QMB Webster Centre for Infectious Diseases QMB Satellite Symposium: Infectious Diseases and Membrane Proteins Abstracts

Q1: Suffocating Mycobacterium tuberculosis: new approaches for anti-TB drug development

Pethe, K.[1]

[1]Nanyang Technological University, Lee Kong Chian School of Medicine and School of Biological Sciences

The rapid emergence and spread of multi-drug resistant *Mycobacterium tuberculosis* and other pathogenic bacteria is a serious concern worldwide that advocates for the development of new classes of antibacterials with a novel mode of action. Current antibiotics derive mainly from natural sources and inhibit a narrow spectrum of cellular processes such as DNA replication, protein synthesis and cell wall biosynthesis. With the spread of drug resistance, there is a renewed interest in the investigation of alternate essential cellular processes, including central metabolic and bioenergetics pathways, as a drug target space for the next generation of antibiotics. However, the validation of those targets is more complex, as essentiality of such targets can be conditional. Interest in targeting central metabolism has also been muted because of a concern about selectivity with human orthologs. Nonetheless, we and others have shown that selective inhibition can be achieved for enzymes that are conserved between bacteria and humans. Oxidative phosphorylation as recently emerged as a relevant target space for the development of new classes of drug for tuberculosis. In this context, I will discuss the relevance of targeting the terminal respiratory oxidases for the development of a rational drug combination for tuberculosis and other mycobacterial diseases.

Q2: Structure and mechanism of the cytochrome bd oxidase

Safarian, S.[1]

[1]Max Planck Institute of Biophysics

The emergence of extensive and multidrug resistant pathogenic bacteria is becoming an ever-growing threat for global healthcare, posing serious clinical and societal challenges. A major survival factor in commensal and pathogenic bacteria is the cytochrome *bd* oxidase. This membrane-embedded enzyme is solely present in prokaryotes and found in almost all bacterial phylae. It acts as a terminal oxidase that catalyzes the reduction of molecular oxygen to water in respiratory chains. Splitting of the dioxygen bond without formation of reactive oxygen species (ROS) and subsequent water formation by *bd*-type oxidases requires four electrons that are extracted from two substrate quinols per catalytic cycle. This exergonic reaction is coupled to the generation of an electrochemical proton gradient across the periplasmic membrane via vectorial release and uptake of protons. Cytochrome *bd* oxidases are structurally unrelated to heme-copper oxidases (HCO) including the well-known cytochrome c oxidases and distinguish themselves by a very high oxygen affinity and insensitivity to a variety of inhibitors as well as agents released by the human immune system. Hence, *bd* oxidases enable aerobic bacteria to colonize hostile environmental niches. Survival of pathogenic bacteria during infection, proliferation and the transition from acute to chronical states is intrinsically linked to bd oxidase abundance. Consequently, the *bd* oxidase is a highly attractive target for the development of a new generation of antimicrobial drugs.

Q3. Next Generation ATP Synthase Inhibitors to Combat Drug Resistant Tuberculosis

Cheung, J.[1], Adolph, C.[1], Buckley, B.J.[2], Majed, H.[2], Aboelela, A.[2], Bujaroski, R.[2], Schillinger, K.[2], Hards, K.[1,3], McNeil, M.M.[1,3], Rhee, K.Y.[4], Kelso, M.J.[2], Meier, T.[5] and Cook, G.M.[1,3]

[1]Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin 9054, New Zealand, [2]School of Chemistry and Molecular Bioscience, University of Wollongong, NSW 2522, Australia and Illawarra Health and Medical Research Institute, Wollongong, NSW, 2522, [3]Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1042, New Zealand, [4]Weill Department of Medicine, Weill Cornell Medical College, New York 10021, United States, [5]Imperial College London, Department of Life Sciences, London SW7 2AZ United Kingdom

The emergence and rapid spread of drug-resistant strains of *Mycobacterium tuberculosis* has created an urgent need to develop antimicrobials with new modes of action. The clinical success of the ATP synthase inhibitor, bedaquiline (BDQ), has validated mycobacterial ATP generation as a high priority drug target. Interaction between a conserved arginine in the stator subunit a and residues within the oligomeric c-ring is essential for proton translocation by the bacterial F_1F_0-ATP synthase. We hypothesize that arginine mimicking compounds would inhibit ATP synthesis by competition with the stator arginine for binding to c-ring sites. Amiloride, a human diuretic that has been used clinically for over forty years, and its derivative 5-(*N*,*N*-hexamethylene)amiloride (HMA), are known arginine mimetics. We show that amiloride and HMA inhibit the growth of *M. tuberculosis* with an MIC of 250 µM and 32 µM respectively, inhibit ATP synthesis in inverted membrane vesicles (IVMs) of *M. tuberculosis* and bind to the isolated c-ring of the mycobacterial ATP synthase. Medicinal chemistry efforts developed a novel derivative, HM2-16F, which has improved potency (MIC 4 µM) and no diuretic properties. HM2-16F had a narrow spectrum of antimicrobial activity and no effect against *Escherichia coli*, *Staphylococcus aureus* or *Enterococcus faecalis* (> 256 µM). HM2-16F was bactericidal against *M. tuberculosis* with similar killing kinetics to BDQ and kills *M. bovis* BCG within THP-1 macrophages. Metabolomics showed dose-dependent depletion of intracellular ATP after treatment with HM2-16F and generated a metabolic profile that was similar to BDQ, supporting our hypothesis that HM2-16F is a novel inhibitor of the *M. tuberculosis* ATP synthase. HM2-16F did not synergize with or antagonize other TB drugs – streptomycin, INH, BDQ, PZA or RIF. A BDQ-resistant mutant of *M. smegmatis* strain R9 ($AtpE^{D32V}$) was still sensitive to growth inhibition by HM2-16F. We isolated four *M. tuberculosis* mutants that were resistant to HM2-16F ($5.1x10^{-6}$ at 5x MIC). All mutations mapped to Rv3066 a DeoR family transcriptional regulator that regulates the expression of Rv3065, a multidrug-transport integral membrane protein suggesting that HM2-16F efflux was the major mechanism of resistance in these mutants.

Q4. Why are HIV-infected individuals failing antiretroviral treatment in Africa?

