2021/2022 GLYCOMICS RESEARCH PROJECTS

A list of selected Glycomics Research Projects is provided on the following pages. These projects are geared towards prospective Honours, Masters and PhD candidates. For more information on these projects please contact the supervisors whose contact details are available on the last page of this document. Undergraduate students are also encouraged to look at the list of projects and contact the appropriate staff member(s) in the projects list, to discuss third year projects and work experience opportunities.

1. Epitope binding investigations of carbohydrate-recognising proteins by NMR spectroscopy

Assoc Prof Thomas Haselhorst, Dr Yun Shi & Prof Mark von Itzstein

Molecular Modelling, Structural Biology

Many carbohydrate-recognising proteins, eg Siglec 2 and *trans*-sialidases, have been implicated in clinically significant diseases such as non-Hodgkin's lymphoma and trypanosomiasis, respectively. Recently, the method STD-NMR was developed to screen compound libraries against various protein targets. This method is suitable for determining an epitope map of a ligand within the protein's binding site as only regions of the ligand that are in contact with the protein's binding site are observed in the NMR spectrum.

Techniques: Computational Chemistry including visualisation and molecular docking; Advanced NMR techniques including STD-NMR; Protein purification

2. From structure to function – rational development of new sialidase inhibitors

Prof Mark von Itzstein, Dr Robin Thomson, Dr Chih-Wei Chang, Dr Andrea Maggioni, Dr Benjamin Bailly, Dr Yun Shi, Dr Thomas Ve & Dr Xing Yu (Hunan Normal University, China)

Molecular Modelling, Medicinal Chemistry, Biochemistry, Molecular Microbiology, Virology, Structural Biology

Sialidases are involved in the infective cycles of a range of viruses, bacteria, and parasites. These include, for example, the causative agents of influenza, cholera, and African sleeping sickness. The essential roles that the sialidases often play in the infection cycle make them interesting targets for drug design. In the case of influenza virus sialidase, development of potent and selective inhibitors of the enzyme, based on knowledge of the enzyme structure, led to a new drug class to treat influenza. In recent years, new structural and mechanistic characteristics of microbial sialidases have been discovered, presenting new opportunities for inhibitor design.

The von Itzstein group works on the development of new sialidase inhibitors against pathogenic organisms using a multidisciplinary approach that includes: computational chemistry and structure-based inhibitor design; synthetic chemistry, working on a range of inhibitor templates; expression and purification of recombinant enzymes; the use of whole virus particles or virus-like particles presenting the enzyme on a non-infectious particle; enzyme assays for evaluation of inhibitor affinity; cell-based evaluation of compounds, and; structural biology studies in solution phase (NMR) or through X-ray crystallography. A student working on this project may specialise in one particular aspect or be involved with a number of the different interconnected aspects of the project.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic carbohydrate chemistry; Protein expression and purification; Virology; Enzyme Assays; Cell-based assays; Advanced NMR techniques including STD-NMR; X-Ray crystallography.

3. Vaccine development against respiratory pathogens

Dr Mehfuz Zaman & Prof Mark von Itzstein

Vaccinology, liposome design and formulation, drug delivery, immunology, virology, microbiology

The upper respiratory tract (URT) is the major entry site for multiple pathogens including Influenza A-B, *Streptococcus pyogenes* (group A streptococci, GAS) and coronaviruses. We are establishing a 'modular' multi-pathogen vaccine platform using liposomes (phospholipid vesicles). The liposomal delivery system allows the incorporation of both viral and bacterial peptide epitopes (a part of a protein recognised by antibodies and cells of the immune system) to prevent URT infection. The liposomal formulation can be stored as a lyophilised powder and reconstituted prior to immunisation, yielding a stable product that potentially does not require a cold-chain from production to needle-free administration. Incorporating lipid-linked sugars (glycolipids) enhances secretory immunoglobulin A (IgA)-mediated mucosal immunity that may reduce infectivity of human secretions and transmission.

This multidisciplinary research project involves liposome formulation, testing in pre-clinical models and immunological and functional assays to examine the mechanisms of protection.

Techniques: Vaccine design, Enzyme Linked Immunosorbent Assays (ELISA), *in-vitro* cell culture assays such as viral propagation and plaque forming assays, *in-vivo* techniques such as viral and bacterial challenges, immunization and sample collection from pre-clinical models.

4. Carbohydrate-based biological probes for the investigation of microbial glycan biosynthesis

Prof Mark von Itzstein & Dr Robin Thomson

Medicinal Chemistry

Bacterial resistance to antibiotics is a growing problem and is driving the search for novel antibacterial therapies. Importantly, bacterial cell membrane components often contain carbohydrate units and structural linkages that are not found in mammalian systems. The biosynthetic pathways to these structures are therefore attractive targets for the development of antimicrobial agents that affect the growth and integrity of, specifically, bacterial cell membranes. This project, as part of a continuing antimicrobial drug discovery programme, will involve the preparation of carbohydrate-based compounds for the investigation of bacterial cell wall biosynthetic enzymes, and their evaluation as inhibitors of bacterial growth.

Techniques: Synthetic carbohydrate chemistry; Bacterial cell growth assays.

5. Multivalent display of carbohydrate structures

Prof Mark von Itzstein, Dr Chih-Wei Chang & Dr Robin Thomson

Medicinal Chemistry

Interactions between cells, and between cells and microorganisms, are often based on multiple, simultaneous or sequential, interactions between protein receptors and their carbohydrate ligands. Mimicking these interactions by the use of multivalent arrays of receptor ligands – for example dendritic structures terminated with biologically relevant molecules or displays of molecules conjugated to nanoparticles or liposomes – has been successful for a number of carbohydrate-recognising proteins. This project involves the design and synthesis of multivalent structures, carrying functionalised carbohydrates, that will then be examined as probes and potentially inhibitors in a range of biological systems, for example in cell-binding studies of human pathogenic viruses.

Techniques: Synthetic carbohydrate chemistry.

6. Chemoenzymatic synthesis of biologically active glycans

Prof Mark von Itzstein, Dr Chi-Hung Lin & Dr Robin Thomson

Medicinal Chemistry

Human cell-surface carbohydrates (glycans) on glycoproteins and glycolipids are involved in important cell–cell and cell–biomolecule interactions. They also often form the initial attachment point for invading pathogenic microorganisms. Studies in glycobiology often require the use of a natural, or specifically modified, glycan to characterise and increase understanding of a specific biological interaction.

However, not all natural glycans are commercially or readily available. While methods of chemical glycan synthesis are advancing, there are significant advantages in the use of enzymes to construct both complex monosaccharides, and the linkages between monosaccharide units to form a glycan. Combining chemical manipulation of monosaccharide residues, or of a final glycan structure, with enzymatic linkage formation, it is possible to produce both natural and specifically modified complex glycan structures. This project will incorporate the use of both traditional carbohydrate chemistry techniques and the use of carbohydrate biosynthetic enzymes, to prepare glycans for use in a range of biological studies.

Techniques: Synthetic chemical and enzymatic carbohydrate chemistry.

7. Investigation of β-glucuronidases

Prof Mark von Itzstein, Dr Chih-Wei Chang, Dr Robin Thomson, Dr Andrea Maggioni, Dr Alpesh Malde, Dr Yun Shi & Dr Xing Yu (Hunan Normal University, China)

Molecular Modelling, Medicinal Chemistry, Biochemistry, Structural Biology

β-Glucuronidases are essential mammalian enzymes, which play a major role in the normal structuring and turnover of components of the extracellular matrix. In addition to their roles in normal human biology, over-expression of, in particular, the endo-β-glucuronidase heparanase can facilitate tumour cell growth and spread.

X-Ray structural information is now available for the important endo-β-glucuronidase human heparanase, as well as for a bacterial heparanase. This structural information can provide new insights into the catalytic mechanism of the enzymes and offers opportunities for inhibitor development.

This project offers a number of avenues for the investigation of β-glucuronidases, which can be either undertaken separately or together; computational chemistry and molecular modelling studies with enzyme X-ray structures; the chemical synthesis of probes to explore enzyme function and activity; biological evaluation of probes and known substrates or inhibitors using enzyme assay and NMR spectroscopy, and; investigation of enzyme–inhibitor complex formation by X-ray crystallography. Each of these aspects will lead to an improved understanding of this important class of enzyme.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry; Protein expression and purification; Enzyme assays; Advanced NMR techniques including STD-NMR; X-ray crystallography.

8. The discovery and characterisation of charged glycans as inhibitors of enterovirus 71 infection

Dr Chi-Wei Chang, Dr Benjamin Bailly, Dr Crystall Swarbrick, Dr Mehfuz Zaman, Dr Robin Thomson & Prof Mark von Itzstein

Medicinal Chemistry, Virology, Structural Biology

The picornavirus Enterovirus 71 (EV71) is a major cause of hand, foot and mouth disease in children less than 5 years old worldwide. While the disease usually presents with mild symptoms, it can sometimes spread to the central nervous system and cause severe neurological complications such as flaccid-like paralysis or encephalitis. There are currently no treatments or vaccines against EV71 infection.

EV71 is thought to infect cells by binding to various cellular receptors including glycosaminoglycans (GAGs) and sialylated glycans. While most efforts in anti-EV71 drug discovery are focussed on inhibiting the various viral proteases, we take advantage of the scaffold of naturally occurring glycan receptors to investigate the potential of functionalised glycans and GAG-mimetics to inhibit the virus binding to cells.[1] This project therefore involves medicinal carbohydrate chemistry for the design and synthesis of glycans and their mimetics, virology techniques for cell-based screening and evaluation of compounds, and X-ray crystallography and STD-NMR technologies for the characterisation of virus/glycan binding events.

1. Earley D; Bailly B *et al*, *ACS Infect. Dis.* 5: 1708−17 (2019). doi: 10.1021/acsinfecdis.9b00070.

Techniques: Synthetic carbohydrate chemistry; Virology; Cell biology; Crystallography; NMR techniques including STD-NMR.

9. The characterisation of enterovirus 71 binding specificity to host cell receptors

Dr Benjamin Bailly, Dr Crystall Swarbrick & Prof Mark von Itzstein

Virology, Structural Biology, Cell Biology

The hand, foot and mouth disease causing agent enterovirus 71 engages a variety of receptors on the surface of host-cells prior to entry. These receptors include the P-selectin glycoprotein ligand-1 (PSGL-1), the scavenger receptor class B member 2 (SCARB2), glycosaminoglycans (GAG) and sialylated glycans. The interplay between these receptors is still poorly understood. The types of GAGs and sialylated glycans the virus binds to have not been fully investigated, and we believe that given our progress with GAG-like binding inhibitors they may be more important than previously reported. Furthermore, in our experience different cell-types have different susceptibilities to glycan-based binding inhibitors, suggesting that cell binding events may be more complicated than previously characterised.

This multidisciplinary research project involves the differentiation of various cell types and subsequent functional assays to investigate virus-cell binding events, glycan-array experiments, cell-based chemical combination assays using glycans, competition STD-NMR experiments and crystallography using purified virus particles.

Techniques: Virology; Cell biology; Crystallography; NMR techniques including STD-NMR; Glycan-Array.

10. Structure Affinity Relationship (SAR) by NMR

Assoc Prof Thomas Haselhorst, Dr Andrea Maggioni & Prof Mark von Itzstein

Structural Biology, Biochemistry

Abbott laboratories have published a new NMR spectroscopic method called “SAR by NMR” to identify binding ligands and simultaneously to detect amino acids within the protein binding sites which play a key role in the binding event. This project will involve the expression and purification of ^{15}N labelled rotavirus VP8* protein in minimal media and the analysis of the purified labelled protein by means of high-resolution NMR spectroscopy. $^{15}N/^{1}H$-HSQC experiments of the *apo* protein and complexed with potential binding ligands are acquired. For amino acids involved in the binding event a change in their chemical shifts is likely. This valuable information can then result in lead structures for the design of new anti-viral drugs.

Techniques: Chemical Characterisation including Proton and Carbon-13 NMR; Advanced NMR techniques including STD-NMR; Protein purification.

11. Parainfluenza virus glycan receptor characterisation and structure-based discovery of anti-parainfluenza viral agents

Prof Mark von Itzstein, Dr Patrice Guillon, Dr Ibrahim El-Deeb, Dr Larissa Dirr, Dr Thomas Ve, Dr Robin Thomson, & Dr Alpesh Malde

Molecular Modelling, Medicinal Chemistry, Molecular Biology, Biochemistry, Structural Biology

Human parainfluenza viruses (hPIV) are important respiratory tract pathogens, second only to respiratory syncytial virus. Infants, young children, the elderly and the immunocompromised are most severely infected, developing croup, pneumonia or bronchiolitis that may need patient hospitalisation. Currently there are neither vaccines nor specific antiviral therapy available to prevent or treat hPIV infections.

Among the hPIV proteins, the haemagglutinin-neuraminidase (HN) glycoprotein represents a promising target for new antiviral drug discoveries. The HN protein is crucial in several steps of the virus life cycle. Firstly, HN recognises and binds sialic acid exposed at the surface of the host cell. Moreover, HN binding is necessary for the activation of the hPIV fusion (F) protein that allows fusion of the cell and virus membranes. HN also has an important action during the viral budding process because it cleaves sialic acid from glycoconjugates to prevent the accumulation of virions at the cell surface and their auto-agglutination. Sialic acid recognition is the trigger of all these HN activities, and the research experiments of this project are focused on the development of high potency inhibitors of the HN–sialic acid interaction.[1]

The X-ray crystal structures of the HN glycoprotein of hPIV types 3 and 5, and of Newcastle Disease Virus are available and can be used as homology models for the study of HN from other hPIV subtypes. While some characterisation of hPIV glycan receptor interaction has been undertaken, a complete systematic study is yet to be done. Furthermore, the combination of molecular modelling, structure-based design, fragment screening, and synthetic chemistry, may provide new inhibitors of viral replication. Using biochemistry and structural biology techniques on whole virus and recombinant HN glycoprotein, the effect of these new inhibitors on the virus/glycan interaction can be investigated. A student working on this project may specialise in one particular aspect or be involved with a number of the different aspects of the project.

1. Reviewed in: Chibanga V *et al*, *Antiviral Res*. 167: 89–97 (2019). doi: 10.1016/j.antiviral.2019.04.001.

Techniques: Computational Chemistry including visualisation and molecular docking; Fragment screening using ^{19}F NMR; Synthetic Chemistry; Protein expression and purification; Virology; Biological Assays; Advanced NMR techniques including STD-NMR, X-ray crystallography.

12. Structural characterisation and inhibition of Nipah and Hendra virus infections

Dr Thomas Ve, Dr Yun Shi, Dr Andrea Maggioni, Prof Johnson Mak & Prof Mark von Itzstein

Structural Biology, Biochemistry, Virology

Nipah virus (NiV) is a highly lethal (risk group 4) zoonotic paramyxovirus causing severe, rapidly progressive encephalitis in humans with the case fatality rate ranging from 40-70%. NiV is closely related to Hendra virus (HeV), another risk group 4 paramyxovirus that is native to Australia and infects both horses and humans. NiV is widely distributed in Southeast Asia, India, and Africa. WHO has earmarked NiV on a priority list of eight pathogens that is expected to cause severe outbreaks in the near future. While a one-health approach of vaccinating the intermediate host (horse in the case of Hendra) is able to block the transmission of Hendra virus, the limited uptake of the Hendra vaccine by horse owners could potentially make such approach ineffective. Furthermore, transmission of NiV to humans may occur after direct contact with infected bats, infected pigs, or from other NiV infected people making a one-health preventive approach not practical to NiV, due to the lack of reliance of an intermediate host.

