

2010 Glycomics Honours Projects - Scientific Disciplines

No	Project Title	Scientific Discipline					
		BC	MC	MB	MM	MMod	SB
1	The synthesis and characterisation of carbohydrate-based compounds as anti-malarial agents		●				
2	New approaches towards anti-Dengue virus agents		●				●
3	Epitope binding investigations of sialidases by NMR spectroscopy					●	●
4	The development of sialidase inhibitors as potential antimicrobial agents		●		●	●	
5	Carbohydrate-based biological probes for the investigation of microbial cell wall synthesis		●				
6	Multivalent carbohydrate structures		●				
7	Investigation of the structure of CMP-sialic acid synthetase					●	●
8	Investigation of β -glucuronidases		●			●	●
9	The development of sialylmimetic nucleosides as probes of sialyltransferase and sialic acid transporter proteins		●				
10	Ganglioside/Micelle protein interaction studied by Saturation Transfer Difference (STD) NMR spectroscopy						●
11	Structure Affinity Relationship (SAR) by NMR						●
12	<i>In silico</i> and NMR-based library screening of the <i>trans</i> -sialidase of <i>Trypanosoma cruzi</i>	●					●
13	Structure-based discovery of anti-parainfluenza viral agents	●	●	●		●	●
14	The molecular pathogenesis of melioidosis: Bacteria-host interactions in the upper respiratory tract				●		
15	Carbohydrate-based compounds as potential anti-bacterial agents		●				●
16	Investigations into bacterial virulence factors - potential drug targets?			●			●
17	Identification of specific amino acid residues responsible for interactions of chemosensory receptor Tlp1 with chemotaxis proteins CheW and CheV of <i>Campylobacter jejuni</i>				●		
18	Identification of <i>Campylobacter jejuni</i> lectins involved in bacteria-host interactions				●		
19	Characterisation of <i>C. jejuni</i> lipooligosaccharides (LOS) and their role in human autoimmune disease				●		●
20	Structural characterisation of <i>C. jejuni</i> lipooligosaccharides (LOS) mutants		●		●		●
21	Regulation of cell surface sialylation by targeting the CMP-sialic acid transporter: Towards the development of anti-metastatic agents	●					●
22	CMP-sialic acid transporter structure elucidation: 3D crystallography	●					●
23	Probing Microbe-Glycan and Primary Cell-Glycan interactions using Glycan Array Technology	●			●		
24	Establishment of a CellFrac microarray	●					
25	Isolation and characterisation of novel lectins from Australian macrofungi	●					
26	Influence of the length of oligosaccharide on the biological activity of the lipooligosaccharide from <i>Moraxella catarrhalis</i>		●		●		
27	The synthesis of novel substrate molecules to probe the function of a unique glycosyltransferase enzyme from <i>Moraxella catarrhalis</i>		●				
28	Analysis of lipopolysaccharide structures from <i>Moraxella bovis</i>		●		●		
29	Development of novel glycosidase inhibitors as potential therapeutics		●				
30	Design of Fluorescent receptors for carbohydrates and related biomolecules		●				
31	Synthesis and drug targeting of antitubercular compounds		●				
32	Bacterial manipulation of the immune system: how <i>Burkholderia pseudomallei</i> alters host cells to survive intracellularly				●		

| BC=Biochemistry | MC=Medicinal Chemistry | MB=Molecular Biology | MM=Molecular Microbiology | MMod=Molecular Modelling | SB=Structural Biology |

33	Bacterial glycans: role of <i>Burkholderia pseudomallei</i> LPS, capsule and glycosylated proteins in virulence			●	●		
34	Evolution of random gene switching mechanisms in bacterial pathogens			●	●		
35	Substrate specificity of phase variable methyltransferases associated with phasevarions			●			
36	A mutagenesis screen to identify key components of post-translational modification pathways bacterial pathogens			●	●		
37	Rotavirus: Structure based drug design		●	●			●
38	The Macrophage-Inducible C-type lectin (Mincle)	●		●		●	●
39	Targets in Cancer: Structure-based investigation of Galectin-1	●		●		●	●
40	Galectins: Targets in Cancer: Synthesis of Galectin-specific Inhibitors		●			●	●
41	Three-Dimensional atomic structure determination of galectin-inhibitor complexes by X-ray crystallography: Design of galectin-specific drugs					●	●
42	Galectin-14: A Recently identified drug target in inflammation	●		●		●	●
43	Metapneumovirus: A major cause of respiratory tract infection	●				●	●

2010 Glycomics Honours Projects

1. The synthesis and characterisation of carbohydrate-based compounds as anti-malarial agents

[Prof Mark von Itzstein](#), Mr Jeff Dyason and Dr Robin Thomson

Medicinal Chemistry

Malaria is the most serious protozoal disease in humans, with 300–500 million acute illnesses and 1.5–2.7 million deaths annually. Resistance to commonly used anti-malarial drugs is now widespread among isolates of *Plasmodium falciparum*, the species responsible for the most severe disease and mortality.

The invasion of red blood cells (erythrocytes) by the merozoite form of the parasite is critical for the survival of the parasite, and a critical stage in the development of disease. It is therefore a logical target for the development of interventions to control malaria. Interaction of the merozoites of *P. falciparum* with erythrocytes is mediated in part by the erythrocyte binding antigen (EBA-175) on the merozoite surface which binds to a sialylated glycoprotein on the erythrocyte surface, glycophorin A. This project will involve the synthesis of carbohydrate-based compounds to probe the interactions between the parasite and host cells, to further our understanding of this important stage of infection.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC.

2. New approaches towards anti-Dengue virus agents

Dr Robin Thomson, Dr Thomas Haselhorst, Mr Jeff Dyason & [Prof Mark von Itzstein](#)

Medicinal Chemistry, Structural Biology

Dengue virus is a mosquito-borne flavivirus that causes both dengue fever and the potentially lethal complication dengue haemorrhagic fever. Dengue virus infection has become a major international public health concern with an estimated 50 million cases, and around 24,000 deaths worldwide per year. There is no specific treatment for dengue fever and development of a suitable vaccine has proven difficult.

The surface of dengue virus is covered by a glycoprotein (the "Envelope" or "E" glycoprotein) that is essential in the life cycle of the virus, being involved in both binding to and fusion with target host cells. Stopping these critical initial stages of the infection process by blocking the interaction of E glycoprotein with the host cells is an attractive approach for therapeutic and/or preventative intervention against dengue virus infection.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Computational Chemistry including visualisation and molecular docking; Advanced NMR techniques including STD-NMR.