Quiñones-Mateu, M.E.[1]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ

While our understanding of HIV-1 pathogenesis and response to antiretroviral treatment (ART) is strongly biased towards subtype B, the predominant HIV-1 subtype in North America and western Europe, response to treatment by non-B subtype HIV-1 strains has been the main concern in the rest of the world, particularly in African and Asian countries with multiple prevalent subtypes. The widespread global access to antiretroviral drugs has led to considerable reductions in morbidity and mortality but, unfortunately, has also increased the risk of virologic failure. Poor adherence to ART is one of the main causes for treatment failure in most necessitated LMICs, mainly due to the emergence, and potential transmission, of drug resistant viruses. On the other hand, the impact of naturally occurring polymorphisms on drug resistance and treatment success within the context of non-B HIV-1 strains is not well understood. Finally, current HIV-1 cure strategies are also focused in subtype B HIV-infected patients from industrialized countries. Here we will discuss our efforts to address these issues in Uganda. We are working on several projects aimed to better understand the role of (i) drug resistant minority variants and/or (ii) mutations outside the classical ART targets on virologic failure in this population. We will also discuss our most recent findings: a reduced peripheral HIV-1 reservoir in virally-suppressed Ugandan patients infected with non-B HIV-1 strains. Our main goal is to determine whether drug resistant minority variants in circulation and/or in the peripheral CD_4^+ T cell population contribute to treatment failure in Africa.

Q5. Evaluating immune modulation of dendritic cells by extracellular vesicles shed from human papillomavirus type 16-E7 expressing keratinocytes

Hibma, M.[1]

[1]Department of Pathology, School of Medicine, University of Otago, Dunedin.

Human papillomavirus (HPV) type 16 infections are typically cleared within two years of infection, however some lesions persist and may progress to cervical cancer. Antigen presenting cells (APCs) initiate a protective adaptive immune response, but the importance of skin and lymph node APC populations in the response to HPV and how the virus regulates their function is not well understood. Here we report the effects of extracellular vesicles from HPV E7-expressing cells on APC activation and function in a mouse model. From these studies we conclude that extracellular vesicles from E7-expressing cells suppress T cell responses at least in part by impairing dendritic cell activation and cytokine secretion. These data support blocking suppression of APCs as a novel immunotherapeutic strategy for persistent HPV infection.

Q6. Molecular Details of Dengue Virus Conformational Changes and Interactions with Antibodies

Marzinek, J.K.[1] and Bond, P.J.[1,2]

[1]Bioinformatics Institute (A*STAR), 30 Biopolis Street, #07-01 Matrix, 138671 Singapore, [2]Department of Biological Sciences, National University of Singapore, 16 Science Drive 4, Singapore 117558

A primary causative agent of infectious disease is the positive single-stranded RNA family of flaviviruses, which includes dengue (DENV), tick-borne encephalitis, West Nile virus, Japanese encephalitis, yellow fever, and Zika virus. In this work, a multiscale simulation approach has been employed to investigate the conformational changes associated with the virion envelope (E) protein during the DENV life cycle. Based on cryo-electron microscopy maps, we constructed a near-atomic resolution model of the complete viral envelope, containing E and membrane (M) proteins embedded within a lipid bilayer vesicle. We leveraged this model to probe the "breathing" dynamics of the viral envelope triggered by the host microenvironment, such as changes in pH, salt, and temperature. We also investigated the molecular details of interactions between antibodies and the virus particle under different states of maturation, and supported by diverse biophysical data, rationalize the occurrence of the antibody-dependent enhancement, which can lead to the most serious forms of DENV infection including dengue hemorrhagic fever and shock syndrome. The combinations of multiscale simulation and experiments have allowed us to gain valuable insights into the structural basis for DENV neutralization, maturation, and pathogenesis, and provide possible routes to novel therapeutics development.

Q7. Secreted forms of a nonstructural glycoprotein encoded by rotavirus activate proinflammtory signaling *via* Toll-Like Receptor 2; A novel virotoxin-based pathophysiologic mechanism in viral gastroenteritis

Taylor, J.A.[1], Bugarcic, A.[1], Didsbury, A.[1], Ge, R.[1], Wang, C.J.[1]

[1.]School of Biological Sciences, University of Auckland, Auckland, New Zealand.

NSP4 is a nonstructural glycoprotein encoded by rotavirus, a major cause of viral gastroenteritis. The predicted topology of NSP4 suggests a transmembrane location but infection of polarized Caco-2 cells with rotavirus results in the secretion of a portion of hyper-glycosylated NSP4 in a soluble, non-virus associated form despite retention of its predicted transmembrane domain. We have examined the structure, solubility and cell-binding properties of this secreted form of NSP4 to further understand the biochemical basis for its proposed enterotoxic function. Our studies reveal the ability of secreted NSP4 to bind to multiple cell type through interactions with glycoaminoglycans and a novel TLR2 agonist property resulting in the activation of intracellular signaling pathways in immune cells. NSP4 represents a novel type of secreted virotoxin, capable of amplifying pathophysiologic effects beyond the infected cells through engagement with innate immune cells and stimulating a pro-inflammatory environment in the intestine that may contribute to the clinical presentation of disease.

Q8. Glycointeractons: new opportunities to cure and prevent infectious disease

Day, C.J.[1], Edwards, J.L.[2], Poole, J.[1], Mak, J.[1], Jennings, M.P.[1]

[1]Institute for Glycomics, Griffith University, Gold Coast Campus, Qld 4222, Australia, [2]The Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital and The Department of Pediatrics, The Ohio State University, Columbus, OH 43205, USA

Many important interactions between bacterial pathogens and their hosts are highly specific binding events that involve host or pathogen carbohydrate structures called glycans[1]. Bacterial adhesins and toxins can exploit host glycans[2]. Host lectins may recognize bacterial glycans in innate immune processes or may be targeted by bacterial glycan adhesins as receptors[3]. The molecular details of many bacterial - host interactions remain to be discovered. Understanding these processes is key for the development of novel strategies for the prevention and treatment of disease. In recent years new, high-throughput glycomic technologies have identified new bacterial-host glycointeractions.

Here we present examples of novel host-glycan interactions and a label-free high-throughput screening strategy for the identification of drugs that can block these interactions. We present a case study that demonstrates the process, resulting in the identification of a drug that can be repurposed to prevent and treat multi drug resistant gonococcal cervical infection in women.

