treatment for chemotherapy-induced thrombocytopenia. Recently, we identified a new role for IL-11 signalling as a potent driver of gastrointestinal cancers, and demonstrated that it is a novel therapeutic target for these diseases. Cell signalling by IL-11 is initiated by binding of soluble IL-11 to its membrane bound, specific receptor, IL-11Rα. This binary complex subsequently engages with the signal transducing receptor, GP130, inducing GP130 dimerisation and resulting in activation of the transcription factor Signal Transducer and Activator of Transcription (STAT)-3.

Despite its importance, our understanding of the structure of the IL-11 signalling complex and the interactions between its components remains rudimentary. Our recent work has produced the first crystal structure and biophysical characterization of IL-11, and we are currently investigating the structural details of the complex between IL-11 and IL-11Rα. This project will use biophysical techniques, including NMR and X-ray crystallography, to understand the interactions between IL-11, IL-11Rα, and GP130 that lead to the formation of the active signaling complex. The ultimate goal is to use this information to design inhibitors of IL-11 signalling that may be useful as cancer therapeutics.
Honours/Masters/PhD

THE STRUCTURE AND FUNCTION OF *Coxiella burnetii* EFFECTOR PROTEIN CIG57: HOW DOES IT SUBVERT ENDOCYTOSIS WITHIN THE HUMAN HOST CELL?

Dr Michael Griffin, Dr Hayley Newton (PDI)

Coxiella burnetii is an intracellular bacterium that causes the human disease Q fever. C. *burnetii* infects alveolar macrophages, and replicates within a spacious, lysosome-derived vacuole that matures to feature an acidic internal environment characteristic of a lysosome. During infection, over 150 effector proteins are translocated into the host cytoplasm to facilitate intracellular survival of the bacteria. One of these effector proteins, Cig57, is essential for replication of C. *burnetii.*

We have discovered that Cig57 has a role in exploiting clathrin-mediated endocytosis of the eukaryotic host by interacting with the host cell nucleators of clathrin coated vesicles, FCHO1 and FCHO2. However, the functional mechanisms of this effector protein have not yet been determined. Our structural studies of Cig57 indicate that the protein consists of 3 domains. While we have solved the crystal structure of the central domain, the structural and functional properties and interactions of the other domains are unknown. This project will use biophysical and structural approaches to understand the structure of the individual domain components of Cig57 and their interactions with FCHO proteins, with a view to exploiting this information for development of therapeutic agents against Q fever.

Honours/Masters/PhD

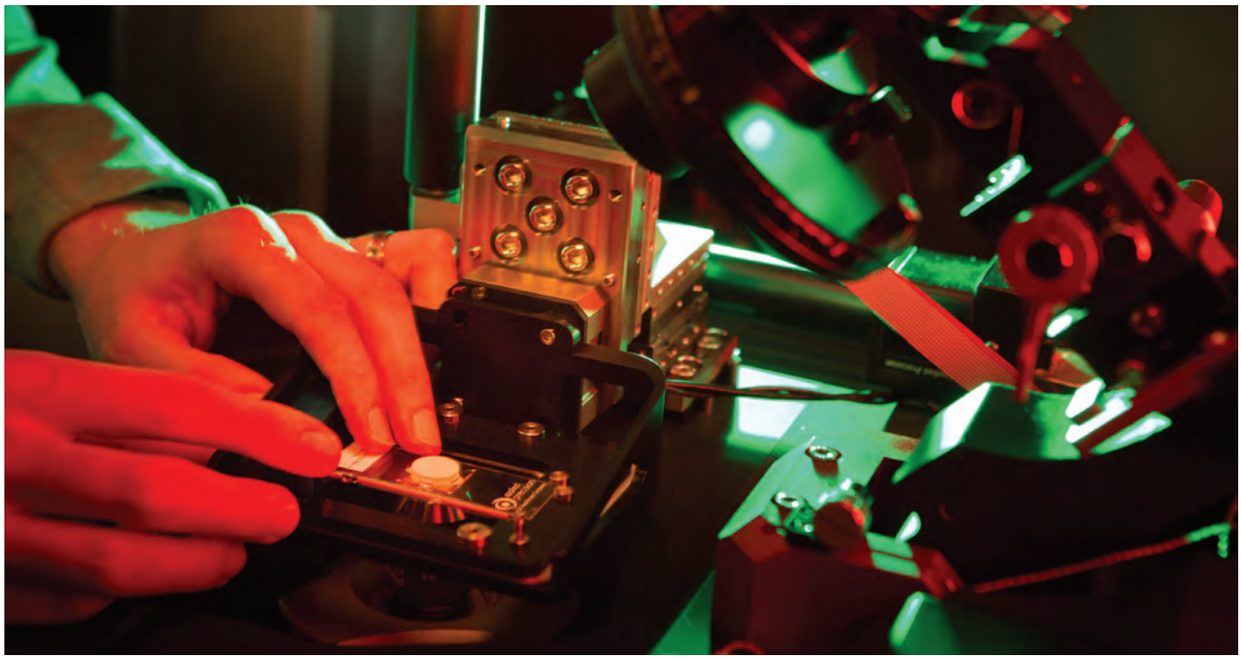
STRUCTURAL CHARACTERISATION OF THE α1A-ADRENOCEPTOR

Dr Michael Griffin, Dr Daniel Scott

The α1A-adrenoceptor (α1A-AR) is a G protein-coupled receptor that regulates the cardiovascular and central nervous systems in response to binding adrenaline and noradrenaline. Other members of the α1-AR sub-family, α1B-AR and α1D-AR, are stimulated by the same endogenous ligands but mediate different physiological roles in the body. Evidence suggests that in disease states such as heart failure and epilepsy, stimulation of α1A-AR is beneficial, whereas stimulation of α1B-AR is detrimental. The therapeutic targeting of α1A-AR is hindered, however, due to a lack of selective drugs. A deeper understanding of the structure of α1A-AR would facilitate structure-based drug design (SBDD) and further validate α1A-AR as a drug target in these diseases.

As with most integral membrane proteins, α1A-AR is highly unstable upon purification from cell membranes with detergents, making structural studies a challenge. Using directed evolution, we have engineered highly stable variants of human α1A-AR that are prime candidates for structural studies. This project involves purifying optimized α1A-AR constructs, testing the stability of these proteins when bound to various prototypical ligands and screening for conditions under which their structural and biophysical properties of can be determined. The ultimate aim of this project is to solve the crystal structure of α1A-AR to not only further our understanding of this important receptor, but also to facilitate the design of novel α1A-AR selective drugs.

Honours/Masters/PhD



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OPTOGENETIC CONTROL OF STREPTAVIDIN

A/Prof Danny Hatters

Optogenetics involves the use of light to control activities of cellular functioning or enzyme functioning. It has most famously been applied to control neuronal action potentials. Indeed this application was nominated as "Method of the year" by the *Nature Methods* journal in 2010. In this project, the student will build an optogenetic tool in the Hatters lab using protein engineering and mutagenesis. The broader objective is to make a light-controllable streptavidin for controlling ligand (biotin) interactions in cells. This will enable us to perform proteomics in a spatially controllable manner inside live cells and is expected to be useful for other researchers internationally.

