Honours projects on offer in the Charles Perkins Centre Hub 2019-2020

Level 3 East – Heart Research Institute projects

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Honours project details

CARDIOMETABOLIC DISEASE
Dr John O’Sullivan
Research interest: Obesity-driven metabolic disease such as insulin resistance, diabetes, fatty liver disease, hyperlipidaemia, and hypertension are the major drivers of atherosclerotic cardiovascular disease in the modern era. Despite our best primary prevention efforts, this trend is continuing and expected to worsen. These complex diseases are the consequence of gene-environment interactions. Therefore, in order to tackle the cardiovascular consequences of the obesity epidemic, we must improve our understanding of the various levels of dysregulation. To do this, both genomic data and environmental data must be captured. The aim of our studies is to discover better diagnostic markers, predictors, and therapies for cardiometabolic disease.

Research projects:
We will probe carefully-phenotyped patient cohorts using genome scanning and metabolomic profiling to discover novel disease markers that may have clinical utility, e.g., by providing better diagnostic markers of disease, and allowing earlier intervention by predicting future disease. Furthermore, integration of genetic and metabolomic data allows delineation of disease pathways, which we then study in animal and cell models of disease. This allows us to determine disease-specific functional regulation, and potential for therapeutic intervention.

Project 1. Giving the failing heart the nutrients that it needs
The aim of this project is to determine the key cardiac substrates depleted in the failing heart, and to determine if replacing them can restore normal heart function. Heart failure (HF) associated with obesity and type 2 diabetes (leading to “stiff hearts”, termed “Heart Failure preserved Ejection Fraction”, or HFpEF), has exploded in prevalence, has no specific treatment, and is driven in large part by altered metabolism. Therefore, our specific aims are to:

1. Measure cardiac substrate changes in human HFpEF and determine association with heart function and clinical outcome;
2. Determine stages of metabolic alteration, and substrate turnover, during the natural history of HFpEF using model systems;
3. Investigate the effects, and underlying mechanism, of key substrate administration on cardiac function.

Project 2. Probing microbiome-metabolome-cardiovascular disease interactions
Recent research has shown significant health benefits deriving from high-dietary fiber/microbiome-accessible carbohydrate (MAC) consumption. Compared with native starch, dietary resistant starch is a high-MAC starch that significantly alters the gut microbiome. In recent studies, we determined the systemic metabolic effects in male C57BL/6 mice fed either a native- or resistant-starch diet for 18 weeks (n = 20/group). Metabolomic analyses revealed that the plasma levels of numerous metabolites were significantly different between these diets, many of which were microbiome-derived. Most strikingly, we observed
a 22-fold increase in the gut microbiome-derived tryptophan metabolite indole-3-propionate (IPA), which was positively correlated with several gut microbiota including Clostridiales, Allobaculum, Bifidobacterium and Prevotella. Major changes were also observed for metabolites solely or primarily metabolized in the gut, e.g., trimethylamine-N-oxide; metabolites that have a significant entero-hepatic circulation, i.e. bile acids; lipid metabolites, e.g. cholesterol sulfate; metabolites indicating increased energy turnover, e.g. tricarboxylic acid cycle (TCA) intermediates and ketone bodies; and increased anti-oxidants such as reduced glutathione. Our findings reveal potentially novel mediators of high MAC-derived health benefits. We will now extend this analysis and examine the role of IPA, and related indoles, as THE major mediators of the cardiometabolic benefits of high-fibre diets.

**Project 3 – Sleep apnoea and hypertension: What goes wrong in the brain?**

*Working with Dr Melissa Farnham [CARDIOVASCULAR NEUROSCIENCE]*

Repetitive hypoxia is a feature of obstructive sleep apnoea (OSA); a condition characterised by intermittent airways obstruction. Patients with OSA present with persistent increases in sympathetic activity and commonly develop hypertension, which is precursor to the more malignant cardiovascular disease. Diabetes is also commonly associated with OSA. Work from our lab has shown that the persistent increases in nerve activity, following a protocol of acute intermittent hypoxia, are dependent upon activation of PACAP receptors in the spinal cord. PACAP (pituitary adenylate cyclase activating polypeptide) is an excitatory neuropeptide found throughout the sympathetic nervous system. PACAP also acts to elevate blood glucose by stimulating adrenaline release. Other groups have shown, in both rats and humans, that intermittent hypoxia elevates blood glucose. We hypothesize that PACAP is driving the increase in nerve activity, leading to hypertension and increased adrenaline release and elevated glucose. These early changes could initiate triggers that promote insulin resistance and development of type 2 diabetes in human OSA conditions. Our goal is to uncover the mechanisms driving the cardiometabolic effects of OSA to spearhead the development of new strategies to treat OSA.