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Q9. Metal-dependent dynamic equilibrium: *A mechanism for regulation of the Plasmodium M17 aminopeptidases?*

Malcolm, T.[1], Drinkwater, N.[1], Belousoff, M.[1], Venugopal, H.[2], Atkinson, S.[3], Borg, N.[3], McGowan, S.[1]

[1]Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, 3800, Australia, [2]Ramaciotti Centre for Electron Microscopy, Monash University, Clayton, 3800, Australia, [3]Biomedicine Discovery Institute, Department of Biochemistry, Monash University, Clayton, 3800, Australia.

M17 leucyl aminopeptidases are metal-dependent hexameric enzymes found in almost all kingdoms of life. A major function of M17 aminopeptidases is the cleavage of single amino acids from peptide substrates. The M17 aminopeptidases from malaria-causing parasites *Plasmodium falciparum* (*Pf*-M17) and *Plasmodium vivax* (*Pv*-M17), are postulated to liberate free amino acids from short haemoglobin peptides for use in parasite protein production[1,2]. *Pf*-M17 has been validated as a potential drug target for the design of new anti-malarial treatments, and high sequence identity between *Pf*- and *Pv*-M17 suggest *Pv*-M17 may also present as an attractive target[3,4].

To further understand these aminopeptidases, we investigated the relationship between oligomeric state and aminopeptidase activity, and the role metal ions play in these different molecular mechanisms. Using AUC, we found both *Pf*- and *Pv*-M17 exist in a metal-dependent equilibrium between an active hexamer and inactive small oligomers, a process we found to be moderated by both metal ion identity and concentration. The structural details of the various assemblies observed using AUC (dimers, trimers and hexamers) are being explored further using cryo-electron microscopy, with the hexameric structure solved to 2.4 Å. To elucidate the impact of metal ion environment on aminopeptidase activity, we kinetically characterised *Pf*- and *Pv*-M17 activity against two distinct substrates and determined that the metal ion environment can moderate enzyme substrate specificity and catalytic activity. Mutation of two of the metal-binding residues resulted in compromised catalytic activity and disruption of the metal-dependent equilibrium system. During the *Plasmodium* life cycle the identity and concentration of metal ions fluctuates [5]. Therefore, we propose parasites may be utilizing the M17 metal-dependent dynamic equilibrium as a biological regulator of haemoglobin proteolysis in parasites.

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Q10. Unravelling the molecular basis for vivax malaria's unhealthy attraction to human reticulocytes

Russell, B.R.[1], Malleret, B.J.[2,3], Han, J.H.[1], Ong, J.J.Y.[1], Ward, K.[1], Chua A.[1,2], Bifani P.[2,3], Chandramohanadas, R.[4], Suwanarusk, R.[1], Snounou, G.[5], Nosten, F.[6,7], Renia L.[2]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ, [2]Singapore Immunology Network (SIgN), A*STAR, Singapore, [3]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, [4]Singapore University of Technology & Design, Singapore, [5]CEA-Université Paris Sud 11-INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases (IMVA), IDMIT Department, IBJF, DRF, Fontenay-aux-Roses, France, [6]Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand, [7]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, UK.

Plasmodium vivax is the most widely distributed and difficult to cure form of human malaria. The ability of *P. vivax* to cause disease is dependent on invasion of immature red blood cells (reticulocytes). How *P. vivax* identifies and invades nascent reticulocytes remains unknown. We aimed to identify the specific blood cell membrane receptors and corresponding parasite proteins used to detect/cytoadhere to human reticulocytes. To do this, we developed a proteomic shortlist of reticulocyte receptors (CD71, CD98, and Small Integral Membrane Protein 1) which were targeted by neutralizing antibodies/knockdowns in *P. vivax* invasion assays. To help confirm the specific roles of the putative receptor ligand pairs we are currently utilising a newly developed culture system for *P. cynomolgi* (*P. vivax*'s sister species) to conduct reverse genetics on the *P. vivax* merozoite ligand orthologues. The identification of the reticulocyte specific receptors and corresponding ligands will aid in the development of vaccines against vivax malaria.

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Q11. Essentiality of succinate metabolism in *Mycobacterium tuberculosis*

McNeil, M.[1,2], Adolph, C.[1], Cheung, C.-Y.[1], Hards, K.[1,2], Jinich, A.[3], Rhee, K.[3] and Cook, G.M.[1,2]

[1]Department of Microbiology and Immunology, University of Otago, New Zealand, [2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand, [3]Weill Department of Medicine, Weill Cornell Medical College, New York, United States

Succinate metabolism is a major focal point in both the central carbon metabolism and the respiratory chain of *Mycobacterium tuberculosis*. *M. tuberculosis* encodes two succinate dehydrogenases (Sdh) (*sdh1*, *Rv0249c-Rv0247c* and *sdh2*, *Rv3316-Rv3319*), as well as a separate fumarate reductase (Frd) with possible bidirectional behaviour (Rv1552-Rv1555). The Sdh and Frd enzymes encoded by mycobacteria have distinct phylogeny, prosthetic groups and predicted biochemistry. The essentiality of individual Sdh and Frd enzymes has previously been investigated using single deletion mutants raising the possibility that remaining enzymes could catalyze succinate oxidation in the absence of the other. To address this and to advance succinate metabolism as a potential drug target, we report on the use of an optimized mycobacterial CRISPR interference (CRIPSRi) system to construct single, double and triple transcriptional repression constructs of Sdh1, Sdh2 and Frd. Sdh1 was non-essential for growth on either OADC, glycerol or glucose, but growth was slowed with succinate as the sole carbon source. No growth phenotypes were observed for Sdh2 and Frd single knockdowns on the same growth media. Both the Sdh1-Sdh2 double knockdown and the triple Sdh1-Sdh2-Frd knockdown had a significantly impaired growth when grown on OADC or with succinate as a sole carbon source. Impaired succinate metabolism could be partially rescued when using glycerol or glucose as the sole carbon source. The loss of succinate metabolism was bacteriostatic and resulted in an impaired mycobacterial electron chain. These results demonstrate that the loss of Sdh1 and Sdh2 is required to impair succinate metabolism in *M. tuberculosis* and that succinate metabolism is required for optimal growth under a range of conditions.