The NiV envelope proteins, glycoprotein G and fusion protein F, are the determinants of viral entry. G and F achieve this via their recognition of the host- cell surface proteins Ephrin-B2 and -B3, and the glycosaminoglycan heparan sulfate (HS). Although detailed structural information is available for the G/Ephrin-B2/B3 interactions, the structural basis for how the G protein coordinates selective binding to specific glycans, such as HS is completely unknown. Furthermore, the mechanistic details of how any of the host-cell receptors trigger viral fusion are poorly defined.

This project will involve a combination of biophysical and cell-biology approaches and aims to define the molecular basis of NiV/HeV interaction with host-cell glycans (glycointeractome), define the molecular mechanisms underlying fusion activation and identify inhibitors targeting these processes. A student working on this project may specialise in one particular aspect or be involved with a number of the different aspects of the project.

Techniques: X-ray crystallography, cryo electron microscopy (cryo-EM), saturation transfer difference nuclear magnetic resonance (STD NMR), library screening, surface plasmon resonance (SPR), glycan arrays, and viral infection assays using pseudotyped particles.

13. Investigating sialic acid metabolism

Prof Mark von Itzstein, Dr Andrea Maggioni, Dr Robin Thomson, Dr Yun Shi & Dr Xing Yu (Hunan Normal University, China)

Medicinal Chemistry, Molecular Biology, Biochemistry, Cell Biology, Structural Biology, Molecular modelling

Sialic acids are 9-carbon acidic amino-sugars, which are found predominantly at the ends of mammalian glycoproteins and glycolipids. The terminal location of the sialic acid residues on these cell-surface sialo-glycoconjugates results in their essential involvement in processes of cell–cell, cell–microorganism, and cell–biomolecule interactions. The amount of sialic acid expressed on a cell's surface, and sialic acid modifications such as *O*-acetylation, vary throughout development, and in diseases such as some cancers. A number of microorganisms also express sialic acids on their surface, in some cases mimicking human sialo-glycoconjugate structures which can help the microbe to avoid detection by the host immune system.

We have a number of projects that examine the steps in the sialic acid biosynthetic pathway, to give natural and modified sialo-glycoconjugates. Non-natural substrates for enzymes of this pathway, or the use of inhibitors, can be used to change the nature and/or level of sialic acid expressed at the cell surface, and so to alter subsequent biological interactions. In the case of pathogenic bacteria which express surface sialic acids, reduction in the level of sialic acid expression may leave the bacteria more vulnerable to attack by the immune system.

These projects cross a number of disciplines. Aspects of the projects, which can be undertaken either separately or together, include; computational chemistry and molecular modelling studies with enzyme X-ray structures; the chemical synthesis of enzyme probes and inhibitors; biological evaluation of probes and inhibitors using enzyme assay and/or NMR spectroscopy; study of changes of cell surface sialic acid and modifications; and investigation of

enzyme–inhibitor complex formation by X-ray crystallography. Each of these aspects will help us gain to an improved understanding of the enzymes of sialic acid metabolism.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry; Protein expression; Cell-based studies; NMR-based enzyme assays; Advanced NMR techniques including STD-NMR; X-ray crystallography.

14. Design and synthesis of a Glycosaminoglycan (GAG) fragment library

Dr Chih-Wei Chang & Prof Mark von Itzstein

Medicinal Chemistry

Glycosaminoglycans (GAGs), found either on cell membranes or in the extracellular matrix, are classes of large linear polysaccharides carrying negatively charged groups, that are involved in a wide range of physiological processes. The GAG family includes heparan sulfate (HS) and chondroitin sulfate, among others. Most of the roles of GAGs in interacting with proteins, and modulating the host of diverse biological activities, are still poorly understood. Better understanding of the biological interactions between GAGs and GAG-binding proteins requires the use of pure GAG fragments.

The synthesis of complex GAGs in a pure form is not trivial. In this project, we aim to develop new synthetic strategies to access a discrete heparan sulfate (HS) fragment library. The homogeneous HS fragments, that will incorporate 2-*O*-, 6-*O*- and *N*-sulfate groups in a defined manner, will be used to elucidate the interactions between the specific GAG sequences and proteins associated with a variety of diseases including cancer, virus infection and diabetes.

Techniques: Synthetic carbohydrate chemistry

15. Synthesis of novel glycosaminoglycan (GAG) mimetics as GAG alternatives

Dr Chih-Wei Chang & Prof Mark von Itzstein

Medicinal Chemistry

Glycosaminoglycans (GAGs), large negatively-charged polysaccharides, exist universally on the cell surface and have various functions that include sustaining the integrity of the extracellular matrix (ECM), and acting as ligands for recognition and binding of biological molecules. In particular, their role as biological ligands has received significant attention in the fields of glycobiology and biomaterials. Variation in length, sequence, sulfation degree, and conformational flexibility of the GAG polysaccharide chains give rise to a large number of complex GAG sequences. Detailed study of GAG functions requires homogenous GAG fragments, however, due to this structural complexity, syntheses can be long and challenging, and reliable production of GAGs for *in vitro* and subsequent *in vivo* experiments can be a limiting factor.

In this project our aim is to look for alternative GAG-*mimetic* scaffolds that can replace native GAG sequences. We will explore these new scaffolds in interactions with specific GAG-recognising proteins and look at their effect(s) on GAG biological functions.

Techniques: Synthetic carbohydrate chemistry

16. Synthesis and biological evaluation of novel anti-cancer agents

Dr Ibraham El-Deeb, Dr Andrea Maggioni & Prof Mark von Itzstein

Medicinal Chemistry, Cell Biology

We have developed[1] a versatile synthesis of a class of potent anti-cancer agents known as the duocarmycins. We are now using a further optimised synthesis of this class of compound to discover novel anti-cancer agents that contain added carbohydrate residues to potentially improve biological function (creating glycoconjugates of duocarmycins). Our preliminary biological evaluation of some of these compounds, in cell-based assays, provides us with optimism that such compounds may have good anti-cancer activity. This project will look at the further development of these glycoconjugates as potential anti-cancer drugs.

1. El-Deeb IM *et al*, *Org Biomol Chem*. 12(24):4260-4 (2014). doi: 10.1039/c4ob00842a.

Techniques: Synthetic carbohydrate chemistry, Cell biology

17. Development of ionophores as novel antimicrobial therapies

Dr Ibraham El-Deeb & Prof Mark von Itzstein

Medicinal Chemistry

The increase in bacteria acquiring resistance to current antibiotics, and a reduction in development of new antibiotics by the pharmaceutical industry over the past years, is placing a significant burden on global health care, with the World Health Organization noting that antibiotic-resistant pathogens represent an imminent global health disaster for the 21st century. Our research is focussed on investigating alternative therapeutic strategies to break antibiotic resistance. Metal-ion homeostasis is critical for bacterial survival, and elevated metal ion levels can be toxic to bacterial pathogens. Ionophores are chemical compounds that facilitate transport of metal ions across biological membranes. Together with our collaborators, we have identified ionophores that are able to break antibiotic resistance by destabilizing bacterial metal homeostasis.[1] This project will extend our work in this area, through development and evaluation of new ionophores.

1. Bohlmann L *et al.*, *mBio* 9:e02391-18 (2018). doi: 10.1128/mBio.02391-18.

Techniques: Synthetic chemistry

18. Exosomes as cancer biomarkers and therapeutics

Dr Andrea Maggioni & Prof Mark von Itzstein

Cancer Biology, Biochemistry

Exosomes are vesicles that are secreted from cells and appear to have roles in the tumour microenvironment, including in metastasis. These vesicles are therefore thought to be invaluable in both a diagnosis setting as well as therapeutic targets. Little is known about the cell surface changes in glycans and glycan-recognising proteins. This project will explore these changes using a multidisciplinary approach that may identify potential biomarkers and therapeutic targets that could be used in diagnosis and drug discovery, respectively.

Techniques: Cell biology, Biochemistry, Glycoanalytics

19. Rotavirus: Structure Based Drug design

Prof Mark von Itzstein, Dr Robin Thomson & Assoc Prof Thomas Haselhorst

Molecular modelling, Medicinal Chemistry, Structural Biology

Rotaviruses are double stranded RNA viruses that are the leading cause of infantile gastroenteritis globally. The resulting dehydrating diarrhoea following infection is responsible for 33% of all hospitalisation of infants.

The triple layered Rotavirus virion must be delivered across host cell membranes into the cytoplasm in order to initiate viral gene expression. Cell-attachment and entry mechanisms are promising targets for therapeutic and preventative interventions against rotavirus diarrhoea.

Rotavirus outer capsids comprise a coat glycoprotein and a spike protein that mediate infection. VP8* is the 18 kDa protein fragment forming the spike tip and binds a cell-surface carbohydrate (sialic acid) during virus attachment to cells. The overall aim of this project is design of carbohydrate based compounds that could bind and block the active site of VP8* thus preventing the virus particle from attaching to the host cell and causing infection. X-Ray crystal structures of VP8* proteins alone and in complex with natural sialic acids are available for use in structure-based design of synthetic ligands, and potential inhibitors of VP8* interactions.

This project offers the potential to combine computational structure-based modelling and design, with chemical synthesis of carbohydrate-based compounds as potential VP8* inhibitors, and evaluation of the VP8*–compound interactions using the technique of STD NMR spectroscopy.

Techniques: Computational Chemistry including visualisation and molecular docking; Synthetic carbohydrate chemistry; Advanced NMR techniques including STD-NMR.

Sialic acid dependence in rotavirus host cell invasion. T. Haselhorst, F.E. Fleming, J.C. Dyason, R.D. Hartnell, X.Yu, G. Holloway, K. Santegoets, M.J. Kiefel, H. Blanchard, B.S. Coulson, M. von Itzstein. (2009) *Nat. Chem. Biol.* Feb;5(2):91-93

20. The development of a glycoenzyme (ZymeBank) data bank with supporting bioinformatics databases

Dr Matthew Campbell, Dr Chi-Hung Lin & Prof Mark von Itzstein

Bioinformatics and Biochemistry

Glycoenzymes are responsible for the biosynthesis of all glycans and glycoconjugates and as such provide a rich source of biocatalysts for industrial applications. Over 350,000 sequences (CAZY.com) have already been identified in genomic databases and the number is growing exponentially, but few glycoenzymes are readily available for the glycomics and biotechnology community. This project aims to develop an international glycoenzyme bank (called ZymeBank) that can be used to synthesis complex glycans for use in biological studies.

The project will focus on expressing and purifying human glycoenzymes and building a new database for storing this data along with a web-app interface. This will address a number of challenges: (i) identification of specific glycoenzyme activities for which there is a need in the community; (ii) establish screening protocols that are benchmarked against existing methods; and (iii) set standards and develop an open access databases for glycoenzyme activites and associated biological pathways.

Techniques: Bioinformatics; Databases; Protein Expression; Molecular Biology

21. Development of MALDI imaging mass spectrometry analysis of glycosphingolipids derived glycans

Dr Arun Everest-Dass & Prof Mark von Itzstein

Analytical Glycomics, Biochemistry

Given the universal presence of glycans on all cell surfaces, it is not surprising that several human diseases display changes in glycosylation of proteins and lipids. For example, cancer cells frequently display aberrant glycans than those observed on normal cells. Mass spectrometry (MS) based glycomic methodologies are now regularly used for the reliable profiling of glycans from clinical samples. Although, routine mass spectrometric glycan analysis is well established and reliable, the analysis of whole tissues destroys any information relating to the spatial distribution of the analytes. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is an emerging technique that seeks to utilize the analytical advantages of mass spectrometry whilst preserving the spatial information of the biological molecule of interest inherent in the sample. The unambiguous correlation between histopathology and MALDI–MSI allows the mass measurement of glycans directly from tissue regions [1].

This project aims to develop novel MALDI MSI based imaging technology to characterise glycosphingolipid derived glycans directly from tissue sections.

Techniques: glycomics, MALDI imaging, histopathology.

[1] Everest-Dass AV *et al*, Mol Cell Proteomics. 2016 Sep;15(9):3003-16. doi: 10.1074/mcp.M116.059816.

22. Inhibition of TIR domain assembly formation in Toll-like receptor signalling

Dr Thomas Ve & Assoc Prof Haselhorst

Innate Immunity, Biochemistry, Structural Biology, Molecular Modelling

Toll-like receptors (TLRs) detect pathogens and endogenous danger, initiating innate immune responses that lead to the production of pro-inflammatory cytokines. At the same time, TLR- mediated inflammation is associated with a number of pathological states, including infectious, autoimmune, inflammatory, cardiovascular and cancer-related disorders. This dual role of the pathways in protecting against infection and contributing to pathological conditions has attracted widespread interest from pharmaceutical and biotechnology industries.

Cytoplasmic signaling by TLRs starts by their TIR (Toll/interleukin-1 receptor) domain interacting with TIR-containing adaptor proteins MyD88 (myeloid differentiation primary response gene 88), MAL (MyD88 adaptor-like/TIRAP), TRIF (TIR-containing adaptor inducing interferon-β/IFNβ), and TRAM (TRIF-related adaptor molecule) Combinatorial recruitment of these adaptors via TIR:TIR interactions orchestrates downstream signaling pathways, leading to induction of the pro-inflammatory genes. Although TLR pathways have been well characterized, molecular information on the signaling proteins is limited, impeding the development of therapeutic strategies and the understanding of the effects of polymorphic variants on human disease

This project aim to identify new inhibitors of TLR4 signalling, which will involve screening of small molecule compound libraries using an *in vitro* TIR domain assembly assay established for the TLR4 adaptor proteins MAL and MyD88. Compounds shown to inhibit assembly formation will be characterised in more detail for protein interaction using Saturation Transfer Difference (STD) NMR, surface plasmon/isothermal titration calorimetry, and X-ray

Crystallography. This information will then be used with molecular modelling and structure analysis to generate more effective small-molecule inhibitors as potential leads for drugs.

Techniques: Production and purification of the TIR domains from the TLR adaptor proteins MAL and MyD88 using established protocols, screening of small molecule libraries using an established biochemical TIR domain assembly assay, STD-NMR, surface plasmon resonance or isothermal titration calorimetry, X-ray crystallography, *in silico* structure analysis and molecular modelling.

23. Novel Antibody-independent Targeted Therapy for the Treatment of B cell Lymphomas

Associate Professor Thomas Haselhorst and Dr Santosh Rudrawar

Research areas: Medicinal Chemistry, Structural Biology, NMR spectroscopy, Cell culture,

Project details: Lymphoma is the most common lymphoid malignancy and is among the 10th most prevalent cancers worldwide. Non-Hodgkin's Lymphoma (NHL) accounts for 80–85% of all lymphomas, including the common B-cell NHLs (B-NHLs). Current standard of care for relapsed/refractory NHLs are anthracyclines that are associated with cumulative cardiotoxicity with limited repeated clinical use. Rituximab-based therapy relies on complement and antibody dependent cell-mediated cytotoxicity to effect cell killing and is associated with severe side effects and in some cases form tumour lysis syndrome.