3. Epitope binding investigations of sialidases by NMR spectroscopy

Dr Thomas Haselhorst, Mr Jeff Dyason and [Prof Mark von Itzstein](#)

Molecular Modelling, Structural Biology

Sialidases (also known as neuraminidases) are enzymes that catalyze the hydrolytic cleavage of terminal alpha-ketosidically linked sialic acids from sialoglycoconjugates such as glycoproteins and glycolipids. Sialidases have been implicated in the pathogenesis of many microorganisms such as bacteria, viruses, and parasites that are associated with serious disease states.

Recently, the method STD-NMR was developed to screen compound libraries against a protein target. This method is suitable for determining an epitope mapping of a ligand within the binding site of the protein since only regions of the ligand that are in contact with the active site of the enzyme are saturated by the protein.

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

4. The development of sialidase inhibitors as potential antimicrobial agents

Dr Robin Thomson, Mr Jeff Dyason and [Prof Mark von Itzstein](#)

Medicinal Chemistry, Molecular Modelling, Molecular Microbiology

Sialidases are involved in the infective cycles of a number of viral, bacterial, and parasitic infections, such as influenza, cholera, and African sleeping sickness. As these enzymes also fulfil important roles in mammalian biology, the development of drugs based on the inhibition of microbial sialidases relies on our ability to design inhibitors with strict specificity for the microbial enzyme. This has been accomplished for influenza virus sialidase, where specific inhibitors were developed based on a knowledge of the structure and mechanism of action of that enzyme. One of the potent sialidase inhibitors developed is now marketed as the anti-influenza drug Relenza™.

The influenza virus sialidase inhibitors were based on the natural substrate of the enzyme, sialic acid. There is now interest in the design of mimetics of these inhibitors, with simplified structures, which can be prepared from less expensive starting materials, and which may have different and/or improved pharmacological properties. This project encompasses exploration of sialidase crystal structures using molecular modelling and computational chemistry and/or the preparation of carbohydrate-based compounds to be used as probes of the activities of several different sialidases.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Computational Chemistry including visualisation and molecular docking

5. Carbohydrate-based biological probes for the investigation of microbial cell wall synthesis

[Prof Mark von Itzstein](#) and 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; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Bacterial Cell growth Assays.

6. **Multivalent carbohydrate structures**

Dr Robin Thomson and [Prof Mark von Itzstein](#)

Medicinal Chemistry

Interactions between cells, and between cells and microorganisms, are believed to be based on simultaneous, multiple interactions between receptors and their ligands. Attempts to mimic these interactions by the use of multivalent arrays of receptor ligands, for example dendritic structures terminated with biologically relevant molecules, have been successful for a number of carbohydrate-recognising proteins. This project involves the design and synthesis of small multivalent structures, carrying functionalised carbohydrate residues, to be used as biological probes in a number of physiological systems.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC.

7. **Investigation of the structure of CMP-sialic acid synthetase**

Mr Jeff Dyason, Dr Thomas Haselhorst, Dr Robin Thomson and [Prof Mark von Itzstein](#)

Structural Biology, Molecular Modelling

Sialic acids are 9-carbon amino-sugars which are found predominantly at the termini of mammalian glycoproteins and glycolipids. Their terminal location on these cell-surface structures results in their intimate involvement in processes of cell-cell, cell-microorganism, and cell-biomolecule interaction. The level of sialic acids expressed on a cell's surface varies throughout development, and in diseases such as some cancers. A number of microorganisms also express sialic acids on their surface, and it has been suggested that these sialylated glycoconjugates mimic mammalian host structures and allow the microbe to avoid detection by the host immune system.

Nucleotide synthetases, like CMP-Neu5Ac and CMP-Kdn-synthetase, play an essential role in the activation of key carbohydrates for incorporation into carbohydrate-bearing components of various glycoconjugates associated with eukaryotes and prokaryotes. The crystal structure of the CMP-Neu5Ac enzyme from *Neisseria meningitidis*, was reported in 2001. This project will involve a study of the crystal structure using computational chemistry and molecular modelling techniques with a view to a better understanding of the reaction mechanism for the formation of CMP-sialic acids, and the substrate/inhibitor specificity of the enzyme. Furthermore a high-resolution NMR spectroscopic study of the function and mechanism of the CMP-Neu5Ac and CMP-Kdn synthetase will be undertaken.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC;

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

8. Investigation of beta-glucuronidases

Mr Jeff Dyason, Dr Robin Thomson, Dr Thomas Haselhorst and [Prof Mark von Itzstein](#)

Molecular Modelling, Medicinal Chemistry, Structural Biology

Beta-Glucuronidases are a family of enzymes that catalyse the cleavage of glycosides of glucuronic acid. They have been identified in a number of mammalian and bacterial species. In man they are essential enzymes, with a major role being the normal structuring and turnover of components of the extracellular matrix. While structural information is available for one human exo-beta-glucuronidase, there is less known about other mammalian and microbial enzymes with similar catalytic function. This project offers a number of avenues for the investigation of beta-glucuronidases; from computational chemistry and molecular modelling studies with the structure of the human beta-glucuronidase, to the synthesis of probes to explore enzyme function and activity, to biological evaluation of these probes and known substrates/inhibitors using enzyme assay and NMR spectroscopy, all of which will lead to an improved understanding of this important class of enzyme.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Computational Chemistry including visualisation and molecular docking; Advanced NMR techniques including STD-NMR.

9. The development of sialylmimetic nucleosides as probes of sialyltransferase and sialic acid transporter proteins

[Prof Mark von Itzstein](#) and [Dr Darren Grice](#)

Medicinal Chemistry

Inexpensive carbohydrates provide exciting potential for the synthesis of novel mimetics of biologically active carbohydrates. One such example is the common carbohydrate fructose. This project will involve the synthesis of a number of carbohydrate mimetics based on fructose, more specifically the synthesis of potential inhibitors of sialyltransferase and sialic acid transporter proteins. Sialyltransferase and sialic acid transporter proteins are indicated as playing a vital role in the progression of cancer metastasis in some cancers. Development of such inhibitors will allow us to develop a better understanding of their role in cancer metastasis and insight into substrate/inhibitor specificity. The ultimate outcome from this work is to produce a chemotherapeutic agent or glycopharmaceutical for the treatment of certain cancers.

Techniques: Synthetic Carbohydrate Chemistry; Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC.

10. **Ganglioside/Micelle protein interaction studied by Saturation Transfer Difference (STD) NMR spectroscopy**

Dr Thomas Haselhorst and [Prof Mark von Itzstein](#)

Structural Biology

Gangliosides, glycosphingolipids, characterised by the presence of sialic acid residues are components of vertebrates cell plasma membrane where they play an important role in a variety of surface events such as recognition of external ligands, biotransduction of membrane-membrane information and host-cell interactions. This project involves the investigation of the interaction of gangliosides with target proteins and lectins. Due to the hydrophobic ceramide tail of the ganglioside and therefore the broad ¹H NMR spectra, it is planned to form ganglioside/micelles in solution and to analyse by NMR spectroscopy. To date, no protein ganglioside/micelle interactions have been studied by NMR methods. Especially the transferred NOESY and the STD NMR experiments are the methods of choice to investigate ganglioside/micelle-protein interactions in solution.