Q12. Drug discovery using the antifungal target lanosterol 14α-demethylase

Monk, B.C.[1], Sagatova, A.A.[1], Wilson, R.K.[1], Tyndall, J.D.A.[2], Lackner, M.[3], Ruma, Y.N.[1], Hosseini, P.[1], Keniya, M.V.[1]

[1]Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand[2]School of Pharmacy, University of Otago, Dunedin, New Zealand; [3]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Azole drugs are used widely to treat fungal infections in humans, other mammals and crops. As a consequence the incidence of azole resistance is increasing. This is driven by mechanisms including: (a) acquisition of amino acid changes in the targeted lanosterol 14α-demethylases, (b) isoforms of lanosterol 14α-demethylase that confer intrinsic azole resistance, (c) overexpression of the target enzyme, (d) bypassing of toxic metabolites using alternate pathways, and (e) drug efflux. We have used phenotypic analysis and a library of crystal structures of full-length lanosterol 14α-demethylases from *Saccharomyces cerevisiae* and two major fungal pathogens in complex with azole drugs to better understand drug susceptibility, assess the impact of resistance-conferring substitutions/mutations and direct the discovery of novel antifungals.

Lanosterol 14α-demethylases from evolutionary divergent fungal species, including human and agricultural pathogens were functionally overexpressed in *S. cerevisiae*, with some phenotypes significantly enhanced by co-expression of demethylases with their cognate NADPH-cytochrome P450 reductase. The short-tailed triazoles fluconazole and voriconazole and the tetrazole VT-1161 bind within the lanosterol 14α-demethylase active site and showed resistance conferred by a common active site mutation (tyrosine [Y] to phenylalanine [F]). In contrast, this mutation did not affect susceptibility to long-tailed triazoles, such as posaconazole, that have additional interactions with the substrate entry channel. Expression of lanosterol 14α-demethylase F1 and F5 isoforms from the mucormycete *Rhizopus arrhizus* in *S. cerevisiae* gave functional enzymes, with the isoform that has an equivalent Y to F substitution conferring intrinsic resistance to voriconazole but not posaconazole. Resistance conferred by the Y to F mutation or substitution, which alters a water-mediated hydrogen bond network in the active site, can be substantially overcome by modifying the linker between the head and tail of the azole drug. Phenotypic analysis and crystallographic studies of recombinant sterol 14α-demethylases expressed in yeast provide insights that facilitate structure-directed antifungal discovery.

Q13. Bacterial Secretins - Versatile outer membrane pores

Hay, I.D.[1]

[1]School of Biological Sciences, Th University of Auckland, Auckland

Bacteria can assemble a wide array of complex nanomachines and appendages within their cellular envelopes. These include long filaments allowing bacteria to attach to surfaces and host tissue; pores which pump out antibiotics to allow bacteria to resist treatment; complex secretion machines which pump enzymes and toxins in to the extracellular environment where they break down host tissue and cause life threatening diseases; channels which exude slime that protects bacteria from antibiotics and immune cells while clogging up host tissues; or syringe-like nanomachines which inject macromolecules directly into host cells to hijack the core cellular functions of the host. How these complex multi-component nanomachines spanning the multifaceted bacterial cell envelope localise and assemble with the correct arrangement and topology remains unclear.

Here I will discuss recent structural insights into the bacterial secretin family of proteins responsible for the outer membrane component of a number of these trans envelope secretion nanomachines.

Q14. From Chaperones to the Membrane with a BAM!

Fleming, K.G.[1]

[1]Johns Hopkins University, T. C. Jenkins Department of Biophysics, 3400 North Charles Street, Baltimore, MD 21218

Bacteria have an amazing ability to directionally sort outer membrane proteins (OMPs) in the absence of an external energy source such as ATP. We gained insight into this process through a holistic computational model. Our OMP Biogenesis Model – termed OmpBioM – integrates parameters from experiments both in vivo and in vitro. It incorporates all major periplasmic chaperones at their cellular concentrations, interaction rate constants and considers biological oligomeric states to predict the periplasmic lifetimes, copy numbers and sorting trajectories for OMPs. Using deterministic and stochastic methods we simulated OMP biogenesis under varying conditions replicating biochemical and genetic findings. OmpBioM stochastic simulations reveal that there are hundreds of binding and unbinding events between periplasmic chaperones and unfolded OMPs within an OMP lifetime. These interactions are thermodynamically favored yet kinetically fast suggesting that the periplasmic conditions are near equilibrium with OMPs being "tossed" from chaperone to chaperone. Following this equilibration in the periplasm, the ultimate, rate-limiting step for OMP incorporation into bacterial outer membranes is folding catalyzed by the essential and conserved BAM complex. OmpBioM provides unique insight into this process by predicting the ranges of rates that are possible for this BAM-stimulated reaction. Overall, a finely tuned balance between thermodynamic and kinetic potentials maximizes OMP folding flux through the periplasm, directs them to the native membrane, and minimizes unnecessary degradation. A kinetic "push" prevents OMPs from incorporation into the wrong membrane; OMP sorting is random in the aqueous periplasm; and – once folded with a BAM! – OMPs are thermodynamically favored and kinetically trapped in their native conformations.

Q15. Insane in the membrane: Biology of bacterial sialic acid metabolism.

Dobson, R.[1,2]

[1]Biomolecular Interactions Centre, School of Biological Sciences, University of Canterbury, [2]Department of Biochemistry and Molecular Biology, University of Melbourne

Sialic acids comprise a varied group of nine-carbon amino sugars that are widely distributed among mammals and higher metazoans. Commensal and pathogenic bacteria that colonise heavily sialylated niches (e.g. the mammalian respiratory tract and gut) can scavenge sialic acid from their surrounding environment and use it as a carbon, nitrogen and energy source—that is, they eat your glycogonjuates for breakfast. Sequestration and degradation of sialic acid involves specific amino sugar transporters responsible for the import into the bacterial cell and five catabolic enzymes that successively degrade sialic acid. Regulation of this pathway is achieved at the transcription level by specific repressor proteins.

In this talk I will present the first crystal structure of a sialic acid specific sodium solute symporter at 1.95 Å resolution in its outward-open conformation. Happily, this structure was determined in complex with sodium and sialic acid bound, providing insight into how this transporter mediates the movement of sialic acid across the membrane. I will then describe our structural studies on the mechanism by which the pathway is regulated at the gene level.

Overall, the work provides new data that enriches our understanding of the import and degradation of sialic acid in clinically important human bacterial pathogens.

Q16. Pore forming proteins of the immune system: What happens when there are no target-recognition domains?