We use rodent models of OSA, both anaesthetised and conscious to measure blood pressure, heart rate and various sympathetic nerve activities following pharmacological manipulations of the central nervous system. The physiology experiments are combined with immunohistochemistry and molecular experiments to assess the signaling changes within the brain and spinal cord. We also study how OSA causes diabetes and are trying to identify the central pathways that lead to glucose handling dysfunction in OSA.

CARDIOVASCULAR MEDICAL DEVICES

Dr Anna Waterhouse

Research interest: Our Research focuses on how medical devices – such as artificial hearts, stents and bypass machines – interact with the body. We apply cutting-edge bioengineering tools to develop new methodologies to assess and understand the interplay of events at the biointerface, where the devices interact with the patient, and manipulate this interplay to improve medical device function, create novel medical devices and diagnostics and both drug and non-drug-based avenues for therapies.

Research projects:

Project 1. Developing models of biomaterial-device thrombosis.
Advances in micro and nanotechnology have revolutionised bioengineering, allowing high precision manipulation of materials for modelling medical devices in the lab. Using bioengineering strategies, increasingly sophisticated devices are being constructed. However, protein and cellular interactions with materials are still poorly understood. One such example where this lack of understanding causes detrimental outcomes is blood-material interactions causing medical device failure. Blood is one of the most complex biological fluids containing multiple proteins and cell types. When blood contacts foreign materials in medical devices, it can cause fatal thrombosis (blood clots). The aim of this project is to develop novel bioengineering solutions to study how material properties and blood flow dynamics govern the initiation of biomaterial-induced thrombosis, with the ultimate aim of improving medical device function.

The majority of experimental systems to test biomaterial-induced thrombosis in vitro rely only on traditional in vitro clotting assays which are done in test tubes using solutions of individual enzymes, purified fibrinogen or platelet-free plasma. These systems do not account for the reaction dynamics of cellular components or physiological blood flow, both of which are integral to thrombosis. Microfluidic systems provide sophisticated, real-time analysis of proteins and blood components that drive thrombosis, combined with the ability to manipulate blood flow at physiologically relevant rates. Utilizing state-of-the-art facilities at Australian Institute of Nanoscale Science and Technology (AINST) (University Sydney), we aim to develop bioengineering solutions using microfluidics to investigate protein/cellular interactions at the biointerface. Different medical device materials will be assessed for their mechanism of thrombosis initiation. Furthermore, this platform system could be used to evaluate novel bioengineered surfaces, such as repellent, immobilized liquid surfaces or tissue engineered materials.

Project 2. Investigating the mechanism by which super repellent surface coatings reduce thrombosis of medical devices
Blood clots (Thrombosis) forming in medical devices can be costly and fatal for a number of reasons. First, these clots can cause failure of the device requiring costly replacement, or
cessation of blood flow, which can be fatal. Second, embolism of thrombi formed in devices can lodge in the lungs or brain, causing pulmonary embolism or stroke. Progress in this field has been achieved through the development of slippery, liquid immobilized surfaces, of Tethered-Liquid Perfluorocarbon (TLP) coatings, which have been demonstrated as effective to prevent thrombosis and biofouling by preventing surface adhesion of blood and pathogens.

The aim of this project is to determine the mechanism by which TLP coating can reduce the thrombogenicity of medical devices. Tethered-liquid perfluorocarbon (TLP) coatings reduce fibrin polymerization and platelet adhesion and activation in vitro under static and blood flow conditions. In vivo, an extracorporeal circuit consisting of TLP coated medically approved tubing and cannulae, remained patent for at least 8 hours at 15L/hr of blood flow in a swine arteriovenous shunt model without the use of any antithrombotic medication (Leslie et al., 2014). However, the mechanism by which proteins and cells are repelled by TLP remains poorly understood. Here we aim to explore how plasma proteins, such as fibrinogen, and blood cells interact with TLP surfaces. This will have implications for how thrombus propagation is induced on TLP surfaces. Utilizing this system, the contribution of adhesion and local accumulation of blood components vs. protein and cellular activation to thrombosis and prevention of thrombosis could be elucidated. Understanding the mechanism of the low-thrombogenic, repellent properties of TLP coatings will enable improved application to medical devices and provides insights for design improvements.