The project will involve modification of a prototype light activated streptavidin system we have developed to improve its responsiveness. This will include modelling and design, mutagenesis, expression and purification and analysis of the mutants using binding assays and other biochemical tools.
Honours/Masters/PhD

PROBING PROTEOSTASIS STRESS IN NEURODEGENERATIVE DISEASE

A/Prof Danny Hatters Prof Gavin Reid, and Dr Yuning Hong

Proteostasis involves the maintenance of the proteome in a folded state by an extensive quality control network. When proteostasis becomes unbalanced, unfolded proteins accumulate and protein aggregation is observed. However, probes are lacking that can quantitate the effectiveness of proteostasis. We have made a dye, tetraphenylethene maleimide that can do this. In this project, the student will use this dye to investigate how cellular stresses alter proteostasis. Methods will involve growing cells including neurons from stem cells, imaging by confocal microscopy, transfections and flow cytometry. The project will also involve making variants of the dye using organic synthesis methods, for enabling new capacity. A key goal is to adapt the dye as a proteomic probe for defining which proteins in the proteome become unfolded under stress. Methods for this aspect of the project will include quantitative proteomics and cell culture.
Honours/Masters/PhD

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MOBILE GENETIC ELEMENTS IN THE EVOLUTION OF ANTIBIOTIC-RESISTANT *Klebsiella pneumoniae*

A/Prof Kathryn Holt, Dr Kelly Wyres

Klebsiella pneumoniae is an opportunistic bacterial pathogen that frequently causes healthcare-associated infections and is recognised as an emerging public health threat. Antibiotic resistance is also a major concern and rates have been increasing globally. Recent comparative genomic studies have highlighted the extensive genomic diversity of *K. pneumoniae,* which can readily acquire and lose mobile genetic elements (MGEs), including plasmids, transposons and others. Plasmids are highly mobile and distinct from the bacterial chromosome, and have been the subject of many epidemiological and evolutionary studies. In contrast, less is known about MGEs such as phage and transposons, which integrate directly into the chromosome. However, these elements can also carry important genes and there is emerging evidence that they can play a role in the evolution of globally distributed, multi-drug resistant clones.

This project will use comparative genomics techniques to characterise a range of chromosomally integrated MGEs among *K. pneumoniae* genomes from our large collection. Protein domain and sequence homology searches will be used to predict the functions of novel genes that are transferred into *K. pneumoniae* chromosomes by the MGEs. The distribution of the different MGEs across the *K. pneumoniae* population will be investigated, and any associations with different types of disease or drug-resistance will be explored.
Honours/Masters/PhD

SINGLE MOLECULE SEQUENCING OF *Mycobacterium tuberculosis*

A/Prof Kathryn Holt

A third of the world's population is infected with *Mycobacterium tuberculosis (Mtb)* bacteria. Each year millions of people with latent *Mtb* infections will develop active pulmonary tuberculosis, which is increasingly difficult to treat due to increasing rates of resistance to antibiotics. Some unlucky individuals also develop extra-pulmonary tuberculosis, including meningitis.

Whole genome sequencing is now being applied on a large scale (i.e. sequencing thousands of genomes) in order to investigate *Mtb* evolution and transmission. However these projects rely on the Illumina sequencing platform, which shatters the genome into very small fragments (~500 bp). This approach is highly effective in identifying substitution mutations that are needed to study transmission and antibiotic resistance, but can't be used to investigate genetic variation in expanded multi-copy gene families that are important to *Mtb* pathogenicity.

This project will test whether long-read, single-molecule sequencing of *Mtb* isolates using the new Oxford Nanopore MinION device can be used to uncover genetic variation in these important multi-copy gene families. In the lab, the student will use the MinION hand-held sequencer to generate long sequencing reads on a collection of *Mtb* strains that have already been sequenced on the Illumina short-read sequencing platform. Using computational bioinformatics analysis, the student will (i) compare the data from the two genome sequencing approaches and assess their ability to identify substitution mutations important for transmission and antibiotic resistance and identify genetic variation in the multi-copy gene families; and (ii) investigate whether these variations are associated with different forms of tuberculosis including meningitis.
Honours/Masters/PhD

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IDENTIFICATION OF NOVEL METABOLIC PATHWAYS IN LEISHMANIA PARASITES

Prof Malcolm McConville, Dr Fleur Sernee, Dr Julie Ralton

Leishmania spp are sandfly-transmitted protozoan parasites that cause a spectrum of serious and potentially lethal diseases in more than 12 million people world-wide. Existing therapies are limited and there is an urgent need to identify new drug targets in these parasites. We have shown that these parasites synthesize a novel carbohydrate reserve material, termed mannogen, that is required for parasite growth in macrophages, the major target cell in the human host. We have now identified a novel family of enzymes involved in mannogen synthesis and catabolism that are essential for virulence. This project will utilize a range of genetic and biochemical approaches to define the function of a new gene/protein that is unrelated to this family of enzymes, and appears to have a key role in initiating (priming) mannogen synthesis. The project will utilize a range of genetic, cell biology and biochemical approaches to investigate whether this protein is directly involved in mannogen priming (catalytic function), or whether it regulates the activity of the mannogen 'synthases' through protein-protein interactions (chaperone function). It will use in vivo proximity-tagging approaches (to measure protein-protein interactions), expression of recombinant protein, mutagenesis and enzyme assays and analysis of parasite central carbon metabolism using mass spectrometry and the Seahorse Metabolic Analyzer.
Honours/Masters

GENOME WIDE ANALYSIS OF HOST RESPONSES TO LEISHMANIA INFECTION

Prof Malcolm McConville, Dr Michael Dagley, Dr Eleanor Saunders

Intracellular pathogens such as *Leishmania* are dependent on host metabolism for growth and survival. As such it may be possible to develop new anti-microbial therapies that target host cell metabolism, and kill intracellular pathogens by restricting their access to essential nutrients. Such an approach may be useful against multiple pathogens and would be difficult to develop resistance against. In order to test the feasibility of this approach, we have undertaken a genome wide siRNA screen in macrophages for genes that either increase or decrease *Leishmania* growth in intracellular vacuoles. This screen has led to the identification of >100 host genes that regulate the outcome of *Leishmania* infection and represent potential host-directed anti-microbial drug targets. This project will further validate these observations and define how individual host genes impact on Leishmania infection. Macrophage genes will be knocked- down using siRNA and the effects on Leishmania-macrophage infection and intracellular growth kinetics studied. Detailed analysis of macrophage and parasite metabolism will also be investigated, providing fundamental information on host-parasite interactions. This project will utilize an array of cell biological (fluorescence microscopy) and metabolomics (mass spectrometry, Seahorse Metabolic Analyzer) approaches.