This PhD project aims to develop novel therapies with an alternative mechanism for B cell killing and improved outcome by synthesising novel carbohydrate-based ligands and conjugating ligands to toxin-loaded liposomes.

Techniques: Synthetic carbohydrate chemistry; Lipid and peptide chemistry, Medicinal chemistry, Cell biology, Cell culture, Computational Chemistry including visualisation and molecular docking, NMR spectroscopy, drug discovery and design.

24. Carbohydrate-based compounds as potential anti-bacterial agents

Dr Milton J Kiefel & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Structural Biology

The prevalence of drug-resistant bacteria is becoming one of the major global health problems. Of particular concern is the emergence of multidrug-resistant Gram-negative bacteria, which are particularly difficult to treat with current therapeutics. In addition to their resistance to many common anti-bacterial agents, Gram-negative bacteria have evolved many virulence factors that are essential for them to infect host organisms. This project aims to develop carbohydrate-based inhibitors of enzymes directly involved in bacterial virulence. Significantly, many of the chemical messengers used by Gram-negative bacteria as virulence factors are unique to these organisms. This means that the compounds developed in this project have the potential to disrupt bacterial virulence without causing damage to the host. In addition to the synthetic chemistry component of this project, high field NMR spectroscopy will be used to undertake substrate-specificity studies with the enzymes of interest. Compounds prepared will also be evaluated for their antibacterial activity using standard assays. Students undertaking this project will learn modern synthetic chemistry methodology in state-of-the-art chemistry research laboratories, will gain "hands-on" experience with the use of high field NMR spectroscopy, and will have the opportunity to undertake antibacterial assays.

25. Investigations into synthesis of ulosonic acids

Dr Milton J Kiefel

Medicinal Chemistry

Ulosonic acids are a family of higher order sugars the are intimately associated with a number of human diseases. Keto-deoxy octulosonic acids are key components of the outer membrane of Gram-negative bacteria, whilst nonulosonic acids include the sialic acids, pseudaminic acids, and legionaminic acids, all of which are known to be associated with human disease and bacterial virulence. Whilst the role of these sugars as virulence factors is generally well understood, much remains to be discovered. One of the current limitations in this field of research is the limited availability of synthetic derivatives and analogues of these important sugars. This project aims to expand on some of our preliminary work into developing a new highly efficient synthesis of ulosonic acids using an aldol condensation as the key step. Students undertaking this project will learn modern synthetic chemistry methodology

in state-of-the-art chemistry research laboratories. They will also gain "hands-on" experience with the use of high field NMR spectroscopy and will produce compounds that will ultimately be used as biological probes.

26. Synthesis of novel natural product analogues

Dr Milton J Kiefel & Prof Tony Carroll

Medicinal Chemistry

Natural products represent an important source of novel chemical entities with unique biological activity. This project involves the synthesis of compounds that are structurally related to specific classes of natural products that have biological activity (e.g. anticancer activity). The aim of the synthetic chemistry is to provide novel compounds with potentially improved pharmacological profiles in comparison to the natural compounds. The specific types of compounds to be made will be determined upon discussion with the student. Students undertaking this project will learn modern synthetic chemistry methodology in state-of-the-art chemistry research laboratories, as well as gaining hands-on experience with a number of important spectroscopic instrumentation.

27. Synthesis of butenolides with anticancer or antimicrobial properties

Dr Milton J. Kiefel & A/Prof Shai Anoopkumar-Dukie
Medicinal Chemistry

Butenolides are naturally occurring molecules characterised by a central 5-membered lactone ring. There is vast structural diversity and biological activity within this group of naturally occurring compounds. We have recently developed a highly efficient and flexible synthesis of this important class of natural products. This project will focus on expanding our synthetic chemistry method to allow the synthesis of novel butenolides, and then evaluate the synthesised compounds for their biological activity. Currently we have research looking at the anticancer activity of butenolides, but this can be expanded to include antimicrobial activity as well.

28. Identification of specific amino acid residues responsible for interactions of chemosensory receptor Tlp1 with chemotaxis proteins CheW and CheV of *Campylobacter jejuni*

Prof Victoria Korolik & Dr Christopher J Day

Molecular Microbiology

The natural habitat of campylobacters is the intestine of warm-blooded animals, and therefore chemotactic motility is an important mechanism involved in the colonisation and pathogenicity of this microorganism. Bacterial motility is subject to sensory control mechanisms that introduce a bias into the swimming direction of the organism towards beneficial environments and away from unfavourable conditions. Although chemotaxis has been demonstrated for *Campylobacter* the chemical substrates, mechanisms involved in the sensory control of motility and the role of chemotaxis in disease, are poorly understood. We, therefore, hypothesise that the chemosensory receptor proteins play a key role in chemotaxis and are involved in the pathogenicity of this organism as the first line of bacterial – host interaction and thus provide rational targets for the design of novel antimicrobial agents.

This project involves characterisation of interactions of the signaling domain of one of the chemoreceptors of *C. jejuni,* named Tlp1 with CheW and CheV chemotaxis proteins. The major aim of this project is to identify which amino acids in the signaling domain of Tlp1 are responsible for binding with CheW and CheV though systematic site-specific mutagenesis followed by analysis of the mutated proteins using yeast 2-hybrid protein-protein interaction system.

This project will further the studies to elucidate the role of Tlp1 chemoreceptor in chemotaxis and pathogenicity of *C. jejuni*, which can potentially provide a tremendous insight into the mechanisms of chemotaxis of this organism.

29.* Understanding the role of chemosenory perception in pathogenicity of *Campylobacter jejuni

Prof Victoria Korolik & Dr Bassam Elgamoudi

Molecular Microbiology

The natural habitat of campylobacters is the intestine of warm-blooded animals, and therefore chemotactic motility is an important mechanism involved in the colonisation and pathogenicity of this microorganism. Bacterial motility is subject to sensory control mechanisms that introduce a bias into the swimming direction of the organism towards beneficial environments and away from unfavourable conditions. Although chemotaxis has been demonstrated for Campylobacter the chemical substrates, mechanisms involved in the sensory control of motility and the role of chemotaxis in disease, are poorly understood.

We, therefore, hypothesise that the chemosensory receptor proteins play a key role in chemotaxis and are involved in the pathogenicity of this organism as the first line of bacterial – host interaction and thus provide rational targets for the design of novel antimicrobial agents.

This project involves characterisation of interactions of one of the chemoreceptors of *C. jejuni*, named Tlp7 with environmental molecules. The major aim of this project is to identify which chemicals are responsible for triggering chemotactic responses governed by Tlp7. This will be determined using site-specific mutagenesis followed by analysis of the wild type and mutated proteins using small molecule and glycan arrays, chemotaxis assays and mammalian cell culture.

This project will further the studies to elucidate the role of Tlp7 chemoreceptor in chemotaxis and pathogenicity of *C. jejuni*, which can potentially provide a tremendous insight into the mechanisms of chemotaxis of this organism.

30. Understanding the role of multifunctional periplasmic proteins in bacterial sensory perception.
Prof Victoria Korolik & Dr Bassam Elgamoudi
Molecular Microbiology

The natural habitat of campylobacters is the intestine of warm-blooded animals, and therefore chemotactic motility is an important mechanism involved in the colonisation and pathogenicity of this microorganism. Bacterial motility is subject to sensory control mechanisms that introduce a bias into the swimming direction of the organism towards beneficial environments and away from unfavourable conditions. Although chemotaxis has been demonstrated for *Campylobacter* the chemical substrates, mechanisms involved in the sensory control of motility and the role of chemotaxis in disease, are poorly understood. We, therefore, hypothesise that the chemosensory receptor proteins play a key role in chemotaxis and are involved in the pathogenicity of this organism as the first line of bacterial – host interaction and thus provide rational targets for the design of novel antimicrobial agents.

This project involves characterisation of interactions of the chemoreceptors of *C. jejuni,* named Tlps, with periplasmic ligand-binding proteins The major aim of this project is to identify how periplasmic ligand binding proteins induce directed bacterial motility to nutrients and host targets though small molecule arrays, chemotaxis assays, systematic mutagenesis followed by analysis of the mutated proteins using yeast 2-hybrid protein-protein interaction system, mammalian cell culture and animal models.

This project will further the studies to elucidate the role of chemosensors in in chemotaxis and pathogenicity of *C. jejuni*, which can potentially provide a tremendous insight into the mechanisms of chemotaxis of this organism.

31. Role of multispecies biofilms in transmission of bacterial pathogen *Campylobacter jejuni*
Prof Victoria Korolik & Dr Bassam Elgamoudi
Molecular Microbiology

Biofilms and complex multi-species communities are a preferred manner by which bacteria exist within the host or grow on various surfaces. In this mode, the cells exude gelatinous exopolymeric substances that are mostly polysaccharides, proteins and DNA. Progressively, a structured biofilm matrix or a gel, consisting of cells and exuded elements, is formed in which cells are protected from physical trauma, immune clearance mechanisms, desiccation and antimicrobial agents. In nature, most biofilms are complex and are formed by many different microbes in a multi-species community. *Campylobacter jejuni*, a ubiquitous pathogen, is a known to be associated with complex gel and surface biofilms and is an ideal model for studies of naturally occurring multi-species biofilms. *C. jejuni* is recognised as one of the most important human food-borne pathogens to date and is the leading cause of acute human bacterial gastroenteritis worldwide. *C. jejuni* survives in foods, animal products and aquatic environments. We hypothesise that it does so by integration into complex microbial communities and biofilms. The formation of complex multi-species communities involving *C. jejuni*, and their role in transmission of infection, are poorly understood. We aim to use a comprehensive, biologically valid multidisciplinary approach to quantify the composition, both microbial and host-related, of naturally occurring complex communities involving *C. jejuni*. We will use transcriptome and mass spectroscopy analysis with wild type and mutated *C. jejuni* strains contained in chicken caeca and in laboratory created biofilms. This will allow us to understand how this pathogen integrates into complex microbial communities and biofilms, how to break the chain of transmission of *C. jejuni* from animals and animal food sources, how to reduce

incidence of campylobacteriosis in human population, and ultimately, how to develop novel strategies for biological intervention in disease transmission to humans.

32. When Glycobiology meets Nanotechnology

Assoc Prof Joe Tiralongo

Materials Science, Nanotechnology, Interaction Biology, Glycobiology, Biochemistry

Micro-technologies in the form of Micro-Electro-Mechanical Systems (MEMS) and micro-plasmonics platforms offer the potential for high-resolution, high-throughput label-free sensing of biological and chemical analytes. Silicon carbide (SiC) is an ideal material for augmenting both MEMS and plasmonics routes, however such inorganic surfaces need to appropriately and efficiently functionalised to allow subsequent immobilization of functional biomolecules. To this end we trialled various organosilane-based self-assembled monolayers for the covalent functionalization of 2-dimensional SiC films, and have now developed an affordable, facile one-step method. Using high-throughput glycan arrays as our model system a novel platform that has the potential to combine established array technology with the label-free capabilities of MEMS or plasmonic systems is one step closer. Using a similar functionalisation route, we have extended the use of organosilanes to biofunctionalise the surface of 3-dimensional nanoparticles, specifically carbon dots. Carbon dots are cheap, biocompatible, chemically stable, heavy-metal free quantum dots, of low toxicity that offer an alternative approach for bio-imaging and -sensing applications. Again, employing glycans as our model system, we are now using our biofunctionalization approach to generate glycan-coated carbon dots that we are using to explore complex glyco-interactions.

Techniques: Organic chemistry; surface plasmon resonance, microarray technology, flow cytometry, microscopy

33. Exploring the immuno-modulatory effect of fungal β-glucans

Assoc Prof Joe Tiralongo & Dr Darren Grice

Separation Chemistry, Immunology, Glycobiology

Mushrooms are increasingly attracting attention for their immuno-modulatory activities, which are primarily due to β-glucans. β-Glucans comprise a group of glucose (Glc) polysaccharides that are chemically diverse, with a common β-glucan being cellulose (β-(1,4)-linked Glc. It is non-cellulosic β-glucans, mainly β-(1,3)-linked Glc that have been shown to be potent immunological stimulators in humans, and some are now used clinically in China and Japan, as well as being commercially available in Australia.

Due to the complexity of β-glucan chemistry and structure a detailed understanding of the mechanism of action, specifically the structural components that dictate specific immunological responses, are yet to be fully resolved. In collaboration with Integria Healthcare, the overall objective of the project is to explore the immuno-modulatory effects of mushroom β-glucans, specifically the project aims to structurally characterise commercially available mushroom polysaccharides rich in β-glucans and correlate this with their associated immuno-modulatory effects The outcomes from this project will lead to a clearer understanding of the properties of β-glucans associated with commercially available mushroom polysaccharides that induce specific immuno-modulatory effects.

Techniques: Carbohydrate chemistry, ELISA assays, separation chemistry, polysaccharide structure determination

34. Novel Agents for Antifungal Drug Development

Associate Professor Thomas Haselhorst and Associate Professor Joe Tiralongo

Structural Biology, Computational Chemistry, Microbiology, Cell Culture, Molecular Biology, NMR spectroscopy

Project details: The opportunistic human pathogenic fungus *Aspergillus fumigatus* causes severe systemic infections including Invasive Aspergillosis (IA), a major cause of life-threatening fungal infections in immuno-compromised patients. An over-whelming number of reports appeared in 2020 demonstrating that COVID-19-associated pulmonary Aspergillosis (CAPA) is one of the leading factors affecting morbidity in critically ill COVID-19 patients [2] with some reports even classifying Aspergillosis as a significantly under-recognized 'Superinfection' in COVID-19.

Drug resistance among fungal pathogens is continuing to develop into an increasingly serious threat to public health and health-care systems worldwide. This PhD projects entails the development of novel antifungal therapies that are urgently needed using our established and unique combined in-silico/SPR drug discovery pipeline evaluating a number of new protein targets.

Techniques: Computational Chemistry including visualisation and molecular docking, *in-silico* screening, microbiology, protein expression, drug discovery and design.

35. Influence of the length of oligosaccharide on the biological activity of the lipooligosaccharide from *Moraxella catarrhalis*

Dr Darren Grice & Dr Jennifer Wilson (School of Medical Science)

Molecular Microbiology, Medicinal Chemistry

Almost all children suffer from middle ear infections (otitis media) at some point in their lives. In Australia, otitis media is particularly prevalent in Aboriginal children, and occurs very early in life. The bacteria most commonly associated with otitis media are *Streptococcus pneumoniae*, *Moraxella catarrhalis* (Mcat), and non-typeable *Haemophilus influenzae* (NTHi).

Gram negative bacteria such as Mcat have a layer at the outer surface that is predominantly made up of carbohydrates (oligosaccharide) attached via a membrane-embedded lipid (lipid A). The carbohydrate-lipidA molecule is known as lipo-oligosaccharide (LOS). The immune system of an infected person recognises and responds to the LOS of many bacteria, (including *M. catarrhalis*).

Previously we have structurally characterised Mcat oligosaccharides from mutant *M. catarrhalis* bacteria. Now we want to assess the biological activity of the lipooligosaccharide produced by the mutant bacteria and investigate whether there is a correlation between the structure of the oligosaccharide and its toxicity. Methods to evaluate the biological activity of the mutant LOS will include growth rate, toxicity and susceptibility to hydrophobic reagents as compared to wildtype.