**HAEMATOLOGY**

**Dr Freda Passam**

**Research Interest:**

The Haematology Research Lab aims to discover novel pathways in blood clotting which can lead to the development of effective and safe drugs to treat thrombosis.

**Research projects:**

**Project 1. Thiol isomerases as novel antithrombotic targets**

It has recently been discovered that thiol isomerases constitute a new clotting pathway. Thiol isomerases are a group of enzymes that regulate the function of blood cell receptors and clotting proteins by reacting with their disulphide bonds. We have identified a thiol isomerase, named ERp5, which is released into the circulation from activated platelets and promotes clot formation in vivo. The aim of this project is to discover alternative pathways in the clotting system that can be targeted to develop efficient and safer antithrombotic drugs.
We will dissect the role of ERp5 in platelet function and clot formation by using mice with genetic deletion of ERp5 in their platelets. We will investigate how this thiol isomerase regulates the interaction of platelets with clotting proteins (fibrinogen, von Willebrand factor) and vascular cells (endothelial cells and neutrophils). We will explore the potential of ERp5 inhibitors to prevent thrombus formation and become candidate antithrombotic drugs.

These studies will employ platelet function tests, cell perfusion assays, flow cytometry and confocal microscopy, and will provide the opportunity to learn the method of intravital microscopy for the study of clot formation in mice.

**Fig1.** In vivo thrombus formation in the cremaster artery of (A) a mouse injected with inactive control compound and (B) a mouse injected with an ERp5 inhibitor. Platelets are labelled in red and fibrin in green.


**Project 2. Redox biomarkers in thrombotic disease**

The redox balance (balance of reduction and oxidation reactions in our blood) is essential for a healthy circulation. Redox imbalance causes alterations of protein function contributing to the development of thrombosis. We are focused on redox modification of disulphide bonds in two proteins critical for thrombus formation: the platelet receptor integrin a2bb3 and the plasma protein von Willebrand factor. We have found that reduced forms of a2bb3 and vWF have decreased thrombotic activity and may therefore protect from thrombotic disease, such as venous clots. The aim of this project is to identify new biological markers that can be used in the monitoring and treatment of patients with thrombotic disease.

We have developed assays which measure the redox balance in blood including tests which measure the disulphide reducing activity of plasma and the production of reactive oxygen species by platelets. We will study the redox modifications of platelet a2bb3 and plasma vWF which occur in patients at high risk for thrombosis to identify those most likely to benefit from drugs which restore the normal redox balance.

This project will employ mass spectrometry to study the posttranslational modifications of clotting proteins using disulphide labels specifically developed for this purpose. It also involves plasma and platelet functional assays.

Alterations of disulphide bonds in clotting proteins which cause decreased clotting activity in (A) the platelet receptor α2β3, bond Cys177-Cys184 and in (B) von Willebrand factor, bond Cys1669-Cys1670.

Project 3. Developing biochips for the study of haemostasis and thrombosis

Many patients with bleeding and clotting disorders go undetected by routine laboratory tests in part because the available assays do not reflect the conditions in the circulation. The Haematology Research Group uses biochips in a microfluidic system that allows blood to flow through passages under controlled conditions. The passages are designed to mimic blood vessels and include features e.g. stenosis, that simulate the circulation in stenosed vessels. The flow of blood through these biochips generates thrombi that can be visualized by real-time microscopy and quantified. The aim of this project is to develop microfluidic devices which can detect the thrombotic or bleeding tendency in patients with clotting problems.

This project will study blood cell adhesion and thrombus formation in the microfluidic devices to assess for persisting thrombotic tendency in patients with a history of venous clots, who have completed treatment. Samples from patients with bleeding disorders on treatment will be assessed for haemostatic potential. A range of parameters, which participate in clot formation, will be measured in the microfluidics system including platelets, fibrin, neutrophil extracellular traps, von Willebrand factor. This project will involve the preparation of microfluidic chips, microscopy and image analysis.