Honours/Masters

METABOLIC SENSING AND VIRULENCE IN *Toxosplama gondii*

Prof Malcolm McConville, Dr Martin Blume

Toxoplasma gondii is transmitted through contaminated food and water and infects nearly one third of the world's population. This protozoa parasite can cause serious disease in babies and healthy adults, as well as life-threatening encephalitis in immuno-compromised individuals. *T. gondii* is remarkable in being able to invade and grow within any nucleated host cell indicating a high degree of metabolic flexibility. We have recently identified novel proteins that appear to be involved in nutrient sensing and are essential for intracellular growth and both acute and long term chronic infections, respectively. This project will focus on defining the metabolic and virulence phenotype of *T. gondii* mutants lacking these proteins. It will also investigate how these proteins are flexibly recruited to different subcellular locations (amylopectin granules and mitochondrion) and their capacity to interact with other proteins under different nutrient conditions using fluorescence microscopy, *in vivo* tagging approaches and proteomic approaches.

Honours

SYNTHESIZING THE UNIQUE CELL WALL OF MYCOBACTERIA

Prof Malcolm McConville, Dr Stephen Klatt

Tuberculosis, caused by *Mycobacteria tuberculosis,* remains the leading cause of death from any bacterial infection worldwide. This bacterium is protected by a unique cell wall that confers resistance to many antibiotics and host microbicidal processes and *M. tuberculosis* enzymes involved in cell wall synthesis are attractive drug targets. We study Mycobacterial cell wall synthesis in genetically tractable species of mycobacteria and corynebacteria and are building new models for how different wall components are synthesized and transported to the appropriate location in the wall during bacterial growth. We have generated a large panel of *Mycobacteria* and *Corynebacteria* mutants lacking genes associated with putative 'cell wall operons' but otherwise have unknown function. In this project advanced mass spectrometry 'lipidomic' approaches will be used to profile all of the lipids in these mutant strains with the view of identifying which cell wall pathway (and potentially enzymatic step) is affected, as well as building a detailed picture of how these different pathways are co-regulated. The project will exploit the world-leading mass spectrometry and lipidomic facilities at the Bio21 Institute, and an array of complementary biochemical/analytical approaches.

Honours

What have been the highlights of your Honours year so far?

Becoming independent in the lab and knowing how to do things. Making nanoparticles is really cool.

Charlee Bickers

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DESIGNING EFFECTIVE VACCINES TO FIGHT INFECTION AND TUMOURS

Dr Justine Mintern, Prof Jose Villadangos

Vaccination currently represents the most effective strategy for eliminating infectious disease. While many vaccines are in use worldwide, for several pathogens our current vaccines fail with ensuing uncontrolled disease. This is the case for HIV, malaria and tuberculosis resulting in disease and devastation worldwide. Vaccines also have the potential to prevent and/or treat cancer, however this is currently not a clinical reality. Therefore, vaccine design must be advanced, and to do so, we require a more comprehensive understanding of the cell biology involved. A key question in vaccine biology is how are the proteins involved in this response trafficked to and from specialised immune cell compartments. This project use CRISPR/Cas9 methodology to investigate the consequence of targeting specific components of the molecular machinery that participate in immune cell protein trafficking.

Honours/Masters/PhD

EATING ONESELF TO PROMOTE IMMUNITY

Dr Justine Mintern, Prof Jose Villadangos

Autophagy is a critical cellular pathway with important implications for human disease. Autophagy involves trafficking of cytoplasmic content to lysosomal compartments where it is degraded. Initially described as a process induced only in nutrient-deprived cells, autophagy is now known to occur constitutively in many cell types, including dendritic cells (DC). This project will investigate how autophagy impacts DC function. This is important as autophagy represents a viable pathway to target to improve vaccine efficiency.

Honours/Masters/PhD

EXPLOITING NANOPARTICLES AS VACCINES

Dr Justine Mintern, Dr Angus Johnston (Monash Institute of Pharmaceutical Sciences)

Incorporating drugs inside nanoparticles (NPs) (Fig. 1) is a promising approach to treat a range of diseases, from HIV to cancer. Encapsulating a drug inside a nanoparticle has a number of advantages over the conventional delivery of naked therapeutics, particularly when delivering delicate therapeutic cargos such as peptides or siRNA. 1) The NP can protect the drug from degradation by the body, ensuring the therapeutic is delivered in an active form. 2) By preventing premature release of the drug, unintentional side effects can be limited. 3) Multiple drugs can be delivered together (such as an antigen and an adjuvant), maximizing the therapeutic response. 4) NPs functionalized with targeted molecules such as antibodies (Abs) can target the specific cells that require the therapeutic. Here, we will examine the potential of nanoparticles as carriers that enable effective vaccination. We will investigate how different nanoparticle formulations can be used to elicit immunity to infection and tumours.

Honours/Masters/PhD

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ELUCIDATING THE RABIES VIRUS P PROTEIN AXIS

Dr Greg Moseley, Prof Paul Gooley

Rabies is a currently incurable lethal disease, which has a 100% case-fatality rate, the highest of known infectious diseases. Rabies is caused by lyssaviruses, including rabies virus and Australian bat lyssavirus. Although lyssaviruses make only five proteins, they are able to mediate viral replication, assembly and budding, as well as arresting potent control over the biology of the infected host cell and the host immune system. Central to this is the multifunctional 'P protein' that is positioned at the core of the virus-host interface where it forms a myriad of interactions with viral and host proteins critical to replication and disease progression. We have shown that by preventing such interactions, we can prevent an otherwise invariable lethal disease *in vivo*, identifying the P protein axis as an important target for antiviral approaches. However, the mechanisms by which the small P protein is able to coordinate and regulate its interactions, and the structural basis of these interactions, remain unresolved. This leaves major gaps in knowledge concerning the fundamental biology of a lethal human disease, as well as strategies to design vaccines and new inhibitors as therapeutics against rabies. We are using structural biology, quantitative proteomics, live cell imaging, cell signalling assays, and viral reverse genetics, to understand the structural organisation of the P protein axis, and to define the molecular mechanisms regulating key interactions at the virus-host interface. The project will seek to define these interactions through the characterisation of the specific molecular surfaces involved, and mutagenic analysis of their function, with the aim of defining novel mechanisms by which virus efficiently co-regulates host cell subversion, immune evasion and genome replication. The findings will redefine understanding of the virus-host interface and fundamental processes in disease.

Honours/Masters/PhD

USING RABIES TO CURE ALZHEIMER'S?!

Dr Greg Moseley, Dr Peter Crack, Prof Paul Gooley

Neuroinflammation is a major cause of human pathologies such as stroke, Alzheimer's disease (AD), and traumatic brain injury (TBI). Viruses such as the lyssaviruses rabies virus and Australian bat lyssavirus, and paramyxoviruses Nipah and Hendra virus, have evolved powerful mechanisms to shut down inflammatory signalling as part of their strategies for immune evasion. We aim to discover the molecular 'tricks' used by viruses to subvert host immunity, and then to exploit these mechanisms to develop new methods to efficiently shut-down the inappropriate immune responses underlying neuroinflammatory disorders. We have made major advances in understanding how viruses achieve immune evasion, including defining the specific virus-host interactions involved, and the molecular basis of these interactions. Using this knowledge, we are now investigating the potential of harnessing viral immune evasion to combat immune disorders. Techniques to be employed include live cell imaging and super-resolution microscopy approaches, structural biology, immune signalling analysis, and models of stroke, TBI, AD and Parkinson's disease (PD).