Techniques: Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Advanced NMR techniques including STD-NMR.

11. **Structure Affinity Relationship (SAR) by NMR**

Dr Thomas Haselhorst and [Prof Mark von Itzstein](#)

Structural Biology

Recently, 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 ¹⁵N labelled *rhesus* rotavirus VP8* protein in minimal media and the analysis of the purified labelled protein by means of high resolution NMR spectroscopy. ¹⁵N/¹H-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 into lead structures for the design of new anti-viral drugs.

Techniques: Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Advanced NMR techniques including STD-NMR; Protein purification.

12. **In silico and NMR-based library screening of the *trans*-sialidase of *Trypanosoma cruzi***

Dr Thomas Haselhorst and [Prof Mark von Itzstein](#)

Structural Biology, Biochemistry

Chagas disease is an incurable condition that afflicts millions of individuals in Latin America. The intracellular protozoal parasite *Trypanosoma cruzi* is responsible for this debilitating condition. Recently, the x-ray structure of the *trans*-sialidase has been solved. It is believed that the *trans*-sialidase plays an important role and it is believed that this enzyme is an important target to design novel anti-Chagas drugs.

This project involves the expression and purification of the *trans*-sialidase followed by an extensive NMR-based analysis of Neu5Ac derivatives and mimetics in complex with

this interesting enzyme. STD NMR experiments will be performed with selected Neu5Ac derivatives and mimetics to determine the binding epitope of these ligands. Furthermore, potential ligands with high internal flexibility can also be analyzed by means of transferred NOESY and computational methods to determine the bioactive conformation. The results gained from a NMR-based analysis in solution can eventually contribute to the design of novel drugs in the battle against Chagas disease.

Techniques: Chemical Characterisation including Proton and Carbon-13 NMR, Mass Spectrometry; Purification including HPLC; Advanced NMR techniques including STD-NMR; Protein purification; Biological Assays.

13. Structure-based discovery of anti-parainfluenza viral agents

Dr Patrice Guillon, Dr Robin Thomson, Mr Jeff Dyson, Dr Thomas Haselhorst and [Prof Mark von Itzstein](#)

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 and elderly are preferentially 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 an ideal target for new antiviral drugs discoveries. HN protein is crucial in several steps of the virus life cycle. Firstly, HN protein 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 protein that allows the cell/virus membranes fusion. HN protein has also an important action during the viral budding process because it cleaves sialic acid on 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 are focused on the development of high potency inhibitors.

The X-ray crystal structures of HN glycoprotein of hPIV type 3, 5 and Newcastle Disease Virus are available and can be used as homology models for the study of other hPIV subtypes. The combination of molecular modelling and synthetic chemistry techniques may provide new carbohydrate-based inhibitors of viral replication. Using biochemistry and structural biology techniques on whole virus and recombinant HN glycoprotein, the project will investigate the effect of these new inhibitors on the virus/glycan interaction.

14. The molecular pathogenesis of melioidosis: Bacteria-host interactions in the upper respiratory tract

[Prof Ifor Beacham](#), Prof Alan Mackay-Sim (Eskitis Institute) and Dr Michael Batzloff (QIMR)

Molecular Microbiology

Melioidosis is a disease endemic to tropical Australia and SE Asia, and an emerging disease worldwide. It is caused by the soil dwelling Gram-negative bacterium, *Burkholderia pseudomallei*. Infection, which peaks in the rainy season, is by inhalation or via wounds.

In this project we seek to identify host and bacterial factors enabling infection of the nasal mucosa in mice, principally the nasal associated lymphoid tissue (NALT) and the olfactory epithelium.

The infection of nasal mucosa will be studied using a variety of bacterial mutants (already constructed) to establish the possible role of quorum sensing, type 3 and type 6 secretion systems and motility. Similarly, we will utilise knockout strains of mice deficient in certain toll-like receptors. Certain lectins, which we predict will interfere with the infection of NALT, may also be utilised.

These investigations will involve (see reference below) animal infection, followed by dissection and examination of bacterial load, and also high-resolution immunofluorescence microscopy of appropriate sections. Bioluminescence imaging may also be utilised. The project brings together scientists involved in bacterial pathogenesis (IB and MB) and in the study of the nasal mucosa, neurobiology and immunofluorescent imaging/microscopy (AM-S and AM) and collaboration between The Institute for Glycomics, Eskitis Institute for Cell and Molecular Therapies and the Queensland Institute for Medical Research.

Owen, S J, Batzloff, M, Chehrehasa, F, Meedeniya, A, Casart, Y, Logue, C-A, Hirst, R G, Peak, I R, Mackay-Sim, A and Beacham, I R (2009). Nasal associated lymphoid tissue (NALT) and olfactory epithelium as portals of entry for *Burkholderia pseudomallei* in murine melioidosis. *Journal of Infectious Diseases* **199**: 1761-1770.

15. **Carbohydrate-based compounds as potential anti-bacterial agents**

[Dr Milton J Kiefel](#) and [Dr Jennifer Wilson](#)

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.

16. **Investigations into bacterial virulence factors – potential drug targets?**

[Dr Jennifer Wilson](#) and [Dr Milton J Kiefel](#)

Molecular Biology, Structural Biology

The prevalence of drug-resistant bacteria is becoming a major worldwide health problem. Of particular concern is the emergence of multidrug-resistant Gram-negative

bacteria, which have evolved many virulence factors that are essential for them to infect host organisms. This project aims to investigate specific proteins and enzymes that are known to play important roles in bacterial virulence, with a view to determining if these virulence factors are potential drug targets. Importantly, whilst these proteins and enzymes are known virulence factors, it is not yet known if blocking their function will result in disruption of bacterial virulence. This study aims to determine this by undertaking the expression and purification of proteins and enzymes associated with bacterial virulence. These biomolecules will then be analysed using high field NMR spectroscopy to determine substrate-specificity profiles and provide an insight into the potential of developing small molecule inhibitors of these virulence factors. Students undertaking this project will learn modern protein expression and purification techniques, particularly those relating to the expression and purification of membrane associated proteins. Students will also gain "hands-on" experience with the use of high field NMR spectroscopy, and will have the opportunity to undertake some preliminary drug design techniques.

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

[Assoc Prof Victoria Korolik](#) and 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 through 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.