36. Analysis of lipopolysaccharide structures from *Moraxella bovis*

Dr Darren Grice, Dr Ian Peak & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Molecular Microbiology

The aim of this project is to isolate carbohydrate components from the bacteria *Moraxella bovis* and determine the structures of these carbohydrate molecules. *Moraxella bovis* causes infectious bovine keratoconjunctivitis (IBK) in cattle, causing significant loss of weight and permanent blindness. Vaccine therapies have been unsuccessful in treating these infectious agents. Obtaining structural carbohydrate information will enable future studies to determine the role of these carbohydrates in disease and develop potential new vaccine strategies.

The project will require the development of knowledge and skills in the areas of cell culture, chemical and biochemical extraction and manipulation strategies, nuclear magnetic resonance (NMR) and mass spectrometry (MS) of isolated carbohydrate materials.

Identification of a capsular polysaccharide from *Moraxella bovis*. J.C. Wilson, P.G. Hitchen, M. Frank, I.R. Peak, P.M. Collins, H.R. Morris, A. Dell and I.D. Grice. *Carbohydr. Res*. 2005, 340(4), 765-769.

37. Analysis of surface carbohydrate structures from Gram-negative *Moraxellaceae* bacteria

Dr Darren Grice, Dr Ian Peak & Dr Jennifer Wilson (School of Medical Science)

Medicinal Chemistry, Molecular Microbiology

The aim of this project is to isolate surface carbohydrate components from bacteria in the *Moraxellacae* family, then determine the structures and biological significance of these carbohydrate molecules. Many bacteria in this family are commensals of the human upper respiratory tract and are important in protecting against disease. Obtaining structural carbohydrate information will enable us to determine the role of these carbohydrates, and potentially developing new strategies to promote upper-respiratory tract health.

The project will require the development of knowledge and skills in the areas of cell culture, chemical and biochemical extraction and manipulation strategies, nuclear magnetic resonance (NMR) and mass spectrometry (MS) of isolated carbohydrate materials.

1. De Castro, Grice, Daal, Peak, Molinaro, Wilson. *Carb. Res*. (2014) 388:81-86.
2. Wilson, Hitchen, Frank, Peak, Collins, Morris, Dell and Grice. *Carb. Res*. (2005) 340:4, 765-769.

38. Development of novel phthalic acid diesters as potential human parainfluenza virus therapeutics

Dr Darren Grice, Dr Andrew Pearson (School of Medical Science) & Assoc Prof Evelin Tiralongo (School of Pharmacy)

Medicinal Chemistry

Infectious diseases, such as Dengue (DENV), Chikungunya (CHIKV) and Human parainfluenza virus (hPiV3), are a major cause of avoidable mortality and morbidity, particularly for children in developing countries. However, due to the rapidly mutating nature of viruses, resistance to conventional drug therapies occurs. The proposed research project is ongoing and follows on from our previous work where we isolated a chemical compound from a Bangladeshi mangrove fern *Acrostichum aureum* that showed potent activity particularly against hPiV3, but also moderate activity against DENV2 and CHIKV. This project aims to synthesise the isolated compound and also synthesise analogues where chemical groups have been varied, then test these compounds for potency as potential anti-viral agents against hPiV3, DENV2 and CHIKV. It is hoped that this work will lead to the identification of a new agent with potent antiviral.

Uddin, Bettadapura, Guilon, Grice, Mahalingam, Tiralongo. *J. Antivir. Antiretrovir*. (2013) 5:6.

39. Degradation of cancer-associated proteins using proteolysis targeted chimeras (PROTACs)

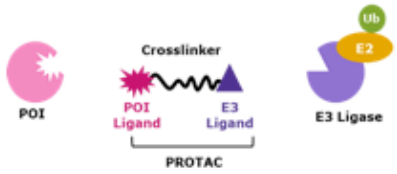
Dr Darren Grice & Prof Mark von Itzstein

Medicinal Chemistry

From previous studies[1,2], it is clear that the use of Proteolysis-targeting chimera (PROTAC) molecules can result in the effective degradation of target-proteins. PROTAC techniques involve the exploitation of normal protein degradation essential for cellular maintenance and hijacking the system to specifically target proteins of interest (POI) for degradation.

To achieve an effective PROTAC design the molecule must provide high affinity binding to both the protein of interest and a suitable ubiquitin ligase and maintain these interactions whilst not inhibiting the overall ubiquitination (or tagging for destruction) process.

Work is underway within the Institute for Glycomics to synthesise novel PROTAC molecules to achieve the successful proteolysis of a cancer-associated protein, which is known to be intimately involved in cancer progression. This research will be further progressed in this ongoing project.



Techniques: The project will involve synthetic organic/carbohydrate chemistry, along with NMR spectroscopy, mass spectrometry and other associated techniques for structural characterisation of the synthesised PROTACs followed by assessment of biological activity.

1. Winter, *et al*. *Science*. (2015) Jun 19;348(6241):1376–81.
2. Gu, Cui, Chen, Xiong, Zhao. (2018). *Bioessays* Apr 40(4), e1700247.

40. Design and Synthesis of Synthetic Receptors for Cell-Surface Carbohydrates

Assoc Prof Todd A Houston, Dr Milton J Kiefel

Medicinal Chemistry

Boronic acids form covalent, but reversible, interactions with polyols such as sugars in aqueous solution. We have developed boronate receptors selective for cell-surface carbohydrates from both mammalian and bacterial cells (specifically sialic acid and KDO). Our receptors display a unique and divergent fluorescent response that can be exploited for selective detection in biological media. Ultimately, these receptors will be developed into boronolectins that target specific cell types and may be used in drug targeting. These synthetic receptors can identify targets complementary to those of nature's antibodies and lectins that normally survey cell surfaces. In addition, we have identified anti-bacterial activity in some of these compounds implying there is potential to develop these into medicinal agents.

Techniques: Synthetic chemistry; fluorescence detection

41. Development of Novel Antibacterial Treatments

Assoc Prof Todd A Houston, Dr Darren Grice, Assoc Prof Nick West (U.Queensland)

Medicinal Chemistry

Deadly infectious bacteria such as *Mycobacterium tuberculosis* and MRSA are able to survive within macrophages in a human host and this makes treatment particularly challenging. Incomplete or ineffective antibiotic treatment leads to development of drug resistant bacteria, a problem of growing global concern. We are currently developing antibacterial compounds that can be formulated into nanoparticles with drug targeting epitopes to improve uptake and drug delivery into macrophages. This allows for improved bactericidal activity and diminishes the opportunity for the development of drug resistance.

Techniques: Synthetic carbohydrate chemistry; liposome formulation; bacterial cell growth assays.

42. Improving Affinity of Glycosidase Inhibitors for Drug Development

Assoc Prof Todd A Houston, Dr Michela Simone (U.Newcastle)

Medicinal Chemistry

Exo-glycosidases are an important family of enzymes involved in a number of vital biological process and pathologies. Glycosidase inhibitors have a wide range of medicinally-relevant activities including anti-cancer, anti-viral and anti-diabetic properties. Unfortunately, these compounds often have a relatively low affinity for their target enzyme. We have identified an important structural motif that improves binding affinity of a number of glycosidase inhibitors, and this can be modified to improve drug targeting to cell surfaces where a number of enzyme targets are displayed. In this way, the inhibitor can be placed in close proximity to the substrates the glycosidase enzyme

Techniques: Synthetic carbohydrate chemistry; molecular modelling; enzyme assays

43. Host immune responses to bacterial signaling molecules

Dr Ian Peak, Dr Christopher J Day & Prof Michael Jennings
Molecular Microbiology, Molecular Biology

The immune system responds to infections after it has recognised infectious agents. All bacteria secrete products, and some of these have profound effects on the host immune system, either acting as recognition molecules for immune attack, or by modifying the immune response to assist the microbe to survive. We are investigating how secreted molecules from pathogenic bacteria are detected. We are characterising host receptors for these secreted products, which will help understand diseases such as cholera, legionnaire's disease, as well as infections caused by *Pseudomonas* in burned, and cystic fibrosis patients.

Techniques: Molecular genetics techniques, immunofluorescence microscopy, protein expression and purification, FACS analysis, cell culture and *in vitro* infections, *in vivo* infections using mouse models of disease, analyzing immune markers such as cytokine and chemokine responses of the host cell, small molecule purification and analysis by Mass Spectrometry and other techniques

44. Improving delivery of antimicrobials across biological barriers

Dr Ian Peak, Dr Matt Zunk (School of Medical Science & Pharmacy), A/ Prof Gary Grant (School of Medical Science & Pharmacy), Prof Vicky Avery (Griffith Institute for Drug Discovery)
Cell Biology, Microbiology, Medicinal Chemistry

Antimicrobial resistance is an increasingly urgent challenge to human health, with a growing number of multidrug resistant (MDR) and extensively resistant (XDR) species. Several antimicrobials considered "last resort" can only be delivered intravenously, as they do not easily cross from the gut into the blood stream if taken orally. However, long term use of intravenous antimicrobials causes higher rates of complications, resulting in more deaths, and contributes to emergence of further resistance. Our approach for improved delivery is to temporarily improve the permeability of the gut, using "permeation enhancer" compounds: by making the gut temporarily more permeable, the oral delivery of "last resort" antimicrobials can be improved.

This multidisciplinary project includes leaders from across Griffith. You can develop skills in one or more of the following: chemical synthesis of the novel permeation enhancer molecules: cell biology and analysis of mucosal cell permeability: analyse delivery & antimicrobial effect on resistant bacteria; high throughput image analysis. In the future, we will collaborate with other Glycomics investigators who are developing novel antimicrobials, and we will investigate using the permeation enhancers to target other diseases such as neurological conditions, and cancers.

Techniques: Cell biology; Synthetic chemistry; Hi-content image analysis microscopy.

45. Glycan-glycan interactions in host-pathogen adherence

Dr Christopher J Day & Prof Michael Jennings

Microbiology; cell assays; array technology; affinity and kinetics measurements

Recently we showed that pathogenic bacteria interact with host cell through direct contact of the carbohydrates expressed by both organisms (Day et al 2015 PNAS 112:E7266). Previously only three glycan-glycan interactions had been described (sea sponges, Lewis antigens and gangliosides) while our study extended this to over 60 new interactions. The role of glycan-glycan interactions in pathobiology and more widely throughout nature has not been fully elucidated. This project will investigate a wide range of bacterial polysaccharides for glycan binding and try to determine the minimal and sufficient structure required for these novel interactions. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC). Cell assays for bacterial adherence will also be performed.

46. Glycan-glycan interactions: Interactions in eukaryotic biology

Dr Christopher J Day & Prof Michael Jennings

Cell assays; array technology; affinity and kinetics measurements

Recently we showed that pathogenic bacteria interact with host cell through direct contact of the carbohydrates expressed by both organisms (Day et al 2015 PNAS 112:E7266). Previously only three glycan-glycan interactions had been described (sea sponges, Lewis antigens and gangliosides) while our study extended this to over 60 new interactions. The role of glycan-glycan interactions in pathobiology and more widely throughout nature has not been fully elucidated. In our studies we noted that bacteria that mimic human glycan structures were still capable of binding human glycan structures indicating that direct interactions between eukaryotic glycans are likely to occur. This project will investigate a range of eukaryotic glycans for their ability to recognise other eukaryotic glycans. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC). Cell assays to observe the binding of labelled glycans to appropriately glycosylated cells.

47. Identification of novel carbohydrate binding proteins

Dr Jessica Poole, Dr Christopher J Day & Prof Michael Jennings

Array technology; affinity and kinetics measurements

Carbohydrate binding proteins (also known as lectins) are a broad range of proteins with a wide specificity for carbohydrate structures. Recently we have found that a large number of bacterial and eukaryotic proteins have the ability to bind to glycans that had not previously been appreciated. This project will investigate a range of proteins from bacterial and eukaryotic sources for their ability to interact with glycans. This study will utilise the glycomics arrays that we produce within the Institute for Glycomics as well as studies of affinity and kinetics using surface plamon resonance (GE Biacore T100) and micro isothermal calorimetry (TA Instruments nanoITC).

48. Investigating epigenetic gene regulation by phase-variable methyltransferases at the promoter level

Dr John Atack and Prof Michael Jennings

Many host-adapted bacterial pathogens contain phase variable methyltransferases, which control expression of multiple genes, and known as phasevarions (phase variable regulons).

Our studies will investigate the specificity, and the mode of gene regulation through differential methylation by these phase-variable methyltransferases. We will clone and over-express newly identified methyltransferases to determine their recognition sequences. We have identified a number of genes in both human and animal pathogens that are differentially expressed in several phasevarions: we will investigate how methylation alters regulation of these genes. We will investigate if these genes are regulated directly or indirectly, and determine the effect of removing any recognition sequences from the promoters of these genes. This project will use protein over-expression and purification methods to allow us to study these methyltransferases *in vitro*. Surface plasmon resonance will be used to conduct kinetic measurements, and gel-shift assays (EMSA) will be used to study binding affinity and ability.

Reporter constructs will be made to observe the effect of methyltransferase phase-variation on the level of expression from individual promoters.

49. *Generation and improvement of an NTHi vaccine*

Dr John Atack, Prof Michael Jennings

Non-typeable *Haemophilus influenzae* is a major human adapted pathogen and causes a number of acute and chronic diseases of the human respiratory tract, including middle ear disease, otitis media (OM) in children, exacerbations in chronic obstructive pulmonary disease (COPD) in the elderly, and pneumoniae. Invasive disease (meningitis and septicaemia) caused by NTHi is increasing annually and is a particular problem in infants under 1 year of age, where the mortality is close to 20%. Antibiotic resistance is increasing each year, resulting in NTHi being on the World Health Organisations list of priority pathogens. There is no currently licensed vaccine available for NTHi. Vaccine design is a problem for NTHi as individual strains show high genetic diversity, and many antigenic proteins are phase-variable – their expression is randomly and reversibly switched on or off. If a vaccine target is able to randomly turn off, the vaccine would lose effectiveness.

This project will: 1) determine the best possible combination of conserved protein antigens to include in a universal NTHi vaccine from both current and putative vaccine candidates; 2) study the role and regulation of a number of uncharacterised NTHi proteins that show high sequence and strain conservation; and 3) determine if know proteins that have been discounted from use in vaccines as they are phase-variable can be used in vaccines as their expression is critical for certain disease stages or colonisation of particular host niches.

50. *Defining the glycointeractome of the major drug resistant pathogen Acinetobacter baumannii*

Dr John Atack

Acinetobacter baumannii is classified by the WHO as a critical top priority pathogen and is resistant to almost all current antibiotics. This is particularly problematic as there are very few new antibiotics currently in development. The rate at which *A. baumannii* acquires resistance to antibiotics means *A. baumannii* infections may soon become impossible to treat. *A. baumannii* is a particular problem in hospitals and aged care units due to its ability to survive harsh environments and cleaning procedures. It is crucial to develop novel treatment methods and therapeutics due to the lack of current new antibiotics in development. Although much is known about *A. baumannii* virulence factors, little is known about the exact host factors *A. baumannii* interacts with during colonisation and disease. Many human adapted pathogens use host glycans (sugars) as to bind to host surfaces and cells. This project will determine which glycans *A. baumannii* interacts with in the human host, i.e., we will define the *A. baumannii* glycointeractome, by leveraging the innovation of glycan array technology and biophysical approaches to precisely determine the glycans *A. baumannii* interacts with. We will utilize multiple *in vitro* and *in vivo* models to determine the ability of these glycans and novel compounds to serve as blocking agents for the treatment of MDR *A. baumannii* infections. This will aid in the development of new treatment strategies and lay the groundwork to develop novel agents to serve as an alternative to, or act in synergy with, existing antibiotics.