Honours/Masters/PhD

NUCLEOLAR TARGETING BY RNA VIRUSES

Dr Greg Moseley, Dr Stephen Rawlinson

Most RNA viruses are known to perform all stages of their replication cycle within the cytoplasm of the infected host cell, with no requirement for the cell nucleus. Nevertheless, several RNA virus proteins have been shown to specifically exploit nuclear trafficking pathways to enter the nucleus during infection, often accumulating in the largest subnuclear structure, the nucleolus. It is becoming increasingly clear that the targeting of subnuclear structures is in fact critical to infection by certain cytoplasmic RNA viruses, representing a fundamental process in viral biology, and providing novel potential targets for therapeutic interventions. Understanding of the nucleolus has undergone a renaissance in recent years, revealing dynamic roles in diverse cellular processes including ribosome production, cell cycle control, stress responses, and apoptosis. Thus, viruses might exploit the multifunctional nucleolus to modulate cellular processes important to their lifecycles. However, the precise function(s) of RNA virus targeting of the nucleolus remain mysterious, leaving this critical aspect of RNA virus biology unresolved. We are investigating this by utilising two medically significant RNA viruses, rabies and Hendra viruses, which express proteins that target the nucleolus; the aim is to uncover the interactions formed by the viral proteins, and to determine their specific roles in viral infection. Techniques to be used include quantitative proteomics/mass spectrometry to identify host interactors, quantitative live-cell confocal imaging, super-resolution microscopy, cellular signalling assays, and protein chemistry.

Honours/Masters/PhD

VIRAL REPROGRAMMING OF HOST CELL SIGNALLING

Dr Greg Moseley, Dr Celine Deffrasnes

Central to replication and spread of pathogenic viruses is the capacity to interfere with the host immune system, in particular the antiviral system mediated by cytokines such as the interferons. It is well known that many viruses target signalling by antiviral type I interferons to shut down the expression of interferon-stimulated genes. However, our recent work has indicated that the interaction of viruses with cytokine signalling pathways is much more complex and intricate than previously assumed.

In particular, we and our collaborators have found that rabies virus, the cause of c. 60,000 human deaths/year, is able to interact with multiple signalling pathways, including those initiated by interleukin (IL-) 6 and interferon-a, and that this uses a number of mechanisms including viral interactions with and remodelling of key cellular structures of the cytoskeleton and sub-nuclear bodies. Importantly, using mutagenic analysis, including viral reverse genetics, we found that altering viral targeting of these pathways profoundly inhibits pathogenesis *in vivo* indicative of critical roles in disease. Furthermore, using sub-diffraction microscopy, we have been able to track viral modulation of cellular structures at super-resolution, finding that these events are vital to disease. We are currently seeking to delineate the precise mechanisms by which viruses interfere with and modulate cellular signalling, not only to inhibit antiviral signalling, but also to reprogram specific signalling pathways toward 'pro-viral' responses. Techniques to be used include functional genomics, quantitative proteomics/mass spectrometry, live cell confocal microscopy, and super-resolution imaging.

Honours/Masters/PhD

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OVERCOMING CANCER DRUG RESISTANCE

Prof Michael Parker, Dr Craig Morton

Conventional cancer chemotherapy kills rapidly growing cells indiscriminately, causing significant side-effects and can lead to disease re-occurrence and resistance to the drugs. One of our interests is the Glutathione S-Transferase (GST) family of proteins that function by recognising foreign small molecule toxins in the body, causing them to be eliminated from the cell. Unfortunately, commonly used anti-cancer drugs are also recognised as toxic by GSTs, which are often overexpressed in cancer tissues and are associated with transformation to malignancy and the adaptive resistance to anti-cancer drugs. There is thus an urgent need for the design of new anti-cancer drugs that circumvent the development of GST-mediated resistance to treatment. Recently, there has been an increasing interest in the development of metal-based drugs as effective and potent protein targeted chemotherapies. We are investigating, though structural and biochemical means, how a range of ruthenium, arsenic and osmium-based drugs and drug-like compounds interact with GSTs. Students will investigate how these compounds work, as well as any drug-like molecules we develop, using X-ray crystallography and a range of biophysical techniques.

Honours/Masters/PhD

IMPROVING CURRENT APPROACHES TO ALZHEIMER'S DISEASE

Prof Michael Parker, Dr Luke Miles

Alzheimer's disease (AD) is the fourth biggest killer in developed countries. Amyloid precursor protein (APP) plays a central role in the development of AD, through generation of the toxic Abeta peptide by proteolytic breakdown of APP. Here we will use X-ray crystallography at the Australian Synchrotron to determine the 3D atomic structures of Abeta bound to therapeutic antibodies currently in clinical trials in order to understand how these molecules recognise Abeta. We use this information to engineer more potent antibodies as treatments for AD. We also have structure-based drug discovery projects on APP itself and other proteins involved in the production and clearance of the Abeta peptide from the brain.

Honours/Masters/PhD

UNDERSTANDING HOW BACTERIAL PORE-FORMING TOXINS PUNCH HOLES IN MEMBRANES

Prof Michael Parker, Dr Craig Morton

The beta-barrel pore-forming toxins constitute the largest group of functionally related protein toxins. They are expressed in many species in the prokaryotic and eukaryotic kingdoms and are related to the membrane attack complex/perforin (MACPF) family of mammalian immune defence proteins. Despite their widespread occurrence and role in bacterial pathogenesis and immune defence, the detailed mechanism by which they form pores remains an enigma. The overall aim here is to visualise the 3D structures of family members as a basis for functional studies to reveal the molecular details of how these toxins insert into membranes to form beta-barrel pores and how the process is regulated. The structures will shed light on one of the most fundamental biological events (namely, protein insertion into cell membranes) and also provide the basis for the design of novel tools with various biotechnology applications and the design of novel antibiotics.

Honours/Masters/PhD

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tRNA SYNTHETASES ENZYMES AS ANTI-MALARIA DRUG TARGETS

Dr Stuart Ralph

We are characterising aminoacyl-tRNA synthetase (aaRS) enzymes as drug targets in *Plasmodium*. These enzymes catalyse the attachment of amino acids to their relevant tRNA molecules and are essential for protein synthesis. They have recently been recognized as promising drug targets across a broad range of microbes, and we have recently identified *Plasmodium* aaRSs that are potential targets for new drugs to treat malaria. *Plasmodium* aaRS enzymes differ from those of humans, so we hope to develop drugs specific for *Plasmodium*. We are using in silico screening methods to identify likely inhibitors of *Plasmodium* tRNA synthetases and developing assays to measure specific inhibition of *Plasmodium* aaRS enzymes. We will also test inhibitors for their ability to kill *Plasmodium* grown in culture.
Honours/Masters/PhD