Figure 3. Microfluidic devices for measuring thrombotic and bleeding tendency. (A) Biochip containing channels for perfusion of blood (B) Schematic of a 90% stenosed channel for the study of thrombus formation (C) Blood sample with increased thrombus formation and (D) decreased thrombus formation at the
Relevant publications:

THROMBOSIS
Prof Shaun Jackson [Contact - A/Prof Simone Schoenwaelder]

Research interest:
Our research is focussed on the haemostatic and innate immune systems and their dysregulation in cardiovascular disease. Our main research focus is on blood cells (platelets, leukocytes), blood coagulation proteases and endothelial cells. These cell types play a fundamental role in the pathogenesis of diseases such as heart attack and ischaemic stroke, but also more broadly, in the context of inflammation, cancer metastasis and vascular development. Whilst our studies are primarily aimed at defining new mechanisms promoting thrombosis and inflammation (termed thromboinflammation), we also actively translate our research discoveries into new therapeutic approaches.

Research projects:
Project 1. Development of novel antiplatelet and anticoagulant drugs for the treatment of stroke
The development of a thrombosis or embolus in the cerebral circulation (ischaemic stroke [IS]) is the third most common cause of death and the most common cause of adult disability globally. Acute stroke therapy requires the prompt re-opening of occluded blood vessels to minimise tissue death - typically achieved through delivery of fibrinolytic agents modelled on tissue-type plasminogen activator (t-PA).

Despite the significant burden stroke has on the community, progress in the management of stroke continues to be unsatisfactory, with only one clinically approved thrombolytic agent for IS therapy. Further to this, rtPA therapy comes with significant limitations, with lysis resistant blood clots, as well as haemorrhage presenting as major complications. One of the main factors delaying reperfusion and increasing the risk of re-occlusion of cerebral vessels is the presence of platelets in arterial thrombi, with numerous preclinical and clinical studies demonstrating the benefits of adjunctive anti-platelet therapy to enhance cerebral reperfusion and reduce re-occlusion following thrombolysis. Unfortunately, in IS patients, the benefits of combined antiplatelet/thrombolytic therapy are partially offset by the increased risk of life-threatening intracerebral bleeding, limiting the widespread use of this approach.

We have identified a novel class of antiplatelet drug that is highly effective at promoting and facilitating thrombus dissolution and complete vascular reperfusion, without markedly increasing tail bleeding times. In this project, we will examine the safety and effectiveness of this novel antiplatelet to facilitate reopening of the blood vessel, examine its impact on clot porosity and dissolution, and examine its impact on end-organ damage, particularly in the stroke context.
These studies will not only provide important insight into our understanding of blood clot formation but may also lead to new approaches to regulate the size and stability of blood clots forming in the body, providing major clinical benefit in the delivery of thrombolytic therapy (blood clot removal).

These studies will use (i) in vivo models of thrombosis and thrombolysis; (ii) genetic mouse models, (iii) state-of-the-art imaging systems (tissue clearing techniques, confocal microscopy, intravital microscopy, laser doppler flowmetry and laser speckle contrast imaging); and (iv) behavioural assessment to determine cerebral damage following recovery from stroke.


Project 2. Investigation of a new thrombosis and inflammation mechanism triggered by ‘death pathways’ in platelets

Ischemia reperfusion (IR) injury commonly occurs in a wide range of human diseases, including acute myocardial infarction (AMI) and ischemic stroke. IR injury is characterised by poor blood flow in the micro-vasculature, exacerbating tissue ischaemic and organ injury. We have identified a new mechanism by which platelets and neutrophils cause microvascular obstruction. This previously unrecognised thrombotic mechanism is induced by the fragile membranes from dying platelets, that physically bridge adjacent neutrophils to facilitate neutrophil aggregation, leading to vessel occlusion. We can demonstrate that dying platelets convert neutrophils to a hyperadhesive inflammatory state. In turn, neutrophils can induce platelet death via production of oxidants. These new findings suggest a bidirectional communication mechanism operating between platelets and neutrophils, that may exacerbate microvasculature dysfunction and inflammation during IR injury. We are investigating this unique communication and its contribution to IR injury.

These studies will use (i) in vitro functional assays to assess platelet death and neutrophil hyperadhesive function; (ii) mouse models of IR injury adopted to investigate microvascular dysfunction; and (iii) real-time in vivo imaging of platelet death and neutrophil-platelet adhesion dynamics in the microvasculature during IR injury.