18. Identification of *Campylobacter jejuni* lectins involved in bacteria-host interactions

[Assoc Prof Victoria Korolik](#) and Dr Christopher J Day

Molecular Microbiology

Carbohydrates (or glycans) that modify proteins and lipids play a key role in numerous cell recognition events, including those involved in the regulation of the immune

system, and in the attachment of pathogenic organisms to host tissue. Protein-carbohydrate interactions have been identified as adherence factors for numerous commensal and pathogenic bacteria. These interactions between carbohydrates and pathogens are dependent on carbohydrate binding proteins known as lectins.

Campylobacter jejuni, the most prevalent cause of gastroenteritis in developed countries, is a highly motile, Gram-negative spiral rod that requires microaerobic conditions for growth. *C. jejuni* is a zoonotic pathogen, being a commensal organism in poultry and other wildlife. *C. jejuni* does not ferment or oxidise carbohydrates as a carbon source, instead relying on amino acids such as aspartate and serine. All carbohydrate binding is attributed to interactions important for adherence and/or colonisation of the host rather than for energy acquisition. *C. jejuni* has recently been shown to recognise a range of carbohydrate structures including Fucose and Sialic acid containing glycans.

C. jejuni lectins are yet to be identified or characterised, however, two putative lectins have been identified through homology to known lectins of other pathogenic organisms.

This project will involve screening for additional lectins expressed by *C. jejuni* which will be performed using additional bioinformatic analysis and affinity chromatography to find glycan binding proteins from *C. jejuni* cell lysates. The putative lectins identified will be characterised using isogenic mutation of the genes and glycan binding analysis of the mutant bacteria and purified protein expressed using an *E. coli* expression system.

The identification and characterisation of *C. jejuni* lectins may provide new targets for the development of novel therapeutics for *C. jejuni* infection.

19. Characterisation of *C. jejuni* lipooligosaccharides (LOS) and their role in human autoimmune disease

[Assoc Prof Victoria Korolik](#), [Dr Jennifer Wilson](#) and Dr Christopher J Day

Molecular Microbiology, Structural Biology

Campylobacter jejuni is now well established to be a world-leading cause of bacterial food-borne gastroenteritis. The symptoms vary in severity and may include nausea, severe or bloody diarrhea, abdominal cramping and fever. The infection is usually self-limiting, however, in rare cases may progress into a debilitating polyneuropathic disorder - Guillain-Barré Syndrome (GBS) or its oculomotor variant the Miller-Fisher Syndrome (MFS). Factors like infection severity; host's predisposition and ganglioside molecular mimicry have been linked to the development of these neuropathies. Mimicry of the host's gangliosides by the *Campylobacter* lipooligosaccharides (LOS) has been extensively studied over the last two decades. Gangliosides are important cell membrane glycosphingolipid structures associated with cell to cell recognition, cell growth and differentiation that profuse in high concentration in the peripheral nervous system (PNS). *C. jejuni* mediated GBS and MFS patients were found to produce antibodies cross reacting with GM₁, GM_{1b}, GD_{1a}, GalNAc-GD_{1a} GT_{1a} and GQ_{1b}, gangliosides

The aim of this project is to generate *C. jejuni* 11168 isogenic mutants in a number of specific genes in the core OS gene cluster. The mutants will be generated by insertional inactivation of each gene using non-polar antibiotic resistance cassettes.

C. jejuni 11168 isogenic mutants will be grown and the modified LOSs harvested. The differences in the biological function (serological, adhesion and colonisation) of the

LOSs produced by wild type and isogenic mutants of *C. jejuni* 11168 will be compared, using various immunoassay formats, human cell culture, as well as avian and mammalian animal models.

20. Structural characterisation of *C. jejuni* lipooligosaccharides (LOS) mutants

[Dr Jennifer Wilson](#), [Dr Darren Grice](#), Dr Christopher J Day and [Assoc Prof Victoria Korolik](#)

Molecular Microbiology, Medicinal Chemistry, Structural Biology

Campylobacter jejuni, is recognized as the most common causative agent of bacterial gastroenteritis worldwide. Infection in humans primarily results from consumption of contaminated poultry products, however sources of sporadic infection can include raw milk and contaminated drinking water. Clinical symptoms may include abdominal pain, diarrhoea and fever but the disease is usually self-limiting. It is now well recognised that the onset of the development of the neuropathies Guillian Barre and Miller Fischer syndromes is preceded by an episode of campylobacteriosis. The lipooligosaccharides of *Campylobacter jejuni* have been implicated in the initiation of the autoimmune response that causes the development of these diseases. In this project *C. jejuni* 11168 mutants will be grown and the modified lipooligosaccharides LOSs harvested. These LOSs will be purified and their core oligosaccharide (OS) structures determined by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. Glycan array will also be used to characterise these oligosaccharides. In addition, the in vitro the biological activity of the LOS harvested from the mutant bacteria will be tested

21. Regulation of cell surface sialylation by targeting the CMP-sialic acid transporter: Towards the development of anti-metastatic agents

[Dr Joe Tiralongo](#) and Dr Thomas Haselhorst

Biochemistry, Structural Biology

A correlation between increased cell surface sialylation and the metastatic potential of various cancers has been extensively reported [1]. Changes in the specific pattern and quantities of sialic acid (Sia) may account for the increased propensity of cancer cells, particularly colorectal cancer, to disseminate and survive in the circulation.

The sialylation of glycoproteins and glycolipids occurs in the Golgi apparatus, and a key step in this process involves the transport of CMP-Sia into the Golgi by the CMP-Sia transporter (CST). On the proposed model of metastasis, inhibition of the CST should result in a reduction in cancer cell surface sialylation and hence metastatic potential, and indeed this has been shown to be the case [2].

With the ultimate aim of developing novel anti-metastatic agents through the regulation/inhibition of cancer cell surface sialylation, we have now synthesised and screened a discrete library of glycosyl- and sialyl-nucleosides as potential inhibitors of the CST. A number of these compounds are showing promising inhibitory activity against the CST, and are now being assessed for their ability to inhibit cancer cell sialylation in a cell-based assay. However, for the development of additional potent inhibitors of the CST a better understanding of the protein-inhibitor interactions critical for CST inhibitor recognition will be critical. To this end, we have developed a novel method for interrogating the binding site of the CST with various ligands and putative inhibitors by Saturation Transfer Difference (STD) NMR spectroscopy [3]. Our methodology utilises Golgi-enriched fractions isolated from *Pichia pastoris* over-

expressing recombinant CST that are directly applied to STD NMR spectroscopy. In this project we will use putative inhibitors and ligands to identify and map the binding epitope of CST ligands/inhibitors, information that will be employed to design more potent CST inhibitors.