Spicer, B.A.[1], Law, R.H.P.[1], Bayly-Jones, C.[1], Kondos, S.[1], Pang, S.S.[1], Venugopal, H.[1], Caradoc-Davies, H.[2], Whisstock, J.C.[1], Dunstone, M.A.[1]

[1]Monash University, Clayton, VIC, Australia, [2]Australian Synchrotron, Clayton, Vic, Australia

The MACPF/CDC family proteins use a common fold to oligomerise into a ring-shaped transmembrane pore capable of either direct cell lysis or passive transport other factors proteins. Members of this family are found in all kingdoms of life with a range of functions including as immune effectors, pathogenicity factors, parasite egress, fungal defense and development.

Decades of structural research on the MACPF/CDC family suggested that oligomer assembly on the target membrane is mediated via a dedicated ancillary domain. This is followed by planar diffusion upon the target membrane into a ring shaped prepore. The prepore undergoes a concerted conformational change to form the final pore. This model of mechanism, however, is only consistent with Cholesterol Dependent Cytolysins (CDCs), pleurotolysin and perforin that all have dedicated membrane binding ancillary domains. It is now emerging that this canonical mechanism does not explain how two important pathogen-targeting systems function: Membrane Attack Complex (MAC) and MPEG-1.

The MAC can target a wide range of eukaryotic and bacterial surfaces. In fact, the MAC can assemble on any surface that triggers the complement pathway and bystander host cells rely on the inhibitor CD59 to prevent unwanted MAC assembly. The MAC is a hetero-oligomer structure that consists of C5b, C6, C7, C8 and 18 units of C9 that assembly and insert in a simultaneous fashion. The MAC components lack any ancillary membrane binding domains; however, the sequential insertion mechanism allows for membrane anchoring once C7 has bound. Our X-ray structure of the soluble C9 component of the MAC in comparison with the near atomic single particle cryo-EM structure of the 22-subunit polyC9 shows how this sequential assembly pathway has evolved and how it is controlled.

Similar to the MAC, MPEG-1 is proposed to target a wide range of bacteria engulfed by macrophage but it is unknown how this broad target recognition is achieved. Moreover, the new SP cryo-EM structures of an MPEG-1 prepore shows an absence of any ancillary domains that can bind specifically to the target surface. Interestingly, the structure of MPEG-1 prepores bound to liposomes alludes to an “upside down” membrane binding orientation which we hypothesise anchors the complex to the vacuole membrane to prevent autolysis. Whilst there is some evidence of a trans-pore mechanism it remains to be discovered how the final MPEG-1 pore is actually formed in the absence of a target specific ancillary domain.

Overall, these new structures of the MAC and MPEG-1 challenge the existing dogma in MACPF/CDC pore assembly. We conclude that MACPF/CDC pores used by the immune system have evolved to be able to target highly variable surfaces by evolving different assembly pathways.

Q17. Genomics to tame Tuberculosis

Dunstan, S.J.[1]

[1]The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia.

Tuberculosis (TB) is a disease of global importance that lacks effective tools for its control and elimination. Diagnosis can be slow, treatment is long, disruption of transmission is labour intensive, and the current vaccine has poor effectiveness. The progression to active TB is thought to depend on environmental, bacterial and host factors.

We are using a genomics approach to characterize the independent roles of host and pathogen genome variation, as well as their interaction, in active TB. The results and impact of our host and pathogen genomic studies in large, clinically complex TB patient cohorts from Vietnam will be presented. Our results demonstrate how pathogen genomics can define the population diversity and spread of *Mtb*, and investigate drug resistance emergence. Furthermore, we have identified TB susceptibility genes, providing insight into the mechanisms of protective immunity.

Scrutinising both host and pathogen genomic variation can advance our discovery of more rapid diagnostics and new host directed therapies, especially relevant for drug resistant TB, and also for vaccine development.

Q18. A Novel Peptide Delivery Platform for Mucosal Vaccination Based on Group A Streptococcus Pili

Tsai, C. J.[1, 2], Loh, J. M.[1, 2], Proft, T. K.[1, 2]

[1]School of Medical Sciences, University of Auckland, Auckland, NZ, [2]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, NZ

Well-defined synthetic vaccines based on individual peptides are specific and safe. However, peptide antigens are usually poorly immunogenic and sensitive to proteolytic degradation, thus require costly conjugation to carrier proteins and administration with potentially toxic adjuvants. Lactic acid bacteria have become promising vehicle for delivering active molecules to mucosal sites. We have developed PilVax, a novel peptide delivery platform that allows the presentation of a stabilised and highly amplified peptide as part of the group A streptococcus (GAS) serotype M1 pilus structure (PilM1) on the surface of the food-grade bacterium *Lactococcus lactis*. Pili (*sing.* Pilus) are hair-like protrusions from the bacterial cell surface. GAS pili are mainly formed by multiple copies of the highly immunogenic, covalently-linked backbone pilin (Spy0128). We have generated prototype constructs by engineering two model peptides, $Ova_{324-339}$ from ovalbumin, and M2e from influenza virus hemagglutinin, into Spy0128. A plasmid harbouring a strong lactococcal promoter was used to express the modified pili on the surface of *L. lactis*. The resulting recombinant *L. lactis* strains were used to immunised mice intranasally. Serum and lung fluid samples were obtained and analysed for peptide-specific antibody responses. Intranasal immunisation of PilVax generated strong mucosal and systemic antibody responses in mice. Interestingly, a construct carrying two copies of M2e peptide in tandem elicited significantly higher antibody responses compared to the strain with a single M2e peptide inserted in the same location. We have further show that it is possible to insert more than one peptide into the same integration site, and peptide epitopes can be incorporated into structurally similar but antigenically different pilus structure. PilVax also provides benefits such as higher safety and lower production and transportation costs. Furthermore, the needle-free mucosal administration route is an additional advantage for the use in developing countries, where efficacious vaccines are most needed.

Q19. Food, sex and genomics

Williamson, D.[1]

[1]Doherty Institute, University of Melbourne

The use of genomics has revolutionised infectious diseases epidemiology. This talk will provide an update on how genomics has been applied to understanding transmission of pathogens in two important areas, namely food-borne diseases and sexually-transmitted infections.

Q20. Understanding the mechanisms of infectious disease: from atomic to system resolution.