51. A mutagenesis screen to identify key components of post-translational modification pathways bacterial pathogens

Dr Freda Jen & Prof Michael Jennings

Molecular Biology, Molecular Microbiology

Many pathogenic bacteria modify proteins after translation. Some of these modification are on proteins on the surface of the bacteria that are key in understanding host:pathogen interactions and in developing vaccines. Recent advances in *Neisseria meningitidis* have identified post-translation modification of virulence factors with glycans and phosphorylcholine. Some key post-translation modification pathway components have also been identified, but the

picture is incomplete. The aim of this project will be to conduct transposon mutagenesis and screen for loss of key post-translation modifications. In this way novel post-translation modification pathway components will be identified and investigated.

52. Determining the differences between bacterial and archaeal type signal peptidase I substrate recognition

Dr Yaramah Zalucki, Dr Christopher Day, Assoc Prof Thomas Haselhorst & Prof Michael Jennings

Structural biology, enzyme assays, antibiotic resistance, microbiology

Signal peptidase I is an essential enzyme in bacteria that cleaves signal peptides as the final step of protein export to the periplasm. We have discovered a novel secreted protein from *Bacillus subtilils*, TasA, whose signal peptide can bind signal peptidase I of *E. coli* (LepB), but is very inefficiently cleaved. In *B. subtilis*, the TasA signal peptide is removed a dedicated signal peptidase I called SipW, whose protein sequence is more similar to archaeal signal peptidase I. However, it is unknown why the TasA signal peptide is inefficiently cleaved by LepB, and requires an archaeal, not bacterial signal peptidase for its efficient removal. To answer this question, the student will purify SipW, and develop an enzyme assay to measure its cleavage of signal peptides. Subsequently both SipW and LepB will be compared in enzyme assays using the same substrates (based off the TasA signal peptide sequence). The project will develop insights into how archaeal and bacterial signal peptidases differ in their ability to process signal peptides.

The techniques involved in this study include enzyme assays, protein purification and protein analysis (coomassie gels and Western blots), cloning of genes (PCR, DNA manipulation) and other general molecular biology techniques.

53. The role of signal sequence non-optimal codons in protein structure

Dr Yaramah Zalucki & Prof Michael Jennings

Microbiology, protein purification and protein structure analysis

Codon usage is biased at the 5' end of secretory genes, with the highest percentage of non-optimal codons found compared to any region of the genome. The exact role for the observed bias is unknown. We have strong evidence that changing signal sequence non-optimal codons to the most optimal codon in the synonymous codon family results in structural changes in the mature region of the protein. However, detailed analysis on the exact nature of the structural change has not been done. In this project, we will alter the codon usage in the signal sequence of two small proteins (>20 kDa), purify them and determine any structural differences by NMR and other techniques. Determining any structural change from altering signal sequence codon usage will be a novel find, and important in the field structural biology and how proteins are targeted for protein export. The techniques used in this project will involve cloning, PCR, protein purification, NMR analysis, protein analysis (Western and coomassie staining techniques, DNA sequencing and phenotypic analysis of any mutants made.

54. Role of promoter mutations in the *mtrCDE* efflux pump in antibiotic resistance in *N. gonorrhoeae*

Dr Yaramah Zalucki & Prof Michael Jennings

Microbiology, molecular genetics and antibiotic resistance

Antibiotic resistance is in *N. gonorrhoeae* is a major public health concern. One of the major determinants of resistance is the MtrCDE efflux pump, which exports compounds from the inner membrane to the extracellular milieu. Expression of the efflux pump is controlled by a repressor, MtrR, and a conditional activator, MtrA. We have identified a number of novel promoter mutations in the *mtrCDE*, whose role in increasing expression of the efflux pump has not been characterised. In this project, we will place these mutations individually, and in conjunction with known promoter mutations, to measure their effect on antibiotic resistance in *N. gonorrhoeae*. These mutations will also be placed in the context of a promoter-less lacZ fusion, to measure their effect on the strength of the promoter. We will also look at how these mutations influence the binding of the two known regulators of the efflux pump, MtrA and MtrR to the promoter region. The techniques used in the project will involve cloning, PCR, MIC assays, RNA extraction and other general microbiology and molecular biology techniques.

55. Investigation of Neu5Gc tumour antigens in cancer

Dr Lucy Shewell, Dr Christopher Day & Prof Michael Jennings

Glycobiology, Biochemistry, Biophysics, Cancer Biology

Approximately half or more of all human proteins carry a carbohydrate moiety through the process of glycosylation and it is well established that one of the universal features of cancer cells is aberrant glycosylation. The changes in glycosylation that occur in cancer cells include loss of expression or excessive expression of certain glycans (carbohydrates attached to proteins or lipids), increased expression of incomplete or truncated glycans, and the appearance of novel glycans. Glycoproteins, therefore, make ideal cancer biomarkers because these molecules are secreted or shed into the circulation from tissues or blood cells allowing them to be detected in the serum. Glycans terminating with the sialic acid Neu5Gc are not expressed at significant levels on healthy human tissues, because humans express an inactive cytidine monophosphate *N*-acetylneuraminic acid (Neu5Ac) hydroxylase (CMAH) enzyme, and thus cannot synthesize Neu5Gc. Nevertheless, Neu5Gc-containing glycans are found in human tumour tissues, tumour cells and tumour secretions, and have been proposed as a tumour biomarker.

The Shiga toxigenic *Escherichia coli* (STEC) Subtilase cytotoxin (SubAB) recognizes α2-3 linked Neu5Gc via its pentameric B-subunit SubB. We purpose-engineered the SubB protein to increase specificity and selectivity for Neu5Gc containing glycans and have demonstrated that this mutant protein, termed SubB2M, recognizes Neu5Gc glycans exclusively and is able to detect Neu5Gc-enriched serum glycoproteins. We showed that SubB2M can detect elevated levels of Neu5Gc in serum samples from patients at all stages of ovarian cancer using only very small volumes of serum (~1µl) via surface plasmon resonance (SPR). SPR is a biophysical technique for measuring the binding of molecules in real-time without the use of labels. This project will investigate whether serum Neu5Gc levels are elevated in patients with other types of cancers compared to normal controls using a SubB2M-SPR assay. This project will also attempt to discover and characterize Neu5Gc-containing cancer biomarkers.

Techniques: SPR, affinity purification, protein gel electrophoresis, western blotting

56. Investigation of the glycan binding sites of cholesterol-dependent cytolysins (CDCs)

Dr Lucy Shewell, Dr Christopher Day & Prof Michael Jennings

Molecular Microbiology, Glycobiology

The cholesterol-dependent cytolysins (CDCs) are a family of toxins produced by a number of Gram-positive human pathogens including *Streptococcus*, *Clostridium*, *Listeria*, *Bacillus* and *Gardnerella*. These toxins form pores in cholesterol-containing membranes, hence it was thought that cholesterol was the cellular receptor. We have found that the CDCs bind with high-affinity to glycan targets and that these glycans serve as cellular receptors. This project aims to further investigate the glycan binding of several of the CDCs by using molecular modeling to identify key residues involved in binding to the glycan targets. Site-directed mutants of these residues will be generated and analysed using a range of techniques, including surface plasmon resonance (SPR) and cell-based assays, to confirm their role in glycan binding. Identifying key residues of the CDCs involved in glycan binding will provide insight into the function and tropism of these toxins and may assist in the development of inhibitors of these toxins.

Techniques: molecular biology, SPR, affinity and kinetics measurements; cell assays

57. Pre-clinical development of a whole parasite liposomal vaccine approach for a Babesia vaccine

Dr Danielle Stanisic & Prof Michael Good

Parasitology, Immunology, Vaccinology

Babesiosis is a tick-borne infectious disease, caused by parasites of the genus Babesia. Human babesiosis is typically asymptomatic, or results in mild symptoms that resolve within a few days in healthy individuals. However, Babesia infection in the very young, the elderly, splenectomized, and immunocompromised individuals can result in acute anemia, multi-organ failure, or death. There is currently no human vaccine available, with prevention strategies focused on controlling the tick vector. Babesia parasites also infect cattle, with bovine babesiosis (or cattle tick fever) having a major economic impact on the livestock industry in South America, Africa, Asia and Australia. The currently used live attenuated cattle vaccine has a number of major drawbacks.

We have previously shown that a whole blood-stage parasite liposomal malaria vaccine is able to induce protective immunity in rodent models of the related Apicomplexan parasite, Plasmodium. This vaccine approach has been shown to induce a broad protective immunity. We have recently applied this same approach to the development of a Babesia vaccine, using a rodent model of *B. microti*. Further work is required to optimise the vaccine formulation to both maximise protective efficacy and to enable the development of a product that is compatible with administration to humans and cattle. In this project, different vaccine candidates will be generated containing the whole Babesia parasite. For some vaccine candidates, recombinant proteins/peptides derived from the parasite will also be included. Pre-clinical development of these vaccine candidates will include characterisation, optimisation and evaluation of the vaccine formulations.

Techniques: Parasitology, Vaccinology, Real-Time PCR, Cellular and Humoral Immunology (including Cell Culture, ELISA, Flow Cytometry and Cytokine Analyses).

58. Development and evaluation of a controlled malaria infection immunization approach for the development of a malaria vaccine.

Dr Danielle Stanisic & Prof Michael Good

Parasitology, Immunology, Vaccinology, Drug Discovery

Malaria is a parasitic disease prevalent in many developing countries, with transmission reported in 90 countries. It is associated with extensive morbidity and mortality, mainly in pregnant women and young children. Currently available control strategies are becoming increasingly less effective; therefore the development of an effective vaccine is considered to be of critical importance. Many researchers have focused on single parasite-derived proteins in their quest to develop a sub-unit vaccine against malaria. However, many of these proteins are highly variable, and are not useful in eliciting responses that can protect against multiple strains of the parasite. A vaccine approach that uses the whole malaria parasite however, would contain multiple parasite antigens including antigens that are not altered by the parasite i.e. are therefore conserved between different parasite strains.

Using rodent models of malaria, it has previously been shown that different whole parasite asexual blood-stage vaccine approaches are able to induce species and strain-transcending protective immune responses. One such approach is controlled infection immunization (CII). This involves administering a malaria infection at the same time as anti-malarial treatment is commenced. So far, these immunization regimens have required either multiple days of anti-malarial treatment (which is not viable for a vaccine strategy) or a single large dose of drug that is not currently clinically indicated in humans and may not be tolerated. This may be overcome by using alternative anti-malarial drugs in the context of CII or by the use of slow release drug formulations

This project will involve further pre-clinical development and evaluation of the CII approach. Using rodent models of malaria, pre-clinical development will initially involve characterising and optimising different anti-malarial drug formulations in the context of CII. Their ability to control parasite growth will be examined. If required, slow release drug formulations may be developed. The optimal drug formulations and parasite combinations will be evaluated for their ability to induce protection against subsequent challenge infection. Immunological and functional assays will be used to assess immunogenicity and to examine the immune mechanisms of protection. Results from this project will inform the transition of this vaccine approach into clinical studies.

Techniques: Parasitology, Vaccinology, Real-Time PCR, Cellular and Humoral Immunology (including Cell Culture, ELISA, Flow Cytometry and Cytokine Analyses).

59. Development and pre-clinical evaluation of a transmission-blocking liposomal malaria vaccine

Dr Danielle Stanisic & Prof Michael Good
Parasitology, Immunology, Vaccinology

Malaria is a global public health problem with transmission still being reported in over 90 countries. It is an infectious disease caused by Plasmodium parasites which are transmitted by female Anopheline mosquitoes. Current control methods are becoming increasingly less effective, therefore the development of an effective vaccine is considered to be of critical importance. The majority of malaria vaccine candidates are based on single malaria proteins, but many of these are highly variable and are not useful in inducing immune responses that will protect against multiple strains of the malaria parasite.

An alternate approach currently being developed, involves using the whole malaria parasite – such a vaccine contains multiple parasite proteins including those that are conserved between different parasite strains.

This study will involve the pre-clinical investigation of a *Plasmodium falciparum* transmission blocking liposomal vaccine. This vaccine type does not prevent an individual from being infected like an asexual blood-stage vaccine aims to do, but rather stops an infected individual from transmitting malaria to other individuals. This is because it targets the parasite life-cycle stage that is infective to mosquitoes. It is thus seen as a community-based vaccine approach.

In this project, different vaccine candidates will be generated containing the *P. falciparum* gametocyte-stage parasite; this is the life-cycle stage that is found in the blood of malaria-infected individuals and is infective to mosquitoes.

For some vaccine candidates, recombinant proteins/peptides derived from the gamete-stage of the parasite, which is the stage of the parasite within the mosquito, will also be included. Pre-clinical development of these vaccine candidates will include characterisation and optimisation of the vaccine formulations. Immunological and functional assays will also be undertaken to characterise the immunogenicity and transmission-blocking activity of the single and multi-component vaccine candidates ie whether the induced immune response impacts on parasite development and/or survival in the mosquito host.

Techniques: Parasitology, Vaccinology, Cellular and Humoral Immunology (including Cell Culture, ELISA, Flow Cytometry and Cytokine Analyses).

60. Developing novel peptide-based vaccines for infectious diseases including COVID-19 and *rheumatic heart disease*

Dr Victoria Ozberk, Prof Michael Good and Dr Manisha Pandey
Molecular Immunology and Vaccinology (MIV)

Infectious diseases account for over 17 million deaths per year. Globally, as of August 2021, there have been over 205 million confirmed cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and over 5 million deaths. SARS-CoV-2 is the causal agent of Coronavirus Disease 2019 (COVID-19). A protein called the Spike (S) protein is attached to the viral surface. The interaction between the S protein and a receptor present on human lung cells (angiotensin converting enzyme 2 receptor; ACE2 receptor) initiates viral entry into human cells. There are a total of 8 SARS-CoV-2 vaccines approved for full human use. These vaccines have high efficacy rates against current SARS-CoV-2 strains; however, their efficacy may be compromised against mutant strains.

Another significant pathogen, *Streptococcus pyogenes* is a Gram-positive bacterium that causes multiple diseases. *S. pyogenes* primarily infects the upper respiratory tract (URT) and the skin. If left untreated, invasive (necrotising fasciitis and streptococcal toxic shock syndrome) and post streptococcal sequalae of diseases (rheumatic fever and rheumatic heart disease) can follow. *S. pyogenes* infections and their sequelae are responsible for more than 500,000 deaths each year. Despite the burden of disease, a vaccine is not yet available. Ideally, a vaccine against SARS-CoV-2 and *S. pyogenes* should be immunogenic and protective at the primary sites of infection and against all strains of the infectious agent.