1. Varki, N.M. & Varki, A., 2007, *Lab. Invest.*, **87**, 851-7.
2. Petrick, A.T., Meterissian, S., Steele, G., Jr., *et al.*, 1994, *Clin. Exp. Metastasis*, **12**, 108-16.
3. Maggioni, A., von Itzstein, M., Tiralongo, J., *et al.*, *ChemBioChem*. **9**, 2784-6

22. CMP-sialic acid transporter structure elucidation: 3D crystallography

[Dr Joe Tiralongo](#)

Biochemistry, Structural Biology

A correlation between increased cell surface sialic acid (Sia) and the metastatic potential of various cancers has been extensively reported [1 and references therein]. The sialylation of glycoconjugates occurs in the Golgi apparatus, and a key step in this process involves the transport of CMP-Sia into the Golgi by the CMP-Sia transporter (CST). On the proposed model of metastasis, inhibition of the CST should result in a reduction in cancer cell surface sialylation and hence metastatic potential, and indeed this has been shown to be the case [2].

Even though the CST, a member of a highly conserved family of type III transmembrane proteins collectively referred to as nucleotide sugar transporters (NST), has been well characterised at the biochemical level, the 3D structure of the CST is yet to be elucidated.

A rate-limiting step in the process of membrane protein structure elucidation is the quantitative production and purification of functional recombinant protein. We have now established an efficient procedure for the expression in *Pichia pastori* and single-step purification of milligram quantities of functional CST that will be used in this project for 3D-crystallography trials.

1. Varki, N.M. & Varki, A., 2007, *Lab. Invest.*, **87**, 851-7.
2. Petrick, A.T., Meterissian, S., Steele, G., Jr., *et al.*, 1994, *Clin. Exp. Metastasis*, **12**, 108-16.

23. Probing Microbe-Glycan and Primary Cell-Glycan interactions using Glycan Array Technology

[Dr Joe Tiralongo](#) and Dr Christopher J Day

Biochemistry, Molecular Microbiology

The identification and characterisation of carbohydrate binding proteins (or lectins) has been greatly enhanced through the development of glycan array technology. Binding specificities can be effectively interpreted by probing arrays, consisting of glycans (carbohydrates) of known structure covalently immobilised onto glass-slides. Typically, glycans are printed using robotic printing technology (similar to that used to prepare DNA microarrays) onto an appropriately functionalised glass-slide allowing covalent attachment to the glass surface. Specific interactions can then be visualised following interrogation of the glycan array with, for example, a fluorescent-labelled bacteria or cultured cells.

Glycan arrays are fast becoming the technique of choice for identifying and elucidating the specificity of lectins, and have been successfully used to identify and characterise the interactions of bacteria with their glycan receptors [1]. However, there are few reports of glycan arrays being used to study carbohydrate recognition by mammalian cultured cells.

1. Day, C.J., Tiralongo, J., Hartnell, R.D., *et al.*, *PLoS One*, **4**, e4927

24. Establishment of a CellFrac microarray

[Dr Joe Tiralongo](#) and Dr Christopher J Day

Biochemistry

Adhesion to host cells is an essential virulence factor for the vast majority of microbial pathogens, with the specificity of these interactions being a major determinant of tissue tropism. Cultured mammalian cells have long provided valuable model systems for investigating host-pathogen interactions. However, different cultured cells possess distinct and diverse cell surface components to which pathogenic microbes are able to bind. Therefore, technologies that enable the interaction of pathogenic microbes with an array of cultured cells, or the corresponding cellular components isolated from those cells, to be evaluated in a high-throughput manner would be a valuable tool for studying tissue tropism. Therefore, our interest in better understanding host-pathogen interactions led us to establish a discrete CellFrac microarray comprising cytosolic and membrane fractions isolated from 15 diverse mammalian cell lines immobilized onto glass slides. This lab-on-a-chip technology has the potential to replace traditional cell-based binding assay systems for the study of not only host-pathogen but also protein-protein interactions.

In this project we will further validate our technology using antibodies and lectins of known specificity, as well as exploring host-pathogen interactions using a number of different microbes, including bacteria and fungal pathogens.

25. Isolation and characterisation of novel lectins from Australian macrofungi

[Dr Joe Tiralongo](#) and Dr Evelin Tiralongo (School of Pharmacy)

Biochemistry

Lectins are important sugar binding proteins that are ubiquitous in nature, occurring in plants, bacteria, viruses, fungi, animals and humans. Lectins can reversibly bind, but do not modify, free sugars, sugar residues of polysaccharides, glycoproteins or glycolipids leading to various physiological effect, including the ability to agglutinate cells. Lectin carbohydrate specificity varies widely and can be highly specific for certain glycan structures. Therefore, lectins can exhibit very specific affinity towards certain cell types depending on glycosylation patterns. Many lectins are quite toxic and are thought in plants to play a crucial role in the defense system; however, lectins from some food sources such as tomatoes, lentils, peas are non-toxic [1].

Lectins have been isolated and purified from plants, mushrooms, animals and microorganisms, and of the 60 or so commercially available, only that from *Agaricus bisporus* is of fungal origin. Australian fungi are greatly under-explored with a considerable number of un-described species of known chemical, genetic and biological profiles. Therefore, investigating fungal species may lead to the identification of novel lectins with unique glycan specificities that may prove to be useful for Glycomics and related biomedical and cancer research.

In this project protein extracts of Australian mushrooms will be generated and putative lectins isolated using well-established techniques including protein precipitation, size exclusion, ion exchange and affinity chromatography. Lectin purification will be monitored using a haemagglutination assay and assessed *via* SDS-PAGE. Isolated lectins will be biochemically characterised, specifically for lectin carbohydrate specificity.

1. Lehmann, F., Tiralongo, E., and Tiralongo, J. (2006) *Cell. Mol. Life Sci.* **63**, 1331-1354

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

[Dr Jennifer Wilson](#) and [Dr Darren Grice](#)

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-lipid A molecule is known as lipooligosaccharide (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.

27. The synthesis of novel substrate molecules to probe the function of a unique glycosyltransferase enzyme from *Moraxella catarrhalis*.

[Dr Jennifer Wilson](#), Assoc Prof Vito Ferro and [Dr Darren Grice](#)

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. *Moraxella catarrhalis* is one of the microorganisms that causes of middle ear infection in children.

In our recent studies of *M. catarrhalis* we have identified an unusual glycosyltransferase enzyme that has a number of features that distinguish it as being unique. It appears that this enzyme is responsible for the addition of three glucose residues to a central core glucose – typically, the addition of each sugar would require a separate enzyme and it is twice the length of a “normal” glycosyltransferase enzyme. To understand how this glycosyltransferase operates in this unique way we need specific “designer” substrate molecules to be synthesised so that we can probe the biological function of this enzyme.

In this project a number of synthetic probes will be synthesised to help us to understand how this enzyme which is crucial to *M. catarrhalis* survival function operates.