Bond, P.J.[1,2]

[1]Bioinformatics Institute (A*STAR), 30 Biopolis Street, #07-01 Matrix, 138671 Singapore, [2]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore

Our bodies are under constant attack from pathogenic bacteria and viruses from our surroundings. In order to cause disease, these microorganisms must enter the human host, bind to and invade specific cell types, and grow and replicate. Some pathogens can make us sick by hijacking the host cellular machinery. For example, dengue virus is responsible for infecting hundreds of millions of people each year, and the severity of disease can be enhanced by interaction with antibodies. In order to fill existing gaps in our molecular understanding of the viral life cycle, we have been integrating structural, biophysical, and genomic experiments with multiscale modelling, towards the “virtual dengue virus”. We recently combined cryo-electron microscopy and simulation data to identify how the membrane envelopes of virus particles interact with antibodies to facilitate infectivity, with important consequences for vaccine development strategies. We are also studying how the release of toxins from pathogens can similarly make us ill through interactions with our host defense systems. For example, mammalian innate immune receptors can detect a broad range of pathogen-associated molecules, but their excessive immunostimulation can lead to systemic inflammation and sepsis, which kills millions of people worldwide. We have thus developed computational models to trace in molecular detail the cascade associated with transfer of endotoxic molecules from bacterial envelopes to host immune receptors, and leveraged these models to establish previously undisclosed modes of action of anti-inflammatory peptides that occur naturally during wound healing. Collectively, our work is helping to unravel the key determinants governing host-pathogen interactions, and should help towards the search for novel therapeutics to tackle ongoing issues of antimicrobial resistance.

Q21. RNase HI activity provides metabolic flexibility under antibiotic stress in the mycobacteria

Alzubaidi, A.,[1] Mansfield, E.,[1] Cheung, J.,[2] Mizrahi, V.,[3] Cook, G.,[2] Lott, J.S.,[1] Dawes, S.S.[1]

[1]School of Biological Sciences, University of Auckland, Auckland, NZ; [2]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ; [3]Department of Pathology, University of Cape Town, Cape Town, RSA.

Antimicrobial resistance (AMR) in bacteria is a crisis both in New Zealand and globally. AMR evolves due to metabolic flexibility that compensates for antibiotic exposure. Enzymes that promote bacterial survival during treatment with current therapeutics are therefore important new drug targets.

RNase HI provides an essential cellular function in mycobacteria by removing R-loops (RNA strands that have invaded the genome and cause genotoxic stress) and is therefore a potential drug target. We showed that RNase HI activity compensates for transcriptional inhibition by the front line anti-tubercular agent, rifampicin. In the model organism *Mycobacterium smegmatis*, which contains two genes encoding RNase HI activity, a knockout of either one increases the number of unresolved R-loops in the cell. Unexpectedly, inhibition of transcription by rifampicin treatment also increases R-loop number, both in wild type and RNase HI-depleted strains. In agreement with this observation, RNase HI depletion enhanced killing by rifampicin by ~100-fold, validating this novel combination for therapy and re-sensitisation of rifampicin resistant strains.

RecA, the master regulator of the bacterial SOS response, is a validated drug target in the mycobacteria. We showed that the SOS response in the mycobacteria, in turn, compensates in part for RNase HI loss in the mycobacteria, suggesting the combination of RecA inhibitors with RNase HI inhibitors could provide a vital component in new combination regimens in the future.

The conserved functions of these enzymes in bacteria suggest that this strategy might be generally applied.

Q22. The hows and whys of cofactor F_{420} biosynthesis in mycobacteria

Greening, C.[1], Grinter, R.[1], Ahmed, F.H.[2], Bashiri, G.[3], Ney, B.[1], Lee, B.M.[2], Hards, K.[4], Scott, C.[5], Warden, A.[5], Oakeshott, J.G.[5], Taylor, M.C.[5], Coppel, R.[1], Cook, G.M.[4], Baker, E.N.[3], Jackson, C.J.[2]

[1]School of Biological Sciences, Monash University, Melbourne, AU, [2]Research School of Chemistry, Australian National University, Canberra, AU, [3]School of Biological Sciences, University of Auckland, Auckland, NZ, [4]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ, [5]CSIRO Land & Water, Canberra, AU.

A unique aspect of the redox metabolism in mycobacteria is the use of the electron carrier, F_{420}. While this cofactor has been implicated in mycobacterial persistence, we lack a systematic understanding of how and why it is synthesised. In this work, we present three advances in this area. First, we show that mycobacteria depend on a membrane-bound $F_{420}H_2$-dependent menaquinone reductase to maintain redox homeostasis under hypoxia. Thereafter, we show that mycobacteria use a suite of promiscuous $F_{420}H_2$-dependent reductases to detoxify various antimicrobial compounds. Finally, we present a revised biosynthetic pathway for the cofactor and prove that its synthesis proceeds through previously unidentified intermediates. To make these advances, we combined mycobacterial genetics and physiology with cutting-edge analytical chemistry, synthetic biology, and structural biology approaches. In addition to covering published work, we will also premiere our latest advances, including high-resolution substrate- and product-bound crystal structures of the central biosynthesis enzyme CofD. These findings transform our understanding of a vital but understudied cofactor and suggest that the F_{420} biosynthesis is a fertile target space for antimycobacterial drug development.

1. Jirapanjawat et al. (2016) *The redox cofactor F_{420} protects mycobacteria from diverse antimicrobial compounds and mediates a reductive detoxification system*. Applied and environmental microbiology 82:6810-6818.

2. Ney et al. (2017). *The methanogenic redox cofactor F_{420} is widely synthesized by aerobic soil bacteria.* The ISME journal. 11:125-137.

3. Greening et al. (2017). *Mycobacterial $F_{420}H_2$-dependent reductases promiscuously reduce diverse compounds through a common mechanism*. Frontiers in microbiology. 8:1000.

4. Bashiri et al. (2019). *A revised biosynthetic pathway for the cofactor F_{420} in prokaryotes*. Nature communications 10:1558.

Q23. Targeting Menaquinone Biosynthesis in the Pathogens *M. tuberculosis* and *S. aureus*

Johnston, J. M.[1], Stanborough T.[1], Ho N.A.T.[1], Nigon L.V.[2], Jirgis, E..[2], Jiao, W.[3], Ferris, S.[2], Furkert, D.P.[2], Brimble, M.A.[2], Lott, J.S.[2], Baker, E.N.[2], Bashiri G.[2,] Dawes, S.S.[2], Bulloch, E.M.[2]

[1]University of Canterbury, Christchurch, New Zealand, [2]University of Auckland, Auckland New Zealand [3]Ferrier Institute, Victoria University of Wellington, Wellington, New Zealand.