ALTERNATIVE SPLICING IN MALARIA AND OTHER PARASITES

Dr Stuart Ralph, Dr Aaron Jex

Next generation sequencing has revealed that an unexpectedly high proportion of mammalian genes undergo alternatively splicing to produce multiple transcript isoforms. We have recently shown that alternative splicing is also widespread in the human parasites *Plasmodium* (causative agent of malaria) and *Toxoplasma*, (causative agent of toxoplasmosis). We have also demonstrated that this mechanism is necessary for parasites differentiating from human to mosquito life-stages. We will now apply novel long-read sequencing techniques to establish the impact of alternative splicing on whole transcripts in a variety of human and veterinary parasites. We will also investigate the implications of alternative splicing on generation of proteome diversity and its consequences for parasite differentiation.
Honours/Masters/PhD

THE METABOLIC RESPONSE OF PLASMODIUM PARASITES TO ANTIMALARIAL DRUGS

Dr Stuart Ralph, Prof Malcolm McConville

We have developed methods to analyse the metabolome of *P. falciparum* using chromatographic separation of small molecule metabolites and mass spectrometry. This methodology will allow us to sample much of the parasite's metabolome before and after treatment with antimalarial drugs. We will explore the mode of action of some well-known antimalarials, as well as potential targets for promising but uncharacterized novel antimalarials.
Honours/Masters/PhD

BIOINFORMATIC ANALYSIS OF PROTEIN SUBCELLULAR LOCALISATION IN HUMAN PARASITES

Dr Stuart Ralph, Prof Karin Verspoor

We are making and validating new bioinformatic tools that predict and classify sub-cellular localisation of proteins. We are interested in automatically extracting information from the literature that specifies the sub-cellular localisation of proteins in the malaria parasite *Plasmodium falciparum*, and in related human parasites. Information about sub-cellular localisation in infectious agents is crucial to prioritising targets for drugs and vaccines. We have built a database that details subcellular localisation of hundreds of Plasmodium proteins (http://apiloc.biochem.unimelb.edu.au/apiloc/apiloc), and will use this as a training set for Biomedical Natural Language Processing. The project will involve construction of a tool to recognise and extract records of cellular localisation for proteins as a means to identify proteins on the surface of malaria parasites, as well as tools to predict localisation for novel, uncharacterised proteins.

Honours/Masters/PhD

What is your Research Project?

My project investigates protein quality control and how these processes are undermined in settings of neurodegenerative disease. There was a new gene discovered for ALS that generates aggregating junk proteins so we're trying to figure out how a junk protein might affect the way a cell functions in protein degradation and synthesis.

Jordan Lilley

Prof Gavin Reid
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'INTEGRATED 'OMICS' TO IMPROVE THE DIAGNOSIS AND TREATMENT OF ACUTE RHEUMATIC FEVER

Prof Gavin Reid

Aboriginal and Torres Strait Islanders are 69 times more likely than non-Aboriginal people to develop acute rheumatic fever (ARF), an autoimmune consequence of Group A streptococcal (GAG) infection, and are 55 times more likely to die from ARF and rheumatic heart disease (RHD) caused by an episode or recurrent episodes of ARF. These high rates are important contributors to the gap in life expectancy between Indigenous and non-Indigenous Australians. The two most obvious deficiencies in the ability to reduce the burden of RHD are (i) the inaccuracy of current approaches used to diagnose ARF, and (ii) limited immune-modulating treatment options to reduce the likelihood of development, or severity, of RHD in AHD patients. Here, as part of a newly established collaboration with Dr Ian Wicks, Dr Willy-John Martin and Dr Andrew Webb at the Walter and Eliza Hall Institute of Medical Research, we will develop and apply mass spectrometry based lipidomic and metabolomic analysis strategies to: (Aim 1) identify novel serum and high-density lipoprotein (HDL) associated candidate lipid and metabolite biomarkers of ARF in an indigenous ARF case-control study cohort, and (Aim 2) evaluate the *in vitro* effect of hydroxychloroquine (HCQ) on GAS-induced immune responses, and its mechanism of action to treat ARF and potentially mitigate progression of ARF to RHD. Students involved in this research will gain experience in the development and application of a variety of analytical biochemistry techniques, including mass spectrometry, lipidome and metabolome profiling, and associated 'omic approaches for biomolecular identification and characterization.

Honours/Masters/PhD

UNCOVERING THE ROLE OF EXOSOME DERIVED LIPIDS IN ALZHEIMER'S DISEASE

Prof Gavin Reid, Dr. Laura Vella, A/Prof Kevin Barnham

Alzheimer's disease (AD) is a progressive dementia affecting more than 40 million people worldwide. The lack of disease-modifying treatments and the knowledge that existing drugs offer only modest delays in symptom onset exacerbate the significant financial and emotional tolls that AD places on families and communities at large. A new approach to the study of Alzheimer's disease is long overdue. In recent years, small extracellular vesicles released by the cell, called exosomes, have emerged as key players in neurodegenerative disease. Exosomes are important for two principal reasons, they contain a selection of proteins, RNA and lipids, which act as key players in cell-to-cell communication, and can be characterised to provide a snapshot of parental cell homeostasis. The proposed project will access the wealth of lipidomic information contained within human brain exosomes. Lipids have been shown to play a fundamental role in influencing the various risk factors of AD and to be closely involved in the pathogenesis of AD. This will involve isolating and characterising exosomes from human control and Alzheimer's disease brain. The aims are to afford the first lipid profile of brain derived exosomes and uncover the role of exosomal lipids in Alzheimer's disease. The student will learn techniques including exosome isolation, western blotting, density gradients, electron microscopy and lipidomic profiling. This will be a collaborative research project with the Florey Institute of Neuroscience and Mental health and The University for Melbourne.

Honours/Masters/PhD

DETERMINING THE EFFECT OF ASPIRIN ON PLASMA RESOLVIN LEVELS IN HEALTHY VOLUNTEERS AND INTENSIVE CARE PATIENTS

Prof Gavin Reid

Inflammation is a major mechanism involved in many human diseases. Recent studies have shown that resolution of inflammation is an active process regulated, in part, by a series of bioactive lipid metabolites derived from omega-3 polyunsaturated fatty acids circulating in the blood, including lipoxins, resolvins, protectins and maresins. Other studies have also shown that low-dose aspirin (acetylsalicylic acid) inhibits excessive inflammation by significantly increasing the synthesis of these specialized pro-resolving mediators (SPM), as well as reducing the levels of pro-inflammatory lipid derived metabolites. SPM's promote resolution of inflammation in both animal and human models of inflammatory activation, inhibit programmed cell death (apoptosis) attenuate white cell-endothelial interactions, enhance microbial phagocytosis, decrease radical oxygen species generation and inhibit cytokine generation and release. Collectively, these experimental observations provide a powerful biologic rationale for studying the effect of aspirin on pro-resolving lipid mediator plasma levels in clinical patient cohorts. Using newly developed mass spectrometry based techniques and processes, it is now possible to measure the levels of these mediators in blood. At the present time, however, the physiological effect of a single aspirin dose on plasma pro-resolving lipid mediator levels in healthy human subjects, or on therapeutic response in intensive care patients, is unclear. The aim of this research project, therefore, is to develop optimized collection, storage, extraction and liquid chromatography–tandem mass spectrometry analysis conditions for the clinical measurement of plasma pro-resolving lipid mediators, or their precursors, in response to aspirin in (i) a pilot study of healthy volunteers, and (ii) a randomized double blind placebo control study to evaluate the effect of aspirin on clinical outcome in intensive care patients. This research will be carried out in collaboration with Professor Rinaldo Bellamo, Director of Intensive Care Research at the Austin Hospital..