28. Analysis of lipopolysaccharide structures from *Moraxella bovis*

[Dr Darren Grice](#), [Dr Ian Peak](#) and [Dr Jennifer Wilson](#)

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.

29. Development of Novel Glycosidase Inhibitors as Potential Therapeutics

[Dr Todd A Houston](#)

Medicinal Chemistry

Glycosidase inhibitors have a wide range of biological activities including anticancer, antiviral (e.g. Relenza), and antidiabetic properties. Our group is involved in the synthesis and testing of novel glycosidase inhibitors. We have identified a unique beta-galactosidase inhibitor by tethering a boronic acid onto a known amine inhibitor. The boronate should increase the affinity of this compound for cell-surfaces and serve as a method of drug targeting. Currently, we are applying this modification to other, more potent glycosidase inhibitors in order to increase the selectivity of these compounds.

30. Design of Fluorescent Receptors for Carbohydrates and Related Biomolecules

[Dr Todd A Houston](#) and [Dr Milton J Kiefel](#)

Medicinal Chemistry

Bis(boronic acids) have been widely used in the development of chemosensors for sugars, most importantly glucose. We have recently reported that bisboronates have an even higher affinity for bis(alpha-hydroxycarboxylates) such as tartrate. We are now designing novel boronate-based receptors for hydroxyacids (sialic acid and KDO) and inositols, an important class of cell-signalling molecules. The latter type may have the biological impact of slowing cell growth, and thus may be coupled with other compounds in cancer therapy. Ultimately, these receptors will be developed into "boronlectins" that target specific cell types and may be used in drug targeting. They can identify targets complementary to those of nature's antibodies and lectins that normally survey cell surfaces.

31. **Synthesis and Drug Targeting of Antitubercular Compounds**

[Dr Todd A Houston](#)

Medicinal Chemistry

Tuberculosis is the most deadly infectious disease in the world today and there is a need for new, more effective, antitubercular compounds. Based on the affinity of *Mycobacterium tuberculosis* for cholesterol-rich membrane regions found in macrophages, we are synthesizing a number of steroid-based amines and aminoglycosidases for testing as inhibitors of mycobacterial growth. The biological testing will be carried out by collaborators at facilities that can deal with drug-resistant strains of the microorganism. This drug targeting protocol is being applied to compounds active against *Leishmania* protozoa, the causative agent of the widespread tropical disease, leishmaniasis.

32. **Bacterial manipulation of the immune system: how *Burkholderia pseudomallei* alters host cells to survive intracellularly**

[Dr Ian Peak](#) and other supervisors will be project-specific, may include [Prof Ifor Beacham](#), Dr Gary Grant (School of Pharmacy), Assoc Prof Nigel Morrison (School of Medical Science)

Molecular Microbiology

The innate immune system's role is to contain infection, including macrophages whose role is phagocytose and kill bacteria. However, the bacterium *Burkholderia pseudomallei* can invade and multiply within macrophages. *B. pseudomallei* causes the disease melioidosis, which can be fatal and is localized to tropical regions: in NT it is the commonest fatal community acquired pneumonia. It is widespread in other areas of SE Asia, and *B. pseudomallei* is considered a potential agent of bioterrorism.

The aim of the project is to investigate how *B. pseudomallei* overcomes the innate immune system and how it manipulates the host cell.

The student will gain experience in a range of the following techniques: Molecular genetics techniques, immunofluorescence and time-lapse 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.

33. **Bacterial glycans: role of *Burkholderia pseudomallei* LPS, capsule and glycosylated proteins in virulence**

[Dr Ian Peak](#) and other supervisors dependent on specific project, may include [Prof Ifor Beacham](#), [Prof Michael Jennings](#) and Gary Grant (School of Pharmacy)

Molecular Microbiology, Molecular Biology

The bacterium *Burkholderia pseudomallei* causes the disease melioidosis, which can be fatal, and is localized to tropical regions: in NT it is the commonest fatal community acquired pneumonia. It is widespread in other areas of SE Asia, and *B. pseudomallei* is considered a potential agent of bioterrorism. Its capsule and LPS are considered primary virulence factors, yet there is evidence to suggest their role may be more complex. In

addition, we believe that glycosylation of bacterial proteins has an important role in the capacity of this organism to survive and within host cells.

The aim of the project is to identify bacterial glycans, and to assess how *B. pseudomallei* glycans contribute to pathogenesis.

The student will gain experience in a range of the following techniques: Molecular genetics techniques, protein expression, protein purification and analysis by Mass Spectrometry, cell culture and *in vitro* infections, *in vivo* infections using mouse knock-outs, immunofluorescence and time-lapse microscopy

34. Evolution of random gene switching mechanisms in bacterial pathogens

[Prof Michael Jennings](#)

Molecular Biology, Molecular Microbiology

Host adapted bacterial pathogens have evolved mechanisms to evade host immune responses. One of these mechanisms is phase variation (high frequency, reversible ON/OFF switching of gene expression). In most cases phase variation is mediated by hypermutagenic DNA repeat sequences in the coding sequence of the phase variable gene. Alternations in DNA repeat number lead to frame shift mutations. The DNA repeats that mediate these ON/OFF switching events are transcribed and translated and encode repeated peptide sequences in the resulting protein. These peptide sequences are not required for protein function, but must be compatible with the function of the protein in which they have evolved. For example, many phase variable genes encode glycosyltransferases that function to synthesize surface oligo- and polysaccharides. The constraints on the type of repeat sequences and position in a coding sequence where it can evolve has not been addressed. The aim of this project is to use experimental evolution to observe the evolution of new high frequency switching mechanisms in bacterial glycosyltransferases. The project will involve construction of selectable/counter-selectable fusions with target genes, to cycle these constructs through multiple round of selection and counter-selection, to identify and characterize the resulting high frequency mechanisms that have evolved in this model system.

Moxon, R., Bayliss, C. & Hood, D. Bacterial contingency loci: the role of simple sequence DNA repeats in bacterial adaptation. *Annu Rev Genet* **40**, 307-33 (2006).