Our laboratory focuses on two main elements of vaccine design and development. These include (i) peptide (immunostimulatory) antigens and (ii) vaccine delivery systems. We have identified highly efficacious peptide antigens for use in vaccines against *S. pyogenes*. These peptide antigens, conjugated to a carrier molecule such as diphtheria toxoid (DT) have been tested with various vaccine delivery/adjuvant systems such as Aluminium hydroxide for intramuscular delivery or liposomal platforms for mucosal delivery. Two vaccine combinations for *S. pyogenes* are now being prepared to enter a Phase I Clinical trial. In parallel, using murine models, immunological and molecular biology investigations will continue to unravel mechanism of vaccine mediated strain transcending immunity. Using our expertise in designing peptide vaccines, we are working towards the rapid development of a vaccine against SARS-CoV-2. We have identified peptide antigens from the receptor binding domain (RBD) of the Spike protein and demonstrated their potential as a vaccine candidate. We will now combine these peptide antigens with various vaccine delivery systems to develop a vaccine that is protective at the primary site of infection (URT) and against upcoming

viral mutants of concern. Success with this project will lead to the development of vaccines which will have real world impact.

Techniques: ELISA, flow cytometry, SDS-PAGE, MCS-conjugation, in vitro neutralisation assay, cell culture, liposome formulation, *in-vivo* techniques such as vaccination and sample collection.

61. Developing of an immunotherapy to treat invasive *Streptococcus pyogenes* infection

Dr Victoria Ozberk, Prof Michael Good and Dr Manisha Pandey
Bacteriology and Immunology

Seemingly mild streptococcal infections can rapidly escalate to serious invasive infections with a high mortality rate. The overall incidence for invasive *Streptococcus pyogenes* disease (ISD) was reported to vary between 2-4 per 100,000 people in developed countries, although in some developed countries, a marked rise in the incidence of ISD has been reported. In developing countries, very high rates are reported amongst the young and the elderly (up to 75 per 100,000) (Steer et al, 2012). In approximately 20% of cases, ISD is accompanied by a streptococcal toxic shock syndrome (STSS) with multi-organ failure and case fatality rates approaching 50% even in the best-equipped facilities. It can occur after any streptococcal infection but most commonly occurs after infections of the skin and is usually associated with necrotising fasciitis, myositis or deep bruising.

Streptococcal 'superantigens' (SAgs) are thought to play the key role in the pathogenesis of STSS (Proft and Fraser, 2016). However, we have recently demonstrated that the M protein also plays a critical role in the pathogenesis of STSS (M. Pandey et al, 2019). We have developed a model for STSS using HLA-humanized mice and showed that these mice became gravely ill when infected with a SpeC+ (a streptococcal superantigen) positive *S. pyogenes* organism that caused STSS in human patients. The project will utilise humanised mice to model STSS caused by SpeA+ organisms and will examine critical roles for both the M protein and SpeA in pathogenesis. It will further assess whether vaccination with our lead vaccine candidate (J8/p*17) can prevent disease and whether passive immunotherapy can rapidly ablate the mitogenic and inflammatory activity of SpeA+ *S. pyogenes* organisms, and clear infection. The project will further advance to test combination therapy utilising monoclonal antibody to J8/p*17 and antibiotics with a view to reduce repeated antibiotic administration and thus antibiotic resistance. Success with this project could quickly lead to novel therapies to treat STSS.

Techniques: Culturing of bacteria, mouse infection model, PCR, Enzyme Linked Immunosorbent Assays (ELISA), *in-vitro* cell culture assays, *in-vivo* techniques such as bacteria challenge, vaccination and sample collection.

62. Modelling development of naturally acquired immunity to *S. pyogenes* to decipher protective immune mechanisms to aid vaccine design

Dr Manisha Pandey, Prof Michael Good
Bacteriology, Immunology and Vaccinology

Streptococcus pyogenes is a Gram-positive bacterial pathogen of humans. It causes a broad spectrum of diseases ranging from self-limiting throat and skin infections to life-threatening streptococcal toxic shock syndrome and rheumatic heart disease. Altogether, these infections result in over 500,000 deaths annually. Naturally acquired immunity to *S. pyogenes* takes several years to develop and its slow acquisition has been attributed specific virulence factors impeding innate immunity and significant antigenic diversity of the type-specific M protein, hindering acquired immunity. There are known to be in excess of 250 different M types and limited evidence suggest that M-type-specific immunity can protect in a type-specific manner. This also poses a significant hinderance to vaccine development, as an effective vaccine will need to protect against most, if not all, existing *S. pyogenes* serotypes. In addition, it is yet to be defined if a single vaccine will protect against both skin and mucosal infections which are the primary infection sites.

To understand protective immune mechanisms against *S. pyogenes* infection, this project will investigate immune responses following natural infection and/or vaccination in mice and humans. *S. pyogenes* can infect via skin or mucosa and it is not clear whether infection at one site would induce immunity to protect at another site. Therefore, understanding the mechanisms of cross-compartment immunity i.e. skin infection protecting against mucosal infection and vice-versa, is critical. These investigations will tease apart the role of specific immune cell populations contributing towards protective immunity against multiple serotypes as well as at various infection sites. Deciphering immune mechanisms involved in site-specific and cross-compartment immunity will have significant implications for vaccine designs and vaccination strategies.

Techniques: Culturing of bacteria, mouse infection models, PCR, Enzyme Linked Immunosorbent Assays (ELISA), *in-vitro* bacterial and cell culture assays, flow cytometry, *in-vivo* techniques such as bacteria challenge, vaccination and sample collection.

63. Reprogramming autoimmunity in rheumatic heart disease with regulatory T-cells

Dr Ailin Lepletier, Dr Manisha Pandey, Prof Michael Good
Immunology, Immunotherapy, Autoimmunity

Rheumatic heart disease (RHD) is an incurable chronic disease associated with an autoimmune mechanism unleashed by group A streptococcus (GAS) infection and not yet fully understood. Global burden of disease estimates performed in 2010 calculated the number of individuals living with RHD was at least 34.2 million, with 10.1 million disability-adjusted life years lost. Together with others, we have identified autoreactive T cells and antibodies sharing common epitopes with GAS strains that are known to irreversibly damage the heart in RHD. Though the protective mechanisms of immune suppressive $CD4^+$T-lymphocytes, known as regulatory T cell (Treg) in the context of autoimmunity is well defined, a role for Treg in modulating autoreactive immune response in RHD has been largely overlooked.

We propose to provide a novel approach and pre-clinical evidence for the efficacy of Treg immunotherapy in controlling rheumatic heart disease (RHD) by enhancing the function of Treg. Towards this, we will conduct several studies to take the first deep dive into Treg contributions to RHD using a rat autoimmune valvulitis (RAV) model. We will further assess samples from patients with RHD to demonstrate proof of concept. It is essential to gain a clear understanding of Treg impact on autoimmunity associated with complicated GAS infections and developing a highly effective and specific therapy to RHD.

Techniques: Immune cell isolation, *ex vivo* expansion of Treg, cell-based *in vitro* assays, flow cytometry analysis, cytometric bead array (CBA), Enzyme Linked Immunosorbent Assays (ELISA), immunohistochemistry, multiplex immunofluorescence.

64. Vaccine development for group A streptococcus to prevent skin and mucosal infection

Prof Michael Good & Dr Manisha Pandey
Molecular Immunology and Vaccinology (MIV)

Streptococcus pyogenes infections and their sequelae are responsible for an estimated 18 million cases of serious disease with >700 million new primary cases and 500,000 deaths per year. *S. pyogenes* infection always commences at the skin or throat and infection at either site can lead to very high rates of serious streptococcal-associated pathology including rheumatic heart disease (RHD), glomerulonephritis and invasive GAS (iGAS) disease. Mortality rates from rheumatic heart disease alone exceed 350,000 per annum with Indigenous Australians reported to suffer the highest rates in the world. Also, in these communities, skin infection (pyoderma) is far more prevalent than pharyngeal disease (≥70% vs <5%) leading to the hypotheses that RHD can follow skin infection in these populations [5, 6] and that skin infections can lead to immunity in the URT.

Broad-spectrum immunity to *S. pyogenes* in humans takes years to develop and this is attributed to serotypic diversity of *S. pyogenes* strains (>250 emm-types). This has severely hindered vaccine development. We have designed a vaccine that is based on a peptide from the conserved region of the M protein. Following intramuscular immunisation this vaccine induces both skin/systemic and mucosal immunity and protects against a panel of streptococci of multiple serotypes. The vaccine does not induce mucosal IgA and we hypothesize that protection is induced by transudative IgG. The project will investigate mechanism of immunity of this vaccine and it's ability to induce enduring memory responses at both the mucosal surface and in the skin.

Techniques: Culturing of bacteria, mouse infection models, PCR, Enzyme Linked Immunosorbent Assays (ELISA), in vitro bacterial and cell culture assays, flow cytometry.

65. Defining the global coverage capacity of a lead Strep A vaccine

Dr Simone Reynolds, Prof Michael Good & Dr Manisha Pandey
Molecular Immunology and Vaccinology (MIV)

Streptococcus pyogenes is responsible for infections such as pharyngitis, sepsis, necrotizing fasciitis and streptococcal toxic shock syndrome. Our lab has been developing a vaccine to combat this disease and are preparing to take the lead vaccine candidates into clinical trials. As part of the pre-clinical research we are interested in defining the global utility or coverage of our vaccine. A recent paper in Nature Genetics (Davies et al, 2019) utilised large-scale comparative genomics to determine the coverage and variation of Strep A antigens that could be potential vaccine candidates/targets. The technological platform described in this paper offers a method to assess the theoretical coverage of a vaccine candidate.

The lead vaccine candidates currently under investigation in our lab are comprised of two group A Streptococcus antigen, one derived from the M-protein and the other from the subtilisin-like protease SpyCEP. Using the information from the Nature Genetics paper as a guide, the project will assess the actual global coverage of a lead Strep A vaccine to determine its global utility. To assess the global coverage, the project will determine whether our vaccine recognizes all variants of SpyCEP and if anti-SpyCEP antibodies neutralize variants of SpyCEP (using an IL-8 protection assay) in bacteria isolates. The project will also determine the allelic variants of the conserved C3 epitope that is present in our lead vaccine candidates (J8/p*17). The efficacy of our vaccine against Strep A harbouring these allelic variants will also be assessed.

Techniques: Bacteria culturing, DNA extraction, PCR, Enzyme Linked Immunosorbent Assays (ELISA), basic Bioinformatics.

66. Role of complement in viral-induced arthritis

Dr Lara Herrero, Dr Penny Rudd & Assoc Prof Thomas Morrison (University of Colorado, Denver, USA)

Virology, Immunology, Cellular & Molecular Biology

Alphaviruses are a group of arboviruses which cause a range of clinical manifestations including encephalitis, arthritis, arthralgia and myalgia. Viruses in this group include the arthritogenic chikungunya virus (CHIKV) and Ross River virus (RRV). CHIKV was originally confined to the African continent but since 2004 has rapidly expanded its global range and is now considered a re-emerging virus of global public health concern. RRV was originally considered endemic however in 2015 a large outbreak occurred in Queensland and Northern New South Wales. Recently, Victoria faced what will be the second Australian RRV outbreak in two years, sparking concern that the

virus has changed its pattern of circulation and causing significant public concern. New evidence suggests that RRV has potential for emergence into new areas of the world in a similar pattern to CHIKV. While the mortality rates associated with alphaviral diseases remain low at >1 per 5,000 cases, the significant economic burden due extensive morbidity remains high. Combatting mosquito-borne diseases is one of our most pressing global health challenges. We recently demonstrated that alphavirus-induced disease is largely driven by activation of the host innate inflammatory response, in particular the complement system. We now aim to define the mechanisms underlying complement-mediated pathology in alphaviral disease. We hypothesise that (i) Specific glycans expressed on alphaviral surface glycoproteins promote complement-mediated activation thereby driving the pathology of alphaviral disease, and (ii) interactions between alphaviral glycans and other (yet to be identified) host lectins also impact alphaviral disease.

Techniques: mouse model of viral arthritis, clinical disease and joint hypersensitivity measurement, ELISA, real-time PCR, viral plaque assays, flow cytometry, histopathology, western blotting, immunohistochemistry.

67. A glycomics approach towards the discovery of novel markers associated with viral inflammatory disease

Dr Penny Rudd, Dr Lara Herrero & Prof Travis Klein (QUT)

Virology, Immunology, Cell Biology

Arthropod-borne arthritogenic alphaviruses such as chikungunya virus (CHIKV) and Ross River virus (RRV) cause large epidemics of severe musculoskeletal disease and have been progressively expanding their global distribution emerging in new regions of the world. The hallmark of alphavirus disease is crippling pain and joint arthritis, which often has an extended duration leaving patients bed-ridden and incapacitated. Interactions between virus and host determine the course of infection and are likely to be critical in understanding viral pathogenesis and control. Alphaviruses display N-linked glycosylated transmembrane glycoproteins with the exact composition of the

glycosylation being dependent on the host species and cell type in which the virion was assembled. Our aim is to use glycan and lectin array technologies to discover interactions between virus and human cells to inform the rational design of therapeutics. The identification of the protein glycosylation status and glycan binding specificities of alphaviruses and human cells of the joints (chondrocytes, bone, fibroblasts, muscles) will provide an understanding of the virus-human host cell interaction and, consequently, potential novel insights into pathobiology.

Techniques: Handling of primary human bone/chondrocyte/joint cells, viral plaque assays, flow cytometry. Experiments will be undertaking in the state-of-the-art glycobioanalytical facility that has equipment to support array printing and analysis.

68. How mosquitoes transmit deadly viruses

A/Prof Lara Herrero, Dr Penny Rudd, Dr Arun Everest-Dass, external supervisors in Australia-wide state health departments

Virology, Viral-ecology, Cell Biology, Molecular Biology

The incidence of human and animal diseases caused by mosquito-borne pathogens has increased at an alarming rate globally. In nature, arboviruses are maintained in continuous transmission cycles between mosquito vectors and susceptible vertebrate hosts. The early interactions between the arbovirus and initially infected mosquitoes is likely to be a crucial step in determining whether the virus is able to successfully establish infection.

Understanding how viruses infect these cells will significantly expand our knowledge of how arboviruses are transmitted and cause disease. This project utilises glyco-analytical approaches, unique mosquito cells and an arbovirus model system to identify new markers associated with virus transmission by mosquitoes. Markers associated with transmission will be identified by establishing global glycome and lectin profiles of the cells derived from a major mosquito species. The overall objective of this proposal is to explore the glycomics of mosquito cells and its role in arbovirus mosquito infection.

Techniques: Handling of primary mosquito cells, cell culture, viral plaque assays, flow cytometry, molecular biology, glyco-analytical techniques using mass spectrometry and liquid chromatography. Experiments will be undertaking in the state-of-the-art glycobioanalytical facility.

69. Drug repurposing for the treatment of alphaviral infections

Dr Penny Rudd & Dr Lara Herrero

Virology, Virus-Host Interactions, Therapeutic Treatments

Ross River (RRV) and chikungunya (CHIKV) viruses are mosquito-borne viruses that cause severe joint and muscle pain in humans, which can last months or even years and may become a cause of chronic pain and disability. CHIKV and RRV can be found in over 100 countries across the globe. In 2015, over 700 000 cases of chikungunya were reported in the Americas alone to the Pan American Health Organization (PAHO) regional office and that same year, Queensland saw the largest epidemic of RRV in 20 years with over 4000 cases in the first quarter.

There are currently no specific treatments for these alphaviruses. Pain relief is prescribed to help alleviate symptoms. This project focuses on examining the repurposing of currently available drugs towards the treatment of viral induced arthritides. Discovering new uses for approved drugs provides the quickest possible transition from bench to bedside and may lead to novel treatments, which could prove beneficial towards pain management for hundreds of thousands of arthritic patients worldwide.