Honours/Masters/PhD

What is the difference between Honours and 3rd year?

Completely different. The practical class gives you some experience, but it's packed into 5 hours. Now I have the whole day to think about what experiments I will do and how. There is lots more lab time rather than class room time.

Molly Parkyn Schneider

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PLAYING GOD WITH GPCRS: ENGINEERING G PROTEIN-COUPLED RECEPTORS FOR STRUCTURAL BIOLOGY AND DRUG DISCOVERY WITH DIRECTED EVOLUTION

Dr Daniel Scott, Prof Ross Bathgate

G protein-coupled receptors (GPCRs) are a large family of signalling proteins (>800 gene members) located on the surface of all cells in the body, particularly in the brain. GPCR signalling controls virtually every physiological process in the body, making these receptors the targets of many current drugs treating conditions like pain, hypertension, schizophrenia and asthma. GPCRs exist as an ensemble of conformational states (inactive, intermediate and active) in equilibrium, with agonist binding shifting this equilibrium shift towards active states to stimulate cell signalling. A deeper understanding of the structural basis underlying GPCR signalling is needed to guide the design of improved therapeutics. To do this the major challenges for GPCR structural biology need to be overcome. These include: low expression and purification yields; low protein stability; and the inability to stabilize relevant receptor conformations for analysis. In the Receptor Structure Drug Discovery lab we engineer GPCRs that overcome these issues using *in vitro* directed evolution methods. Engineered receptors can then be used for crystallography, NMR and other biochemical techniques to further our structural understanding of these proteins and to facilitate structure-based drug design.

This project focuses on developing novel directed evolution methods to enable the engineering of GPCRs that are locked into particular, physiologically relevant conformations for which we do not as yet have crystal structures. Such conformationally stabilized receptors will also give us insights into the protein dynamics that control GPCR signalling and provide tools to design new drugs that target and stabilize specific GPCR conformations.
Honours/Masters/PhD

HOW TO DESIGN A BETTER GPCR DRUG: UNDERSTANDING THE STRUCTURAL BASIS OF LIGAND SELECTIVITY AT α1-ADRENOCEPTORS

Dr Daniel Scott, Prof Paul Gooley

Most G protein-coupled receptors (GPCRs) are activated though extracellular interactions of natural ligands, such as hormones or neurotransmitters, to the GPCR's ligand binding site. Binding induces a conformational change of the GPCR resulting in the transmission of intracellular signals. The GPCR gene super-family is made up of numerous sub-families that are all activated by the same ligands, but often control different physiological processes. This presents a challenge for drug discovery because synthetic compounds that are identified to bind to the natural receptor binding site will often bind to similar sites on other receptor family members (off targets), causing side effects and unwanted physiological responses. To achieve GPCR selectivity we need new ways to identify and design more selective GPCR targeting drugs. To meet this challenge we need to understand how natural ligands, and drug candidates, bind to receptors at the atomic level. Contemporary structure-based drug design (SBDD) uses atomic resolution methods (X-ray, NMR and Molecular Dynamics) coupled with high-throughput screening (NMR, Surface Plasmon Resonance, Isothermal Titration Calorimetry, and Microscale Thermophoresis) of small fragment molecules to discover novel leads. A huge challenge for GPCRs is that they are very unstable and

"fall apart" during the experiments needed to guide SBDD. We have engineered stabilized variants of two closely related GPCR subtypes, the α1A- and α1B-adrenoceptors (α1A-AR and α1B-AR). The stability of these receptors in the purified state has allowed us to probe the binding of non-selective and selective ligands with NMR to further our understanding of the structural basis of ligand selectivity. Projects are available focuses on mapping the binding of selective and non-selective ligands to α1A-AR and α1B-AR with these methods to guide SBDD and increase our knowledge of the structure and function of α1-ARs. Designed, selective α1-AR ligands will be critical tools for understanding the precise roles of these receptors in the body and could be candidates for treating heart disease, epilepsy and neurodegenerative diseases.

Honours/Masters/PhD

TOWARDS IMPLANTABLE NANO-BIOSENSORS: IMMOBILIZING STABILIZED G PROTEIN-COUPLED RECEPTORS ON SILICON NANOWIRES

Dr Daniel Scott, Prof Stan Skafidas (Centre for Neural Engineering)
Prof Ross Bathgate

One of the great challenges in neuroscience research is the monitoring of neuronal activity within deep brain structures with high temporal resolution in living animals. Neurotransmitters (NTs) are the primary chemical messengers released from neuron terminals upon depolarization and thus in principle the monitoring of local NT levels in the brain would give an indication of local neuronal activity. To date however, there are no reliable methods to constantly monitor NT levels in the brains of living animals. G protein-coupled receptors (GPCRs) are a large family of signalling proteins (>800 gene members) located on the surface of all cells in the body, particularly in the brain. GPCRs are nature's own biosensors, sensing stimuli such as light, smells, hormones, metabolites and many NTs to initiate cellular responses to these cues. α1-adrenoceptors are GPCRs that sense the neurotransmitter noradrenaline, which is broadly involved in increasing arousal, alertness, memory formation, memory retrieval, restlessness and anxiety. We have engineered stabilized α1-adrenoceptor proteins that can be purified and immobilized on synthetic surfaces. These proteins are stable over long periods (i.e. can still bind noradrenaline) and thus represent new materials for constructing biosensors.

This project focuses on immobilizing these stabilized receptors on synthetic surfaces that can be used to convert a noradrenaline binding event into an electrical signal, such as silicon nanowires (SiNWs). One-dimensional conductors such as SiNWs act as high-gain field-effect sensors, in which the conductance varies strongly with the surface density. Very recently, biomolecule linked SiNW field-effect transistors (FETs) were fabricated to measure the kinetics of single molecule DNA hybridisation and the single molecule dynamics of the lysozyme enzyme. α1-adrenoceptor linked SiNWs could potentially be used as implantable nanosensors for real-time monitoring of noradrenaline activity in the brains of living animals.

Honours/Masters/PhD

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CHEWING THE FAT: LIPID METABOLISM IN THE ERYTHROCYTE COMPARTMENT

Dr Natalie Spillman, Prof Leann Tilley

The malaria parasite alters the lipid composition of the infected erythrocyte plasma membrane, and establishes several membranous organelles in the host erythrocyte. Generating this extensive exomembrane system in the host cytoplasm is critical for the delivery of parasite virulence proteins to their final destinations throughout the erythrocyte. The regulation of lipid trafficking between parasite and host compartments is poorly understood. This project will use molecular, biochemical and lipidomics approaches to investigate how the parasite regulates lipid distribution, and the biological significance of these modifications.