Project 3. Examination of a new thrombosis mechanism linked to diabetes

Diabetes Mellitus (DM) has become one of the major healthcare challenges of the 21st century and a leading cause of cardiovascular disease worldwide. Up to 70% of all diabetes-related deaths are due to cardiovascular disease, primarily related to atherothrombosis. Diabetic individuals not only develop more extensive atherosclerosis, they also exhibit a prothrombotic phenotype, with an exaggerated accumulation of platelets at sites of plaque disruption. The mechanism(s) by which diabetes causes platelet hyperactivity remains incompletely understood. Our recent studies have defined a new mechanism promoting arterial thrombus formation, termed biomechanical platelet activation. We now have
Evidence that this novel clotting mechanism is dysregulated in diabetes, leading to excessive platelet aggregation and thrombus formation. We believe that the chronic oxidative stress environment in diabetic blood vessels plays a key role in amplifying platelet aggregation by altering the shear-sensitivity of the major platelet adhesion receptor integrin αIIbβ3 (GPIIb-IIIa). Importantly, we have also demonstrated that biomechanical thrombosis associated with alterations in redox-sensitive signal pathways linked to GPIIb-IIIa. Importantly, exaggerated platelet aggregation is not inhibited by conventional antiplatelet agents such as aspirin and clopidogrel, which may partly explain reduced efficacy of antiplatelet therapies in individuals with diabetes.

This project will examine: (i) The impact of DM on the biomechanical adhesive function of GPIIb-IIIa; (ii) How redox-sensitive signalling pathways are dysregulated in DM platelets; (iii) Whether inhibiting platelet redox-sensitive signalling pathways reduces platelet hyperactivity and thrombosis in diabetes. To achieve these aims, we will investigate platelet mechanobiology at a cell-molecular scale, using a 4M’s approaches: Mechanics, Microscopy, Microfabrication & Molecular Mouse Models by combining the live-cell dynamic force spectroscopy BFP system with other complementary technologies including the TIRF/STORM super-resolution imaging, microfluidics, in vivo mouse models of thrombosis.


Project 4. Understanding the mechanisms leading to microvascular dysfunction and poor cerebral perfusion in stroke.

For patients presenting with AMI or stroke, the primary goal of therapy is to promptly re-open blocked arteries (recanalization) to salvage the dying ischemic tissue. Despite successful recanalization of major arteries, blood perfusion in surrounding microvasculature supplying the tissue can remain poor, a common complication of ischemic reperfusion (IR) injury, known as microvascular obstruction (MVO). MVO occurs in 60% AMI patients and persistent MVO can lead to progressive worsening of heart function and infarction. Several pathogenic processes have been implicated in MVO, however targeted therapies have not been effective in improving microvascular perfusion. This is due in part to the lack of suitable animal models and technical difficulties associated with performing real-time imaging on the microvasculature. In order to gain a better understanding of the temporal and spatial events leading to MVO, we have established a mouse model of gut IR injury, allowing access to microvasculature in living animals during IR injury. Using precision imaging systems, including confocal and scanning electron microscopy, we have observed previously unappreciated in vivo changes within the microvasculature during IR, wherein both the ischemia and reper-
fusion phases trigger phenotypically distinct endothelial cell death processes. These are associated with the onset of distinct vessel occlusion mechanisms involving red blood cells during ischemia, and platelets and neutrophils during reperfusion - both ultimately cooperating to lead to MVO in local gut and remote organs. These findings not only demonstrate an intimate spatiotemporal relationship between endothelial injury and vaso-occlusion mechanisms, they also help explain why existing therapies remain ineffective.

This project will examine the pathways by which endothelial cells undergo cell death and the molecular mechanisms which trigger these death pathways during both ischemia and reperfusion. Importantly, we will examine whether inhibiting endothelial cell death represents an effective approach to prevent MVO associated with IR injury.


Project 5 – Coronary microvasculature in health and disease.

working with Dr Keyvan Karimi [CLINICAL RESEARCH]

In collaboration with Professor Jackson, we will use intravital microscopy to identify new pathways for microvascular regulation in physiology, and in disease processes - in particular in ischaemia and reperfusion injury in the heart.

CLINICAL RESEARCH
Dr Keyvan Karimi

Research Interests:
Our mission is to detect the earliest signs of heart and blood vessel damage with a view to preventing serious complications in later life. We have several research projects ongoing in our laboratory, including those which aim to identify changes that occur in pulmonary microvasculature in response to hypoxia, and in disease states such as pulmonary hypertension - a rare but deadly disease. In these instances, our focus is to establish new therapies that could be tested in the clinical setting.