Menaquinone (vitamin K2; MQ) is an important electron carrier essential for the electron transport pathway of many bacteria including pathogens such as *Mycobacterium tuberculosis* (Mtb) and *Staphylococcus aureus* (Sau). MQ has also been variously implicated in processes underlying bacterial persistence, virulence, latency and biofilm formation. The pathway that makes MQ is not present in humans and has become of interest in recent years as a source of drug targets with many groups, including our own, having active inhibitor discovery projects targeting these enzymes. Our work in recent years has been focused on characterizing and finding inhibitors for the enzyme MenD (SHCHC synthase), which carries out the first committed step in the classical MQ biosynthesis pathway. This enzyme relies on a cofactor, thiamine diphosphate (ThDP), for its activity and we have solved a series of structures of Mtb MenD at different points of the catalytic cycle. We have used this information along with information gathered around its cooperative behaviour to develop a collaborative inhibitor design program for the MenD enzyme from Mtb and we have now extended this work into the MenD from Sau. This talk will cover our progress to date in understanding the molecular level workings of this vital biosynthetic pathway from these two human pathogens.

Q24. Commensal Neisseria kill *Neisseria gonorrhoeae* through a DNA-dependent mechanism

So, M.[1], Kim, W.J.[1], Higashi, D.[1,2,3], Goytia, M.[2,4], Rendón, M.A.[1], Pilligua-Lucas, M.[5], Bronnimann, M.[1,6], McLean, J.A.[7], Duncan, J.[8], Trees, D.[7], and Jerse, A.E.[5]

[1]Department of Immunobiology and the BIO5 Institute, University of Arizona, Tucson, AZ 85721, USA; [2]Contributed equally to this work; [3]Present address: Department of Restorative Dentistry, Oregon Health Sciences University, Portland, OR 97239, USA; [4]Department of Biology, Spelman College, Atlanta, GA 30314, USA; [5]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD 20814, USA. [6]Present address: Roche Tissue Diagnostics, Oro Valley, AZ 85755, USA; [7]Centers for Disease Control and Prevention, Atlanta, GA 30329, USA; [8]Department of Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

The mucosa is colonized with commensal *Neisseria*. Some of these niches are sites of infection for the STD pathogen *Neisseria gonorrhoeae* (Ngo). Given the reports on the antagonistic behavior of commensal bacteria towards their pathogenic relatives, we hypothesized that commensal *Neisseria* may negatively affect Ngo colonization. Here, we report that commensal species of *Neisseria* kill Ngo through a mechanism that is based on genetic competence and DNA methylation state. Specifically, commensal-triggered killing occurs when the pathogen takes up commensal DNA that contains a methylation pattern it does not recognize. Indeed, any DNA will kill Ngo provided that it is able to enter the cell, is differentially methylated, and has homology to the pathogen genome. Consistent with these findings, commensal *Neisseria elongata* accelerates the clearance of Ngo from the mouse vagina in a DNA uptake-dependent manner. We propose that commensal *Neisseria* antagonizes Ngo infection through a DNA-mediated mechanism, and that DNA is a potential microbicide against this highly drug resistant pathogen. Finally, epidemiological studies have shown that carriage of commensal *Neisseria* reduces the risk of *Neisseria meningitidis* infection. We present evidence that this DNA-dependent killing mechanism may explain the link between the presence of commensal *Neisseria* in the oropharynx and the lower rates of meningococcal infection.

Q25. Cysteine Biosynthesis; Gonorrhoea's weak link?

Oldham, K.[1], Jiao, W.[2] & Hicks, J.L.[1]

[1]School of Science, Division of Health, Engineering, Computing and Science, University of Waikato, Hamilton, NZ, [2]Ferrier Research Institute, Victoria University of Wellington, Wellington, NZ

Neisseria gonorrhoeae is an obligate human pathogen and the causative agent of the sexually transmitted infection gonorrhoea. Gonorrhoea is now a global health problem due to the increasingly high number of strains resistant to all frontline antibiotics. Targeting amino acid biosynthesis is a new and promising route for the development of new antimicrobials. The amino acid cysteine plays an important role in protein molecules and, in microorganisms, provides protection from oxidative stress via reducing systems such as glutathione. In most bacteria, the cysteine biosynthesis pathways are well conserved. However, unlike other *Neisseria* species, *N. gonorrhoeae* cannot grow on sulphate as the sole source of sulphur, relying solely on thiosulphate for the *de novo* synthesis of cysteine[1]. In addition, the cysteine biosynthesis enzymes are essential for the bacteria's survival[2]. We have characterised one of the two key enzymes that form the cysteine synthase complex, a serine-acetyltransferase. We have determined key kinetic parameters and show competitive inhibition by the end-product of the pathway, cysteine. Interestingly, the enzymes of the cysteine synthase complex from *N. gonorrhoeae* display differences to homologs from other bacteria. We are working on solving the structures of these key enzymes, along with *in vivo* knock down experiments. Together these results will inform the design of inhibitors to these key cysteine biosynthesis enzymes to exploit this 'weak link' in the metabolism of *N. gonorrhoeae*. New antimicrobial inhibitors that affect the growth and ability of *N. gonorrhoeae* to infect and invade epithelial cells and/or enhance the efficacy of existing antibiotics would lead to new treatments to combat the increase in antimicrobial resistance of this human pathogen.

1. Hicks, J.L. and C.V. Mullholland, *Cysteine biosynthesis in Neisseria species.* Microbiology, 2018.
2. Remmele, C.W., Y. Xian, M. Albrecht, et al., *Transcriptional landscape and essential genes of Neisseria gonorrhoeae.* Nucleic Acids Research, 2014. **42**(16): p. 10579-10595.

Q26. Advances towards a *Neisseria gonorrhoeae* vaccine

Semchenko EA[1], Tan A[1], Borrow R[2] , Seib KL[1]

[1]Institute for Glycomics, Griffith University, Gold Coast, Australia, [2] Vaccine Evaluation Unit, Public Health England, Manchester Royal Infirmary, UK

Neisseria gonorrhoeae is an obligate human pathogen and the causative agent of the sexually transmitted infection gonorrhoea. There are over 106 million reported cases of gonorrhoea each year worldwide, and if left undiagnosed or untreated, infection can lead to severe sequelae that include pelvic inflammatory disease, infertility, neonatal complications, and an increased risk of HIV. *N. gonorrhoeae* is recognised by WHO and CDC as an urgent threat to global health due to the emergence of multi-drug resistant gonococcal strains. There is currently no vaccine, and no new antibiotics or new vaccine candidates in late-stage development. However, the outer membrane vesicle (OMV) meningococcal B vaccine MeNZB, that was developed to protect against the closely related pathogen *Neisseria meningitidis,* was recently reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand.