Honours/Masters/PhD

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INVESTIGATING HOW PROTEINS ARE IMPORTED INTO MITOCHONDRIA IN HUMAN CELLS

Dr Diana Stojanovski

Mitochondria cannot be created *de novo* and pre-existing mitochondria are used as templates for mitochondrial biogenesis. This genesis requires the ~1500 different mitochondrial proteins to be **imported** into the organelle via dynamic translocation machines. Our understanding of protein import and import machineries in higher eukaryotes remains poor. Our lab is interested in the elucidating the molecular architecture of these mitochondrial machines and unraveling the mechanisms that govern protein import in mammalian cells. Using proteomics approaches we have identified novel regulators of protein import. This project will investigate these proteins and decipher their roles in protein import using a variety of cell and molecular biology techniques, including cell culture, confocal microscopy, Blue-native PAGE electrophoresis, protein chemistry and proteomics.

Honours/Masters/PhD

THE ROLE OF MITOCHONDRIA IN *Coxiella burnetii* INFECTION

Dr Diana Stojanovski, Dr Hayley Newton (Peter Doherty Institute for Infection and Immunity)

Some pathogenic bacteria replicate to large numbers inside human cells. This strategy requires that the bacteria manipulate the eukaryotic cell biology to create a replicative niche and evade killing. To achieve this, intracellular bacterial pathogens transport virulence proteins, termed **effectors**, into the host cytosol. The intracellular bacterial pathogen *Coxiella burnetii,* the causative agent of Q-fever, delivers a unique cohort of effectors into the host cell. We have identified a number of the *C. burnetii* effector proteins that are targeted to mitochondria. We are interested to characterise how these effector proteins are delivered to the mitochondrion and study how they interact with mitochondrial proteins to allow *C. burnetii* to replicate within a eukaryotic cell. We will do this by employing a range of cell and molecular biology techniques, including tissue culture, microscopy, protein chemistry and proteomics.

Honours/Masters/PhD

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GAMETOCYTOGENESIS: THE SEXY SIDE OF MALARIA

Prof Leann Tilley, Dr Simon Cobbold, Dr Matt Dixon

The malaria parasite *P. falciparum* undergoes a remarkable transformation that allows asexual stage multiplication in a human host and sexual reproduction in a mosquito vector. Gametocyte maturation represents a "bottle neck" in the parasite's development; inhibition of this process would ablate disease transmission. This has led to recent efforts to understand gametocyte biology with a view to targeting this stage. One of the key questions is: *what triggers individual asexual parasites to differentiate to gametocytes?* This project will use transgenic parasites and molecular and cell biology techniques in conjunction with metabolomics and proteomics techniques aims to define the molecular players in sexual differentiation. Understanding of differentiation processes is fundamental to the development of effective means of combating this debilitating disease.
Honours/Masters/PhD

MECHANISMS OF ACTION OF AND RESISTANCE TO ARTEMISININ

Prof Leann Tilley, Dr Susann Herrmann, Dr Stanley Xie

Artemisinin derivatives are recommended, in combination regimens, as first line antimalarials in most countries where malaria is endemic. However the mechanism of action of artemisinins and other endoperoxide antimalarials is not fully understood and their usefulness is compromised by their short in vivo half-lives and by the emergence of resistance of *P. falciparum* to these drugs. Efforts are currently underway to find ways of overcoming artemisinin resistance and to design and implement new endoperoxide antimalarials that will be cheap and effective and active against artemisinin-resistant parasite strains. This project will use molecular biology, live cell imaging, flow cytometry and biochemical methods to investigate the molecular basis of artemisinin resistance and to develop novel antimalarial therapies.
Honours/Masters/PhD

What have been the highlights of your Honours year so far?

Getting a significant result is fantastic. Having everything go right is quite an experience. Just finding out something is pretty amazing, to know you are the first person that's looked at this. It may not be concrete yet but having that idea and having it pay off is incredibly rewarding.

Alex Stojilovic

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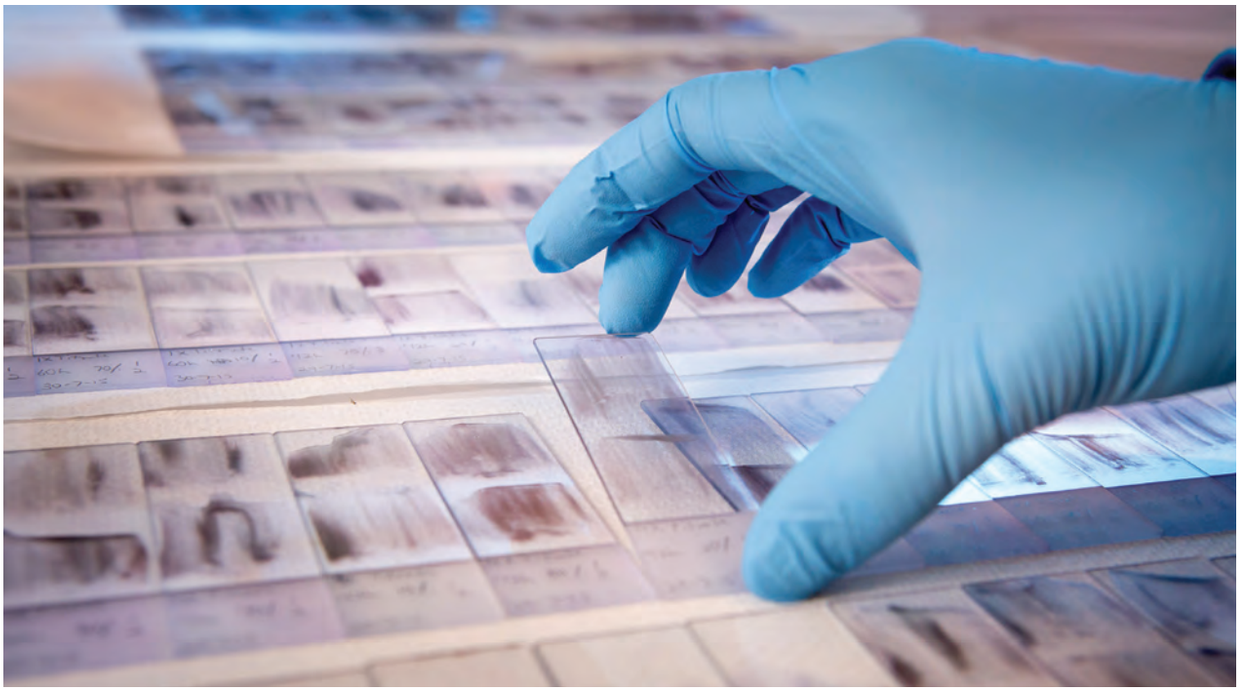
IMMUNE RESPONSES TO PATHOGENIC BACTERIA

Prof Ian van Driel, Dr Andrew Brown, Prof Elizabeth Hartland (Peter Doherty Institute for Infection and Immunity)