Towards this, we will 1) Examine if the drugs reduce joint swelling and ameliorate overall disease outcomes in RRV/CHIKV infected mice 2) Characterise the inflammatory cytokine/chemokine patterns during and after drug treatment and 3) Identify novel biomarkers which may serve as clinical markers for disease monitoring and outcomes.

The resolution of this project may lead to significant advancements for the identification of novel treatment strategies for patients suffering from CHIKV and RRV.

Techniques: mouse models of CHIKV and RRV infection, clinical disease monitoring, *in-vitro* assays, real-time PCR, cell-culture, viral plaque assays, immuno- histochemistry, bioplex assays.

70. Deciphering the mechanisms involved in Chikungunya virus (CHIKV) neuropathogenesis

Dr Penny Rudd & Dr Lara Herrero

Virology, Virus-Host Interactions, Therapeutic Treatments

Alphaviruses are a global health threat to humans and animals alike causing severe disease ranging from lethal encephalitis to debilitating long-lasting arthritis. Classically, the Old World alphaviruses like chikungunya (CHIKV) are primarily associated with painful and chronic arthritis. Yet, in recent years, neurological sequela has been consistently associated with CHIKV infection. Several thousand neuro-virulence cases have been reported especially in the young and elderly.

Very few studies have been undertaken to determine the mechanisms involved in CHIKV neuropathogenesis. We want to answer fundamental questions about the mechanisms involved in CHIKV neurovirulence using a combination of *in vitro*, *in vivo* and *ex vivo* approaches. Towards this, we will look at 1) How CHIKV enters CNS cells and disseminates throughout the brain structures 2) Determine which immune responses are elicited after CHIKV infects brain cells and 3) Assess how inadequate immune responses contribute to CHIKV neuropathologies.

Since there is no current treatment or vaccine, basic knowledge gained about CHIKV-host interactions will play a pivotal role in the discovery of new treatment strategies. These therapies will aim to reduce or circumvent neurological complications involved in CHIKV central nervous system (CNS) disease by preventing viral entry and spread or by counter-acting immune mediated pathology.

Techniques: mouse model of CHIKV infection, clinical disease monitoring, *in-vitro* assays, real-time PCR, cell-culture, viral plaque assays, immunohistochemistry, confocal microscopy.

71. Identifying novel animal reservoirs of Ross River and Barmah Forest viruses

Dr Penny Rudd & Dr Lara Herrero

Virology, Epidemiology, Public Health

Ross River virus (RRV) is a serious disease with no specific treatment or vaccine. It affects thousands of Australians annually, in Queensland with an influenza-like disease and severe debilitating joint pain. It is the most common mosquito-borne virus on our shores. The virus is a burden to Australia and surrounding islands in the South Pacific including Papua New Guinea and Fiji, putting a great number of people in hospital each year.

Most medically important arboviruses are transmitted to humans from other vertebrate species. To be an important reservoir for human infection, the reservoir host must be attractive for arthropod vectors i.e. they must be able to feed on these hosts. The hosts must also develop viraemia that is high enough to allow transmission to susceptible blood-feeding vectors. The ideal hosts must equally have low mortality to the infection and there must be low herd immunity.

Serological studies and laboratory investigations have indicated that several domestic and wild animals serve as RRV reservoirs, including dogs, cats, possums, and horses. However, the primary reservoir hosts for RRV are marsupials with macropods playing a significant role.

In a recent publication, there is evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence. This project aims at looking into what these potential reservoirs could be. Towards this we will 1) Examine susceptibility of various cell lines to RRV infection 2) Determine if Australian animals have antibodies against RRV 3) Assess if the target reservoirs identified and present in Samoa also have antibodies against RRV.

This project will have important ecological, clinical and public health outcomes. It will allow us to better understand disease ecology of RRV and help identify potential outbreaks and reduce the risk global spread.

Techniques: cell culture, *in-vitro* assays, real-time PCR, viral plaque assays, ELISA.

72. Understanding how environmental change impacts vector borne disease

Dr Lara Herrero, Dr Penny Rudd & Prof Brendan Mackey (School of Environment & Sciences)

Bioinformatics, Virology, Modelling

Mosquitoes are considered the most important transmitters of disease globally. The diseases they transmit threaten more than half the world's human population and are reported to have significant health impacts on many domestic species. The ecology of mosquitoes and their capacity to transmit pathogens is complex, involving both intrinsic factors such as virus susceptibility and extrinsic factors such as the effect of environmental pressures. Utilising experimental, statistical and simulation models we will investigate how future climate and land use change affects the spread and infectivity of key mosquito transmitted viruses, and apply scenario based risk assessments to analyse and map modelled risk factors and projected risks.

Techniques: Computer modelling, data mining, Bayesian modelling, bioinformatics

73. Bat Borne Viral Zoonosis; glycans and the host

Assoc Prof Lara Herrero, Dr Penny Rudd, Prof Linfa Wang (Duke NUS), Dr Michelle Baker (CSIRO)
Virology, Epidemiology, Cell Biology, Glycobiology, Public Health

Zoonotic pathogens pose major threats including catastrophic social and economic impacts. Zoonotic infections are triggered by the ability of a pathogen to cross from animal to human. Bats have been shown to carry more than 200 viruses and a significant proportion of these viruses are zoonotic however very little is known about what makes bats unique hosts. This project aims to investigate the mechanisms of viral host interactions focusing on viruses of pandemic potential. Utilising innovative glycobiological technologies this research seeks to be the first ever to identify the "natural" glycome of the bat leading to better prediction and understanding of why bats are uniquely susceptibility to a multitude of important zoonotic viruses. This will fill a significant gap in our knowledge of bat physiology and the unique nature of bats in harbouring viral infections.

Techniques: Handling of primary bat cells, cell culture, viral plaque assays, flow cytometry, molecular biology, glyco-analytical techniques using mass spectrometry and liquid chromatography. Experiments will be undertaking in the state-of-the-art glycobioanalytical facility.

74. Levitating yeast cells in an ion trap

Dr Erik W Streed & Assoc Prof Joe Tiralongo

Biophysics

Techniques from physics have often been adapted to solve problems in the life sciences. Notable examples include microscopy, x-ray diffraction, and fluorescent labelling. We are interested in developing new ways to investigate the properties of cells, subcellular structures, and large biomolecules using ion trapping techniques from quantum physics. Project students will be involved in a subset of the following project aspects: culturing and fluorescent labelling of yeast cells, loading yeast cells into an ion trap, and then measuring the physical properties & manipulating the cell using electrical, hydrodynamic, and laser methods. There are also projects available on mathematical modelling of the particles. Physics or Biological laboratory course experience preferred for in-lab components.

75. Does a licensed meningococcal vaccine protect against *Neisseria gonorrhoeae*

Dr Evgeny Semchenko, Dr Taha & Assoc Prof Kate Seib

Molecular Biology, Microbiology, Vaccine Development, Immunology

There are >100 million cases of gonorrhoea per year, and infection can cause severe sequelae including pelvic inflammatory disease, adverse pregnancy outcomes, neonatal complications, infertility, and increased risk of HIV. Gonorrhoea disproportionally impacts women, particularly in the developing world, and has been recognised by the World Health Organization (WHO), USA Centers for Disease Control (CDC), and Australian National Antimicrobial Resistance Strategy as an urgent threat to global health. There is currently no gonococcal vaccine, and due to multidrug resistance there are concerns that N. gonorrhoeae may become untreatable in the near future.

A recent retrospective case-control study found that individuals vaccinated with the meningococcal serogroup B (MenB) outer membrane vesicle (OMV) vaccine MeNZB were significantly less likely to contract gonorrhoea compared with unvaccinated controls. We have shown that a newer MenB vaccine, induced antibodies in humans that are cross-reactive with *N. gonorrhoeae*. A randomised control trial is now underway to test the efficacy of 4CMenB against gonorrhoea. Human serum samples from this trial will be assessed to understand the vaccine-induced immune response and determine whether antibodies raised to 4CMenB can kill *N. gonorrhoeae* or block its adherence to host epithelial cells.

Techniques: Cloning, protein expression and purification, Western analysis, ELISA, serum bactericidal assays, cell adherence assays, opsonophagocytosis assays.

76. Vaccine development for *Neisseria gonorrhoeae*

Dr Evgeny Semchenko, Dr Taha & Assoc Prof Kate Seib

Molecular Biology, Microbiology, Vaccine Development

Neisseria gonorrhoeae, the causative agent of gonorrhoea, is a significant health problem worldwide. The control of gonorrhoea depends on the development of a vaccine due to the continuing increase of antibiotic resistance and the staggering outcomes of infection, including infertility and increased transmission of HIV. This project aims to characterise potential vaccine candidates to aid in the development of a gonococcal vaccine. The distribution of the identified vaccine candidates will be investigated in a diverse range of *N. gonorrhoeae* strains. The functions of candidates will be examined by generating a mutant strain of *N. gonorrhoeae* that does not express the vaccine candidate and comparing the wild type and mutant strains in a panel of antimicrobial stress assays.

The vaccine potential of these candidates will be assessed by expressing and purifying the protein from *Escherichia coli*, immunising mice to generate antibodies to the protein, and then testing the ability of these antibodies to mediate killing of *N. gonorrhoeae* in various assays (e.g., serum bactericidal assays and neutrophil opsonophagocytic assays to measure killing of the bacteria; adherence assays to test if the antibodies can inhibit association and invasion of human epithelial cells).

Techniques: Cloning, protein expression and purification, construction of gene deletions in *N. gonorrhoeae*, Western analysis, ELISA, serum bactericidal assays, cell adherence assays, opsonophagocytosis assays.

77. Characterising the role of sugars in host-pathogen interactions

Dr Evgeny Semchenko, Dr Taha & Assoc Prof Kate Seib

Microbiology, Glycobiology, Molecular Biology

Human mucosal surfaces, such as the airway, contain a range of carbohydrate structures (glycans) and many bacteria have evolved carbohydrate-binding proteins (lectins) that enable cell attachment, colonisation and invasion of host cells. Our aim is to identify glycans that host-adapted bacterial pathogens bind to during colonisation and disease. This project will focus on bacteria including *Moraxella catarrhalis* (causes middles ear infections and exacerbations of chronic obstructive pulmonary disease), *Neisseria gonorrhoeae* (causes gonorrhoea) and *Neisseria meningitidis* (causes sepsis and meningitis). We will probe Glycan Arrays (consisting of >400 sugars immobilised onto glass-slides) using recombinant proteins and wild type bacteria and a series of mutant strains lacking key outer membrane structures. The affinity and kinetics of interactions will be investigated using surface plasmon resonance. We will also use epithelial cell adherence and invasion assays to investigate the functional role of glycan-based host-pathogen interactions. These findings will contribute to understanding key bacterial and host factors involved in colonisation and disease and may direct development of new drugs and vaccines for these bacteria.

Techniques: Cloning, protein expression and purification, construction of gene deletions in bacteria, array technology, surface plasmon resonance, cell adherence assays.

78. The role of glycosylation in transmission of zoonotic diseases

Dr. Chris Day, Assoc Prof Joe Tiralongo, Dr. Alison Peel & Assoc Prof Daniel Kolarich

Glycomics, glycoproteomics, infection, evolution, zoonotic disease

Animals such as bats are considered prime hosts for zoonotic diseases before they "jump" to humans. Viruses such as influenza or COVID-19 are known to infect different host species, where they can gain novel functions and increase their virulence.

The cell surface of mucosal barriers in the respiratory tract plays a fundamental role in this interplay. This cell surface, but also any body fluid proteins are extensively modified with species specific sugars called glycans. These glycans build a universal language used by cells but also abused by pathogens. Though eukaryotic organisms share one alphabet, evolution made them speak multiple different languages and dialects. We have developed glycomics & glycoproteomics tools to translate these languages and uncover how pathogens learned to speak and interpret glyco-languages between different species. Understanding this relationship is crucial as viral, bacterial and parasite pathogens have developed elaborate strategies to jump between hosts – and many of these strategies involve cell surface glycans. The influenza virus is just one fairly well understood example that uses this strategy.

As part of this project several student projects are available that include 1.) characterisation of the plasma/serum glycome across several vertebrae species; 2.) investigation and understanding cross-species recognition of pathogen adhesins; 3.) novel, cutting-edge science to understand the role of glycosylation and infection in flying foxes;

4.) working in and with an interdisciplinary and international team delivering first-hand knowledge and skills in a variety of biochemical and immunological skills and techniques.

The outcomes of these highly collaborative (across Griffith, national and international partners) projects will provide novel clues how infectious diseases can spread and uncover novel targets to stop their distribution and uncover novel biology in animal species of primary interest for the distribution of zoonotic pathogens. Students will be introduced to biochemistry and immunology laboratory workflows that include (but are not limited to) SDS-PAGE, Western blotting, Proteomics and Glycomics sample preparation, acquisition and data analyses and gain general knowledge in Biochemistry, Glycobiology and Immunology.

Techniques: mass spectrometry, glycomics, proteomics, microarray, Immunoglobulins, protein purification, immunological techniques.

79. Understanding the impact of glycosylation on stem-cell-factor and stem-cell-factor receptor signalling in health and cancer pathogenesis

Dr. Larissa Dirr, Dr. Alpesh Malde, Assoc Prof. Joe Tiralongo, Prof. Mark von Itzstein, Assoc Prof Daniel Kolarich

Glycoproteomics, biochemistry, signaling, protein structure

Receptor glycoproteins are highly important signalling molecules in controlling cell communication and interaction. Dysregulation of these signalling pathways is frequently associated with diseases such as cancer and chronic inflammatory conditions. However, the role their glycosylation plays for protein structure and interaction is still poorly understood.

Type III family of receptor tyrosine kinases such as c-KIT (also known as SCF receptor or CD117 PDGF-receptor-α and β, CSF-1 receptor and the FLT3 receptor play a vital role in the pathogenesis across different types of cancer.

As part of a larger project a variety of student projects are available that include aspects of mass spectrometry applications (proteomics, glycomics and glycoproteomics) next to protein structure, cell culture, Western Blot, electrophoresis and other standard biochemistry techniques. In combination these techniques are being employed to characterise and modulate the glycosylation of these important signalling molecules to understand how protein-specific glycosylation impacts protein function and cell signalling. This knowledge will provide opportunities for developing novel therapeutic strategies targeting these receptor proteins. As part of this project, students will be introduced to biochemistry laboratory workflows that include (but not limited to) SDS-PAGE, Western blotting, Proteomics and Glycomics sample preparation, acquisition and data analyses and gain general knowledge in Biochemistry and Glycobiology.

Techniques: mass spectrometry, glycomics, proteomics, cell culture, Western blotting, protein structure, basic biochemical workflows.