35. Substrate specificity of phase variable methyltransferases associated with phasevarions

[Prof Michael Jennings](#)

Molecular Biology

Many host-adapted bacterial pathogens contain DNA methyltransferases (mod genes) that are subject to phase-variable expression (high-frequency reversible ON/OFF switching of gene expression). In the important human pathogens *Haemophilus influenzae*, in *Neisseria meningitidis* and *Neisseria gonorrhoeae* the random switching of the *modA* gene controls expression of a phase-variable regulon of genes (a “phasevarion”), via differential methylation of the genome in the *modA* ON and OFF states. Many *modA* alleles exist in these organisms, each recognizing a distinct DNA target sequence. In the pathogenic *Neisseria* we have recently shown that *ModA* ON and OFF strains have distinct phenotypes in antimicrobial resistance, in a primary human cervical epithelial cell model of infection, and in biofilm formation. Phasevarions may be a common strategy used by host-adapted bacterial pathogens to

randomly switch between “differentiated” cell types. Although it is clear that phenotypic differences exist in the ModA ON and OFF states, the molecular mechanism whereby differential methylation of the genome alters gene expression at the promoter level is not established. Further examination of this process is not possible in most phasevarion systems as only the DNA target site that is modified by modA1 and modA13 methyltransferases is known. The target site is not known for some systems when we have observed key phenotypes (eg. ModA11, ModA2). The aim of this project will be to identify ModA methylation target sites in these systems using novel approaches.

Srikhanta, Y.N., Maguire, T.L., Stacey, K.J., Grimmond, S.M. and Jennings, M.P. 2005. The Phasevarion: a novel genetic system controlling coordinated, random switching of expression of multiple genes. *PNAS* **102**:5547-5551.

Yogitha N. Srikhanta, Stefanie J. Dowideit, Jennifer L. Edwards, Megan L. Falsetta, Odile B. Harrison, Kate L. Fox, Kate L. Seib, Tina L. Maguire, Martin C. Maiden, Sean M. Grimmond, Michael A. Apicella & Michael P. Jennings. 2009. Phasevarions mediate random switching of gene expression in pathogenic *Neisseria*. *PLoS Pathogens* **5**:e1000400.

Fox K.L., Srikhanta, Y.N., and Jennings M.P. 2007. Phase variable type III restriction-modification systems of host adapted bacterial pathogens. *Molecular Microbiology* **65**:1375-9

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

[Prof Michael Jennings](#) and Dr Freda Jen

Molecular Biology, Molecular Microbiology

Many pathogenic bacteria modify proteins after translation. Some of these modifications 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.

Power, P.M. & Jennings, M.P. 2003. The Genetics of Glycosylation in Gram-negative bacteria. 2003 A microreview for *FEMS Micro Letters* **218**:211-222.

Warren, M J and M P. Jennings. 2003 Identification and Characterization of *pptA*: a Gene Involved in the Phase-Variable Expression of Phosphorylcholine on Pili of *Neisseria meningitidis* *Infection & Immunity* **71**:6892-8.

Ku, S.C., Schulz, B.L., Power, P.M., Jennings, M.P. 2008. The pilin O-glycosylation pathway of pathogenic *Neisseria* is a general system that glycosylates AniA, an outer membrane nitrite reductase. *Biochemical & Biophysical Research Communications* **78**:84-9

37. **Rotavirus: Structure Based Drug design**

[Dr Helen Blanchard](#), [Prof Mark von Itzstein](#), Dr Robin Thomson, Dr Thomas Haselhorst and Mr Jeff Dyason

Molecular Biology, Structural Biology, Medicinal Chemistry

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 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 drugs that could bind and block the active site of VP8* thus preventing the virus particle from attaching to the host cell and causing infection.

This project has the potential to combine the design and chemical synthesis of potential carbohydrate-based drugs with structure based modelling and X-ray crystallographic determination of the structures of VP8* in complex with these potential drugs.

Aims: Structure-based design of potential inhibitors of rotavirus and their synthesis.

Techniques: a) Expression and purification of wild-type and mutant VP8* protein from different strains b) Synthesis of compounds c) Assessment of ligand binding by Saturation Transfer Difference NMR d) Protein crystallisation of VP8* with sialic acid derivatives bound in the active site and elucidation of atomic structure by X-ray crystallography e) Computational Chemistry including visualisation and molecular docking

Effects on sialic acid recognition of amino acid mutations in the carbohydrate-binding cleft of the rotavirus spike protein. Kraschnefski, M.J., Bugarcic, A., Fleming FE., Yu, X., von Itzstein. M., Coulson, BS and Blanchard, H. (2009) *Glycobiology* **19**(3):194-200

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

38. **The Macrophage-Inducible C-type lectin (Mincle)**

[Dr Helen Blanchard](#)

Molecular Biology, Biochemistry, Structural Biology, Molecular Modelling

Mincle is a novel C-type lectin that has been identified as an innate immune receptor for the pathogen *Candida albicans* (yeast), and with a wider role in inflammation. *Candida albicans* presents several molecules to the innate immune system, and these molecules change depending on the virulence of the yeast strain as well as the growth cycle stage of the yeast during an infection. It is not known which yeast molecules are recognised by Mincle. Understanding the nature of ligand-receptor interactions is critical for design of new therapeutics for infections such as *Candida*, and for tissue damage, pain and swelling caused by inflammation.

Aims: a) Structure determination of Mincle by X-ray crystallography b) Investigation of Mincle-ligand interactions c) Structure-based design of potential inhibitors.

Techniques: a) Expression of recombinant Mincle protein. b) Protein crystallisation c) X-ray crystallographic structure determination of Mincle. d) ELISA and saturation transfer difference NMR for assessment of protein-ligand recognition.

This project focuses on elucidating atomic details of Mincle and investigation of the nature of its interactions with small molecule ligands. This will provide information that is critical to the design of potent drugs.

Human and Mouse Macrophage-Inducible C-type Lectin (Mincle) bind *Candida albicans* Bugarcic, A., Hitchens, K., Beckhouse, AG. Wells, CA., Ashman, RB and Blanchard, H. *Glycobiology* (2008) Sep;18(9):679-85

39. Targets in Cancer: Structure-based investigation of Galectin-1

[Dr Helen Blanchard](#) and Assoc Prof Steve Ralph (School of Medical Science)

Molecular Biology, Biochemistry, Molecular Modelling, Structural Biology

To metastasise, malignant cells from primary tumours migrate via the lymphatics to draining lymph nodes, or traverse into the circulatory system to distant sites where they can adhere to endothelial cells lining the blood vessels, proliferating to generate further tumours. Galectins, a family of carbohydrate-binding proteins (lectins) that recognise beta-galactosides in oligosaccharides, are involved in metastasis.

Altered galectin-expression by tumours significantly influences their survival and progression to metastasis. Galectin-1 is important as a potent immunosuppressive factor that inhibits anti-cancer cell immune responses by causing activated T-cell death, facilitating invasiveness and dissemination of tumours. Galectin-1 utilises carbohydrate-recognition during such processes, and targeting this function, is a means for therapeutic design.

Aims: a) Determination of the atomic structure of mutant galectin-1 b) Assess effects of mutation of on galectin-1's function.