Bacterial infectious diseases account for ~10 x 10^6 deaths annually. Our research is directed toward maximizing the chances of developing more effective vaccines and antimicrobial drugs through a better understanding of how the immune system combats bacterial infections. Immune responses **in infected tissues** are essential for controlling invading pathogens in the early phases of infection. Despite having a vital role, the main cells and factors that control innate immune responses in tissues are poorly defined. To gain an integrated understanding of the *in vivo* **innate immune network** in lung tissue, we are investigating the immune response to the intracellular lung bacterial pathogen *Legionella pneumophila*. This important opportunistic pathogen causes Legionnaires' disease, a vastly under diagnosed disease that is associated with high rates of morbidity and mortality. We have revealed a cooperative circuit in the response to *Legionella* infection. Inflammatory dendritic cells induced production of the important cytokine IFNγ by lymphoid. IFNγ, in turn, was necessary for bacterial killing by the inflammatory dendritic cells. Surprisingly, macrophages that engulf bacteria did not respond to IFNγ. We will continue this project by addressing a number of important questions each of which could be the basis of a research student's project. Why are macrophages unable to kill bacteria even in the presence of stimulatory cytokines like IFNγ? We have discovered that another cytokine IL23 plays an important role in combatting *Legionella*. Which cells make IL23 and how does it act to kill bacteria? Macrophages are the cell type inside which *Legionella* bacteria replicate. Why can't Legionella replicate in other similar cells like dendritic cells? IFNγ can convert inflammatory dendritic cells into bacterial killers. What are the key molecular changes that are induced by IFNγ? Techniques commonly used in this laboratory: Flow cytometry, cell culture, use of mice and infection of mice, immunoblotting, immunolocalisation in cells, quantitative PCR, bioinformatic analysis of expression data.

Honours/Masters/PhD



ROLE OF INTERFERON INDUCIBLE GTPases IN IMMUNE DEFENCE

Prof Ian van Driel, Prof Elizabeth Hartland (Peter Doherty Institute for Infection and Immunity)

Legionella pneumophila is a major cause of Legionnaire's Disease, an acute form of pneumonia. As part of its pathogenesis, *L. pneumophila* infects alveolar macrophages and replicates in an intracellular vacuole that avoids fusion with lysosomes. Whereas macrophages propagate bacterial replication, other immune cell types are required for bacterial killing. In particular, we have discovered that monocyte derived cells (MDC) play an important role in controlling *L. pneumophila* infection in the lung. moDC are recruited to the lung in large numbers early during *L. pneumophila* infection and we believe that MDC internalize and kill the bacteria in an interferon gamma (IFNg) dependent manner. Our RNAseq analysis of lung moDC from wild type and IFNg-deficient mice showed that IFNg induced the expression of multiple interferon inducible GTPases (GBPs/IRGs). While most GBPs and IRGs are not yet characterized, some localize to intracellular pathogen vacuoles and may induce killing through novel mechanisms. We hypothesize that moDC utilize GBPs and IRGs to kill intracellular *L. pneumophila*.

The aims of this project is to:

A. Investigate the intracellular localization of selected GBPs and IRGs during *L. pneumophila* infection

B. Identify GBPs and IRGs that restrict *L. pneumophila* intracellular replication

C. Test selected GBP- and IRG-deficient mice for susceptibility to *L. pneumophila* infection

Techniques commonly used in this laboratory:

Bacterial culture, *in vitro* bacterial infection of cultured cells, enumeration of bacterial replication, RNAi knockdown, confocal laser scanning fluorescence microscopy, construction of stable inducible cell lines, molecular biology including primer design, mutagenesis and PCR, western blotting, immunoprecipitation, mouse infection.

Honours/Masters/PhD

How does doing Honours help your future career?

It's been a very big learning experience. I feel like I've learnt so much already, particularly having lab experience. It's really been a good insight into future career possibilities. Now I know I really like working in the lab so that's helped me decide I do want to do something in the lab in the future.

Robyn McConville

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CONTROL OF PLASMA MEMBRANE PROTEOSTASIS BY UBIQUITINATION: ROLE OF THE MARCHS

Prof Jose Villadangos, Dr Justine Mintern

The maintenance of protein localisation and abundance are controlled in eukaryotic cells by a complex network of regulatory pathways which remain poorly understood. These pathways control the distribution of proteins within the cell (proteostasis) and are responsible for changes in protein expression and cell function in response to environmental cues such as the presence of pathogens. Addition of the small protein ubiquitin (Ub) to membrane proteins by Ub-ligases is a major mechanism of control of plasma membrane proteostasis. Therefore this project will (i) contribute to understand the role of ubiquitination in regulation of membrane proteostasis, an issue of fundamental importance in cell biology, and (ii) identify novel target proteins of MARCH ligases in cells of the immune system. These target proteins may be involved in immunoregulation, as other known substrates of the MARCHs. The result of this project will help develop novel therapeutic approaches to fight infection based on manipulation of membrane protein ubiquitination.

Honours/Masters/PhD

THE AMYLOIDOGENIC PROTEASE INHIBITOR CYSTATIN C IN HEALTH AND DISEASE

Prof Jose Villadangos, Dr Justine Mintern

Cystatin C (Cst C) is a secreted protease inhibitor. Its clinical importance as a regulator of extracellular proteolysis in the vascular system is demonstrated by the association between low serum Cst C levels and formation of atherosclerotic plaques, due in part to uncontrolled proteolytic degradation of arterial elastin. A different property of Cst C that makes this protein clinically relevant is that it can form amyloid fibrils, which are found in the cerebral vasculature of patients with neurodegenerative diseases. Such fibrils are believed to originate by a phenomenon known as “domain swapping”, whereby two Cst C monomers associate by “exchanging” subdomains to generate homodimers. These dimers can then be extended by additional rounds of domain swapping and thus form amyloid fibrils. The cells responsible for Cst C production in vascular disease are unknown. Identification of these cells, and characterisation of the mechanisms that control the synthesis and dimerisation of Cst C will lead to the development of therapeutic strategies for the treatment of diseases associated with Cst C.

Honours/Masters/PhD

THE ROLE OF O-GLCNAC GLYCOSYLATION IN REGULATION OF IMMUNITY

Prof Jose Villadangos, Dr Justine Mintern

OGlcNAc glycosylation involves addition of a single sugar, β-N-acetylglucosamine, to serine or threonine residues of proteins. It is a unique type of glycosylation found on nuclear and cytoplasmic proteins. The addition and removal of OGlcNAc is catalysed by OGlcNAc transferase (OGT) and OGlcNAse (OGA) respectively. It is a rapidly reversibly modification akin to phosphorylation. Indeed, OGlcNAc glycosylation occurs in dynamic interplay with phosphorylation, either on the same or adjacent residues. The cross-talk between these two modifications in turn regulates various cellular processes. We are characterising the function of OGlcNAc glycosylation in immune cells by identifying changes in patterns of glycosylation upon encounter of pathogens, and the effect of genetically altering OGT or OGA expression. The function of glycosylated proteins will be further studied to understand the relevance of their OGlcNAc status in various immune cell activities.

Honours/Masters/PhD

CONTACT

For further information regarding individual laboratories, their current staff, recent publications and research themes use the web links below:

- biomedicalsciences.unimelb.edu.au/departments/biochemistry
- www.bio21.unimelb.edu.au
- florey.edu.au/neuropeptides
- svi.edu.au/research_themes/structural_biology

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