80. Cracking the cancer-glycocode to guide novel cancer diagnostics and therapeutics

Dr Arun Everest-Dass, Assoc Prof. Chamindie Punyadeera, Prof. Mark von Itzstein & Assoc Prof Daniel Kolarich

Glycomics, proteomics, cancer, cancer-biomarkers, cancer microenvironment, cancer diagnostics, multi-omics,

Understanding cancer and patient-specific dynamics of protein glycosylation holds enormous yet unmined potential for cancer precision medicine. Glycosylation is a dynamic protein post translational modification in which defined sugars (so called glycans) are attached to proteins by highly individual biosynthetic pathways. Human blood groups are one example of the individuality and clinical relevance of protein glycosylation, as specific glycans form the molecular basis of the human ABO blood group system. About 2 % of human genes are dedicated to biosynthetic pathways of this glycosylation machinery. Genomics and transcriptomics can provide some information about the presence or absence of glycosylation-relevant genes. However, the biosynthetic events that regulate the glycosylation machinery are beyond direct genomic and transcriptional regulation. Glycomics and glycoproteomics approaches thus are the only technologies that can be employed to sequence the cancer glycocode.

In close collaboration with national and international clinical partners we are studying cancer glycocode to understand why cancer forms, what makes individual cancers specific and to identify the weak points that allow us to develop novel strategies to fight cancer. With a focus on cancers such as Leukaemia, Prostate cancer, Melanoma, Ovarian cancer, Head & Neck Cancer or Colon cancer we use highly sensitive and selective glycan/glycoprotein sequencing

tools to study cell surface glycoconjugates and their role in pathological processes. One technology involves cutting-edge Laser capture Microdissection that allows the specific cutting of cancer cells from tissue that has revolutionised how we can read the language of cancer. As part of the Australian Centre for Cancer Glycomics (A2CG) we are now systematically applying our glycan-sequencing technologies to sequence cancer glycomes in a variety of cancers.

Be part of the cancer glyco-revolution. A number of student projects are available supporting this important endeavour that will result in a new generation of diagnostic and prognostic cancer markers. As part of this project, students will be introduced to biochemistry laboratory workflows that include (but not limited to) SDS-PAGE, Western blotting, Proteomics and Glycomics sample preparation, acquisition and data analyses and gain general knowledge in Biochemistry and Glycobiology. They will work in an interdisciplinary and multi-national team at the direct interface between the clinic and the research lab.

Techniques: mass spectrometry, glycomics, proteomics, Western blotting, Laser Capture Microdissection microscopy, basic biochemical workflows

81. The role of glycosylation for immunotherapy

Prof. Nicolle Packer, Prof. Riccardo Dolcetti, Prof. Mark von Itzstein & Assoc Prof Daniel Kolarich

Glycoproteomics, cancer biology, Understanding cancer immunotherapy

Cancer therapies have experienced a tremendous revolution with the introduction of therapies that use monoclonal antibodies that specifically target cancer cell surface targets and immune-checkpoint receptors. More than 95% of the protein receptors targeted by these immunotherapy agents are in fact glycoproteins, but to date the impact of receptor glycosylation in precision medicine is still not understood.

In this close collaboration with colleagues from the Peter MacCallum Cancer Centre students will be introduced to biochemistry and immunology laboratory workflows that include (but not limited to) SDS-PAGE, Western blotting, Proteomics and Glycomics sample preparation, acquisition and data analyses and gain general knowledge in Biochemistry and Glycobiology. Cancers that are being targeted in this project include Leukaemia, prostate cancer, melanoma, Head & Neck Cancer, Hepatocellular carcinoma or Colon cancer are investigated.

Techniques: mass spectrometry, glycomics, proteomics, cell culture, Western blotting, basic biochemical workflows

82. Structural basis and therapeutic targeting of neurodegeneration

Dr Thomas Ve, Dr Yun Shi & Prof Mark von Itzstein

Structural Biology, Biochemistry, Medicinal Chemistry

Axon loss is a common theme in some of the most prevalent neurological diseases, including peripheral neuropathies, traumatic brain injury, Parkinson's disease and glaucoma, but no treatments currently exist that effectively target axonal breakdown. The protein SARM1 is a central player in axon loss. In healthy nerve cells, SARM1 (sterile alpha and TIR motif 1) is present but inactive. Disease and injury activate SARM1, which results in rapid breakdown of the essential “helper molecule” nicotinamide adenine dinucleotide (NAD+) and ultimately destruction of the axon. We have demonstrated that it is SARM1 itself that cleaves NAD+ upon activation through self-association and we hypothesise that detailed structural knowledge of the SARM1 catalytic mechanism and defining the molecular mechanisms upstream and downstream of SARM1 enzyme activity can yield inhibitors as leads to anti-neurodegenerative disease therapeutics. This project can include work in one, or several, areas, including Cryo-EM, X-ray crystallography, NMR and inhibitor design.

Techniques: Cryo-EM, X-ray Crystallography, NMR, Enzyme assays, Computational Chemistry including visualisation and molecular docking; Synthetic Chemistry

83. Molecular basis of nucleotide signalling by bacterial TIR domain containing proteins

Dr Thomas Ve, Dr Yun Shi & Assoc Prof Daniel Kolarich

Structural Biology, Biochemistry, Microbiology, Innate Immunity

In both animals and plants TIR domain enzymes have important immune functions. While bacterial TIR proteins have long been recognised, their biochemistry and function remain poorly understood. Some TIR domain containing proteins with NAD+ cleavage activity have been reported to be involved in (i) subversion of host innate immunity (4) and (ii) in antiphage defence systems, but the mechanism of how these proteins utilise NAD+ and its metabolites to modulate the immune system, or provide resistance against phage infection has not yet been explored. As the bacterial TIR domain family is widespread and highly sequence diverse the characterised NAD+ cleaving bacterial

TIR domains is likely to only comprise a small fraction of this family's enzyme diversity and a kingdom wide analysis of them will allow systematic identification of new bacterial signalling nucleotides as well as potential agonists/antagonists of the innate immune system in animals and plants. Mechanistic understanding of bacterial defence systems has previously led to the development of revolutionary biotechnological tools such as restriction enzymes and CRISPR-Cas. Understanding the mechanism of new defence systems such as the ones containing TIR domains may facilitate strategies for developing new useful molecular tools.

This multidisciplinary project can include work on one, or several, topic, including: (i) Characterise the structural basis of TIR domain NADase activity; (ii) Explore the diversity of nucleotide signals produced by bacterial TIR domain containing proteins (iii) Identify the mechanisms that regulate TIR domain NADase activity; and (iv) Define the interactome of TIR domain produced nucleotide signals.

Techniques: X-ray Crystallography, Cryo-EM, NMR, Enzyme assays, HPLC, mass spectrometry.

84. Pseudotyped Virus-Like-Particles as Platform for Vaccine Development

Dr Belinda de Villiers and Prof Johnson Mak

Pseudotyped virus-like-particles (VLPs) are non-infectious, nano-scale biological materials that can be used for vaccination purposes. VLPs have benefited from the evolutionary processes that impart exceptional efficiency to deliver the payload to animal hosts, thereby eliciting strong immune responses. Our laboratory uses two types of VLP systems for evaluation, which include: (i) lentiviral vector (LV) system; and (ii) vesicular stomatitis viral (VSV) vector system. Both systems have proven successful in clinical settings, including the use of LV system in gene therapy and CART cell therapy; and the use of VSV system as an Ebola vaccine. Our lab explores the potential of using these two systems to develop effective vaccines against viral pathogens. Some of the viral pathogens being evaluated using these systems include HIV, SARS-CoV-2, and African Swine Fever Virus (ASFV). This work requires: molecular virology-based approaches to produce VLPs via tissue culture; biochemical and biophysical evaluation to dissect mechanism; imaging analyses to track the movement of VLPs; structural biology and glycan biology to refine the design of our system; and preclinical animal work to determine the effectiveness of some of these specific vaccine designs. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious materials or PC3 related genetic modified organisms will be used. Successful applicant will be part of a larger research team to work on aspect(s) of the project that are based on: (i) practicality; (ii) project objective; plus (iii) mutual research interest between the appointee and the host lab.

Techniques: Virology, Biochemistry, Biophysics, Molecular Biology, Structural Biology, Glycan Biology, Animal Works

85. *Mechanisms of Assembly and Entry of Viruses that Enhance Virus Transmission*

Dr Belinda de Villiers and Prof Johnson Mak

Viruses are 'obligate parasites' that completely rely on their hosts to propagate. The constant navigation and exploitation of their hosts have allowed viruses to hijack (and to adopt) selected aspects of these host cell systems to facilitate effective virus transmission. These events include: (i) utilising directional trafficking of viral proteins during virus formation to maximise efficiency of cell-cell transmission; and (ii) incorporating glycan-shield on virus surface to block the anti-viral immune response and/or to mediate virus attachment during progeny virus infection. Our lab investigates the directional trafficking and release of viral particles using retrovirus systems, including HIV, Koala retrovirus (KoRV), and Human T-cell-leukemia virus. We also use viral proteins from a wide range of enveloped viruses to study the glycan biology of viral proteins, such as HIV, KoRV, SARS-CoV-2, ASFV, influenza and respiratory syncytia virus (RSV). This work requires: molecular virology-based approaches to produce viruses via tissue culture; biochemical and biophysical evaluation to dissect mechanism; imaging analyses to delineate directional trafficking; structural and glycan biology to reveal molecular interactions. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious materials or PC3 related genetic modified organisms will be used. Successful applicant will be part of a larger research team to work on aspect(s) of the project that are based on: (i) practicality; (ii) project objective; plus (iii) mutual research interest between the appointee and the host lab.

Techniques: Virology, Biochemistry, Biophysics, Cell Biology, Molecular Biology, Glycan Biology, Structural Biology

86. *Mobile Device Compatible Rapid Diagnostics Suitable for Remote Settings*
Dr Belinda de Villiers and Prof Johnson Mak

Accurate and rapid diagnostic assays are critical in controlling the spread of infection events. The current COVID-19 pandemic highlights a major shortcoming of the gold-standard, lab-based, quantitative polymerase chain reaction (qPCR) diagnostics in an evolving-crisis situation. The relative lengthy turn-around time of qPCR plus the requirement of trained staff and high-end equipment in diagnostic processes are the bottlenecks for clinical assessments of patients (subjects) in remote settings. These practical challenges are further amplified in low- to middle-income countries where highly trained staff and pricy infrastructure are often in short supply. Our laboratory is trying to address this technological gap by developing rapid point of care diagnostics, which include the evaluation and development of loop-mediated isothermal amplification (LAMP) and CRISPR-based diagnostics. We are focusing our work on HTLV-1 and ASFV, which have a major impact on the health of our indigenous people within Central Australia and the global pig farming industry respectively. This work requires: molecular biology-based approaches to design and to refine our detection system; molecular virology techniques to generate relevant samples for evaluation; biochemical assessment to evaluate detection system; pre-clinical evaluation of diagnostic system using precious clinical samples. This project has both PC2 and PC3 components, and they complement each other. The PC3 component is reserved to PhD students and post-doctoral fellows, in which live infectious materials or PC3 related genetic modified organisms will be used. Successful applicant will be part of a larger research team to work on aspect(s) of the project that are based on: (i) practicality; (ii) project objective; plus (iii) mutual research interest between the appointee and the host lab.

Techniques*:* Virology, Biochemistry, Biophysics, Molecular Biology, Clinical Integration

87. Development of novel non-antibiotic strategies to ensure food safety in Australia

Associate Professor Thomas Haselhorst and Dr Chris Day

Research areas: Structural Biology, Computational Chemistry, NMR spectroscopy, Microbiology

Project details: The decade long overuse of antibiotics in poultry agriculture and consequently the transferral of antibiotic resistance to humans and the associated health problems underlines the urgent need for novel antibiotic-independent strategies, such as feed supplements (prebiotics) that augment commercial poultry performance and provide food safety. This PhD project aims to develop prebiotic treatment options to reduce the colonisation of Campylobacter jejuni in the chicken intestinal tract. Structural and biophysical investigations of glycan-glycan interactions followed by monitoring the bacterial load in chickens and potential cross-contamination into chicken will form the main part of the thesis. Expected outcomes will be the development of a potentially commercially viable non-antibiotic treatment option for poultry farmers in Australia.

Techniques: Structural investigations on Glycans in solution with NMR spectroscopy, biophysical methods and molecular modelling, developing of a virtual glycan array approach, monitoring Campylobacter bacterial strains.

88. Identification of an anti-tumour agent, the guinea pig serum *L-asparaginase*
Dr Freda Jen, Prof Ifor Beachman, Prof Michael Jennings
Research area: Molecular biology

Project details: Guinea pig serum was serendipitously discovered to be an effective anti-tumour agent over 65 years ago with respect to transplantable lymphomas. It was found that the agent responsible for this remarkable anti-tumour property was L-asparaginase in the sera. There is much evidence that the anti-tumour properties of L-asparaginases are due to depletion of exogenous L-asparagine on which the tumour cells depend for growth, being essentially auxotrophic for L-asparagine. However, the identification of the guinea pig serum L-asparaginase has been problematical since its discovery. L-asparaginase was only found in guinea pig serum and not in the sera of other species investigated, including mouse and rat. The aim of this project is to determine the genetic and biochemical origins of the liver and serum enzymes in guinea pig and in those related species which also have both isozymes.

Techniques: Molecular biology

89. Discovery of CMP-Neu5Ac transporter in pathogenic Neisseria

Dr Freda Jen, Prof Michael Jennings

Research area: Molecular biology

Project details: *Neisseria gonorrhoeae* is a host adapted bacterial pathogen that infects male urethral and female cervical and causes sexually transmitted disease gonorrhoea. Lipooligosaccharides (LOS) is one of the major virulence factors of *N. gonorrhoeae* and is composed of multiple possible glycoforms due to the phase variation (high frequency of ON/OFF switching of gene expression) of the genes involved in LOS biosynthesis. Gonococcal LOS structure is caped with a N-acetyl-5-neuraminic acid (Neu5Ac). However, *N. gonorrhoeae* cannot synthesize the CMP-Neu5Ac required for LOS biosynthesis and must acquire it from the host. In most of the LOS biosynthetic pathway, the core-oligosaccharide is assembled in the cytoplasm. Previously, the alpha-2,3-sialyltransferase, Lst of *N. gonorrhoeae* was proposed to be a surface exposed outer membrane protein. In our unpublished study, we investigated the cellular location of Lst and all our results indicated that Lst is located inside of the cell suggesting that there must be a transport system or a trans-sialidase transport CMP-Neu5Ac from the host to inside of cells. The aim of this project is to discover a novel CMP-Neu5Ac transporter in pathogenic Neisseria.

Techniques: Molecular biology

90. Effect of glycosylation on the structure and dynamics of proteins

Dr Alpesh K Malde & Prof Mark von Itzstein

Molecular Modelling, Computational Medicinal Chemistry, Structural Biology

Sugars (glycans) attached to proteins play an important role in biology, especially in immunity and viral infections. Understanding glycan recognition is crucial for interpretation of the glycan's biological roles, however current experimental approaches are limited in their ability to resolve glycans at an atomic level. This project focuses on integrating experimental glycosylation, glycan array and NMR data with 3D structure of proteins to generate 'physiological' glycoprotein conjugate, protein-protein and protein-glycan complex structures. Computer simulations will be used to investigate glycan interactions at an atomic level and to facilitate design of potential drugs and vaccines. The computational approach involved in the project will be based on molecular dynamics (MD) simulations, which can be used to calculate the time evolution of a system from which various structural, dynamic and thermodynamic properties of interest can be evaluated. The project involves study of receptor-binding proteins from important human pathogenic viruses including but not limited to influenza, parainfluenza, Hendra, Nipah and SARS-CoV-2 viruses.

Techniques: Computational Chemistry and Molecular Dynamics Simulations.

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