Our work is focused on identifying novel gonococcal vaccine target, as well as investigating the cross reactivity to *N. gonorrhoeae* of serum raised to the meningococcal B vaccine Bexsero, which contains the MeNZB OMV component plus three recombinant protein antigens. We have characterised several highly conserved and immunogenic gonococcal candidate vaccine antigens and shown that antibodies to these proteins are bactericidal and can block gonococcal infection of cervical and urethral epithelial cells. In addition, we have found that there is a high level of sequence identity between the MeNZB/Bexsero OMV antigens, and gonococcal proteins. NHBA is the only Bexsero recombinant antigen that is conserved and surfaced exposed in *N. gonorrhoeae*. Furthermore, we have found that Bexsero induces antibodies in humans that recognise and kill *N. gonorrhoeae* in vitro. Work is ongoing to identify the full set of gonococcal targets recognized by Bexsero-induced antibodies, and the functional activity of these antibodies against gonorrhoea.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
Q27	Investigations into a novel hypothetical virulence factor from Group A streptococcus	Haniyeh Aghababa	University of Auckland
Q28	Validation of RNase HI as a drug target in *Mycobacteria*	Al-Zubaidi, A	University of Auckland
Q29	Structural and biophysical characterisation of the human macrophage immune effector, MPEG1	Charles Bayly-Jones	Monash University
Q30	Immunological analysis of New Zealand isolates of *Mycoplasma ovipneumoniae*	Bridgeman, B.J.	Massey University / AgResearch
Q31	Calstabin interactions with RyR1 - a culprit in a complex story?	Sophie Burling	Massey University
Q32	Compounds utilizing the amiloride pharmacophore with antimalarial activity	Christensen, P.R.	University of Otago
Q33	Kauri Dieback Disease: Targeting the Energetics of *Phytophthora agathidicida* to produce new antimicrobials	Donald, C.R.	University of Otago
Q34	The effect of intravenous *Mycobacterium* vaccination on early innate responses in mice	Likhit S. Dukkipati	University of Otago
Q35	Zinc Ionophores as Novel Sanitisers for the Prevention of Bovine Mastitis	Scott A. Ferguson	University of Otago
Q36	Enhancing cellular immune responses for the prevention and treatment of infectious diseases and cancer with innate T cell agonists, proteins and genetic vaccination	Ganley, M.	Victoria University of Wellington
Q37	Identification of a meningococcal adhesin that inhibits epithelial cell migration	Greig, G.	Institute of Environmental Science and Research / Victoria University of Wellington
Q38	Allosteric regulation and inhibition of HisG in *Campylobacter jejuni*	Grout, E.K.	University of Waikato
Q39	Glutamate racemase: a drug target for multi-drug resistant *Acinetobacter baumannii*	Hamilton, K.	University of Otago
Q40	Regulation of two exogenous cell surface signalling (CSS) systems, *foxIR*	Hampton, G. E.	University of Otago

	and *fiuIR* for iron uptake in *Pseudomonas aeruginosa*		
Q41	Zinc ionophores restore oxacillin sensitivity in methicillin-resistant *Staphylococcus aureus* (MRSA)	Nichaela Harbison-Price	University of Otago
Q42	Differential and oxygen-independent roles of the two terminal oxidases in mycobacteria	Kiel Hards	University of Otago
Q43	Investigation of malate metabolism in mycobacteria reveals insight into the energetic role of malate quinone oxidoreductase	Harold, L.K.	University of Otago
Q44	The use of computational modelling to investigate gene-gene interactions in the context of antibiotic resistance	Heywood, A.	University of Otago
Q45	Understanding Antimicrobial and Antiseptic Resistance Relationships in *Staphylococcus aureus*	Krause, A.L.	University of Otago
Q46	Repurposing of the anthelmintic drug niclosamide as an antibiotic to treat Gram negative infections	Hannah R. Lee-Harwood	Victoria University of Wellington
Q47	Characterization of a *Bartonella quintana* effector protein	Alvey J Little	Victoria University of Wellington
Q48	Expression, Purification, and Negative Staining of Candida albicans Plasma Membrane Protein Cdr1	G. Madani	University of Otago
Q49	Investigating the role of RNase HI in antibiotic sensitive Mycobacteria	Mansfield, E.	University of Auckland
Q50	A Validated Assay Platform for Group A Streptococcus Vaccine testing in New Zealand	Reuben Mcgregor	University of Auckland
Q51	Cysteine biosynthesis: characterizing new antibiotic targets in *Neisseria gonorrhoeae*	Oldham, K.E.	University of Waikato
Q52	A chemoenzymatic platform to generate lanthipeptides in the combat against antimicrobial resistance	George Randall	University of Auckland
Q53	TreeVax: A scaffold-based vaccine for group A streptococcus	Saxby, I.	University of Auckland
Q54	The first approval for the release of a genetically modified organism without controls	Strabala, T.J.	Environmental Protection Authority
Q55	Fe-S cluster biogenesis in *Mycobacterium tuberculosis*	Stuteley, S.M.	University of Auckland
Q56	Towards solving the structure of the mycobacterial chromosome	Summers, E.L.	University of Waikato

	condensing protein Lsr2 in complex with DNA		
Q57	Understanding the Innate Immune Response to Group A Streptococcus Pili	Risa Takahashi	University of Auckland
Q58	A Multi-Platform Approach for the Discovery of Autoantibody Targets as New Biomarkers for Acute Rheumatic Fever	Mei Lin Tay	University of Auckland
Q59	Investigating aminoglycoside resistance in *Pseudomonas aeruginosa*	Thacharodi, A.	University of Otago
Q60	Understanding Teixobactin Tolerance in *Enterococcus faecalis*	Todd Rose, F.O	University of Otago
Q61	Smallpox virus chemokine-binding protein: a potential therapeutic approach	Elham Torbati	University of Otago
Q62	Zinc ionophores as novel inhibitors targeting the obligate anaerobe *Fusobacterium nucleatum*	Van Zuylen, E.M.	University of Otago
Q63	Understanding the role of cell envelope-associated proteins in the biology of rumen methanogens	Yeung, J.	AgResearch