Project 6 – Impact of Diabetes on haematopoietic cells linked to atherosclerosis

working with Dr Ashish Misra [ATHEROSCLEROSIS AND VASCULAR REMODELLING]

Obesity and diabetes are major risk factors for a broad range of cardiovascular diseases. With three-times as many people in the world estimated to die from over-nutrition than from starvation or malnutrition in today’s society, the health implications of this "diabesity" epidemic are enormous. Based on current trends, this scenario will get worse, leading to a tsunami of cardiovascular diseases that could overwhelm a healthcare system already struggling to deal with an ageing population. Thus, there is an urgent need to uncover the fundamental mechanisms underlying the development of diabetes, including how cardiovascular risk factors affect atherosclerosis – in order to develop rationale strategies for minimizing the impact of these risk factors on our health and economy.
Bone-marrow derived stem cells (BMDSCs) and progenitor cells are integral to tissue homeostasis and repair and contribute to health through their ability to self-renew and commit to specialized effector cells. Importantly, defects in a variety of progenitor cell populations have been described in both preclinical and human diabetes. The general perception is that diabetes drives defects in bone marrow derived stem cells (BMDSCs) which accrue damage over time, disrupting tissue homeostasis and increasing risk of morbidity. However, the mechanisms by which defective BMDSCs can influence the pathology of individual plaque cells in atherosclerosis, and the subsequent impact this has on diabetes and obesity remains unknown.

In this study, we will be characterizing effects of these BMDSCs on atherosclerotic plaque burden using state-of-the-art transgenic mouse models and cardiovascular genetics. We will be using Cre-LoxP system, genetic knockouts, lineage tracing, clonal analysis, single-cell RNA sequencing, bone marrow transplant and culturing BMDSCs, histology of mouse and human patient samples and general cell and molecular biology techniques.

**ATHEROSCLEROSIS AND VASCULAR REMODELLING**
*Dr Ashish Misra*

**Research Interests:**
Our main objective is to broaden understanding of the cellular and molecular mechanisms involved in blood vessel wall patterning, define the role of these pathways in vascular abnormalities and complications, and then link these insights to translational research to improve the prevention and treatment of human cardiovascular disease. To this end, we employ a unique blending of lab models and cultured cells, as well as human samples, with the aim of unveiling the pathogenesis of cardiovascular diseases. Our ultimate goal is to prevent and reverse vascular disease to prevent heart attack and stroke.

**CARDIOVASCULAR SIGNALLING AND DRUG DISCOVERY**
*Dr Xuyu (Johnny) Liu*

**Research interest:**
Despite the global burden of cardiovascular disease, the development of new cardiovascular drugs has stalled for over two decades. The primary attrition is the intolerance of drug-related side effects. Recently, there is considerable interest in the development of natural supplements for cardiovascular-protective therapeutics owing to the inherent safety profiles and the clinical evidence for ameliorating chemotherapy-induced cardiovascular complication. However, it remains a huge challenge to understand the cardiovascular-protective mechanisms at the molecular level, which impedes pharmacological optimisation of these bioactive agents for therapeutic use. Therefore, we aim to apply cutting-edge chemoproteomic-platforms to understand the intricate signalling interplay in cardiomyocytes.
in response to different natural products and construct a comprehensive chemotype database for cardiovascular drug discovery.

Understand heart-healthy diets at the molecular level
Sulforaphane and alliin are known to be the cardioprotective “ingredients” in broccoli and onion diets. They have been shown to promote cardiomyocyte survival against ischemic injury and exhibit potent anticancer activity by potentiating apoptosis. However, the protein target spectra of these small molecules in cell remain unclear. There is no unified model to explain the cell-type-dependent phenotypes observed in the treatment.

**Project 1:**
(1) Profile the cell-type-specific target spectra of sulforaphane and alliin and forge a molecular link between protein target engagement and phenotypic outcome;
(2) Engineer small-molecule transport proteins targeting specific organelles to enable protein target profiling of sulforaphane and alliin in a spatiotemporally controlled manner.

**Project 2:** In collaboration with the Payne research group (School of Chemistry, USYD),
(1) Optimise the efficacy and mitigate the cardiotoxicity of current chemotherapy through conjugation with cardiovascular-protective natural products;
(2) Apply “click-and-release” chemistry to develop antibody-small-molecule conjugates enabling organ- and tissue-specific release of sulforaphane and alliin.