Techniques: a) Molecular modelling, structure analysis using the atomic structure of galectin-1 b) Protein expression and protein crystallisation of mutant galectin-1 c) X-ray crystallographic structure determination of mutant galectin-1 to assess affects of mutation on structure and ligand-interactions. d) Known galectin-1 inhibitors will be tested as functional inhibitors via *in vitro* based immunological cytotoxic T cell assays using splenic derived lymphocyte cultures prepared from mice immunised against cancers as a source of activated T cells and then grown in the presence of the tumour cells,

This project progresses the design of therapeutics of galectin-1 that has roles in mitogenesis and apoptosis (cell suicide) of immune cells.

40. Galectins: Targets in Cancer: Synthesis of Galectin-specific Inhibitors

[Dr Helen Blanchard](#) and [Dr Todd A Houston](#)

Medicinal Chemistry, Structural Biology, Molecular Modelling

This synthesis project, with computational chemistry and NMR options, forms part of multi-disciplinary research program incorporating structure-based design of drugs targeting proteins with critical roles in cancer.

Tumourigenesis is a complex multi-step process involving changes in cell proliferation and adhesion. To metastasise, malignant cells from the initial tumour migrate into the circulation and adhere to endothelial cells, proliferating to form new tumours. Altered galectin-expression significantly influences this process. Galectin-3 is important in human cancer. These carbohydrate-binding proteins recognise and bind beta-galactosides. This project focuses on synthesis of specific inhibitors of galectin-3, including those based on the carbohydrate taloside framework, as part of an overall goal to develop lead compounds as novel anticancer agents.

Aims: a) Synthesis of carbohydrate based compounds as inhibitors of galectin-3 b) Computational design of potential inhibitors and assessment of protein-ligand interactions.

Techniques: a) Carbohydrate chemistry synthesis of compounds b) Molecular modelling to assist direct the compound design. Compounds prepared will be assessed for binding to galectin-3 via Saturation Transfer Difference (STD) NMR.

Compounds with improved pharmacological profiles over current galactin ligands will be sought. Deoxygenated galactose derivatives that may fit such criteria and serve as precursors to novel taloside derivatives will be synthesized by known methods.

41. Three-Dimensional Atomic Structure Determination of galectin-inhibitor complexes by X-ray crystallography: Design of galectin-specific drugs

[Dr Helen Blanchard](#) and Assoc Prof Ulf Nilsson (Lund University, Sweden)

Structural Biology, Molecular Modelling

Focus is on elucidating atomic structure of the carbohydrate-binding proteins galectins that are involved in inflammation and cancer. Galectin-1 and Galectin-3 are involved in metastasis, where malignant cells migrate away from the initial tumour into the circulation then adhere to endothelial cells at distant sites where they proliferate forming new tumours. Galectin-1 and galectin-3 have functional activities involved in disease that are dependent upon beta-galactoside binding, including apoptosis (cell suicide) of immune cells. These proteins are attractive targets for the development of new therapeutic strategies in oncology.

This project aims to lead to the design of inhibitors of these galectins. X-ray crystallographic determination of the protein structure enables one to see exact atomic details as well as reveal the protein's interactions with inhibitors. This insight into the protein-ligand interactions is critical to, and drives, the design of more effective small-molecule inhibitors as potential leads for drugs.

Aims: a) Crystallographic structure determination of galectin-1 and galectin-3 inhibitor complexes b) Structure analysis to input into the design of more effective inhibitors of these proteins.

Techniques: a) Protein expression and protein crystallisation, using established protocols b) X-ray crystallographic determination of the protein structures in complex with inhibitors provided by Professor Nilsson.

Protein subtype-targeting through ligand epimerization: Talose-selectivity of galectin-4 and galectin-8. Öberg, CT., Blanchard, H., Leffler, H and Nilsson, UJ. *Bioorg. Med. Chem. Lett* (2008) 3691-3694

42. **Galectin-14 : A Recently Identified Drug Target in Inflammation**

[Dr Helen Blanchard](#)

Molecular Biology, Biochemistry, Structural Biology, Molecular Modelling

Galectins are proteins involved in diverse physiological and pathological processes including immune and inflammatory responses, tumour progression and neural degeneration. Galectins are important therapeutic targets for a number of serious diseases. Galectin-14 is a recently identified carbohydrate-recognising protein implicated in allergic inflammation due to its release into the lumen of the lung in a sheep asthma model. Galectin-14 likely regulates the activity of eosinophils (a type of white blood cell) during allergic responses.

Galectin-14 is under-explored. Its atomic structure and carbohydrate specificity are not yet characterised. This project focuses on gaining knowledge of galectin-14 structure and recognition of carbohydrate-based inhibitors, and subsequent application of this information to the design of inhibitors to regulate its activity.

Aims: a) Progress crystallographic structure determination and in parallel undertake molecular modelling to generate and analyse structures of galectin-14 b) Assessment of protein-inhibitor interactions for design of more effective inhibitors of these proteins.

Techniques: a) Galectin-14 protein expression and purification using established protocols b) Protein crystallisation to generate crystals suitable for X-ray diffraction studies c) X-ray crystallographic determination of the structure of galectin-14 d) Saturation Transfer Difference (STD) NMR and ELISA methods for evaluation of the inhibitory ability of proposed inhibitors with galectin-14.

43. **Metapneumovirus : A major cause of Respiratory Tract Infection**

[Dr Helen Blanchard](#) and Prof David Gordon (Flinders University)

Biochemistry, Structural Biology, Molecular Modelling

Human metapneumovirus (hMPV) is a recently identified virus belonging to the *Metapneumovirus* genus of the *Paramyxoviridae* family. hMPV is a major cause of respiratory tract infection (RTI) in children, and also causes severe infection in the elderly and immunosuppressed. The initial step in infections is mediated by attachment of virus to cellular receptors, including carbohydrates. This research focuses on characterisation of the carbohydrate recognition domain, G protein, of hMPV and aims to provide the first information on this virus in relation to its atomic structure and its recognition of carbohydrate cellular receptors.

Aims: a) Determination of the three-dimensional atomic structure of G protein by X-ray crystallography b) Examination of G protein interactions with sugars, toward elucidation of carbohydrate specificity.

Techniques: a) Expression, purification of recombinant hMPV G protein following established protocols. b) Dynamic light scattering (DLS) to ascertain homogeneity of protein samples and characterisation of the G protein. c) Protein crystallisation d) X-ray crystallographic structure determination d) Molecular modeling in association with structure prediction techniques and Saturation Transfer Difference (STD) NMR to assess protein-sugar recognition. e) Progress structure determination of hMPV in complex with various carbohydrates, providing atomic information critical to the design of potent drugs.

Role of Cellular Glycosaminoglycans and Charged Regions of Viral G Protein in Human Metapneumovirus Infection. Thammawat, S., Sadlon, TA., Hallsworth, PG and Gordon, DL. *Journal of Virology* (2008) **82**: 11767-11774