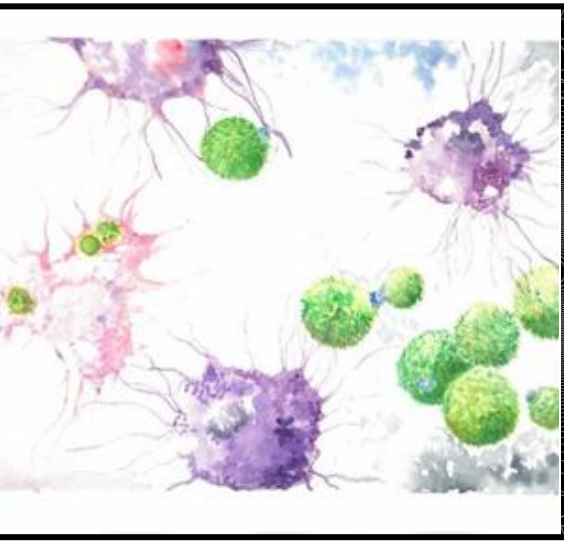
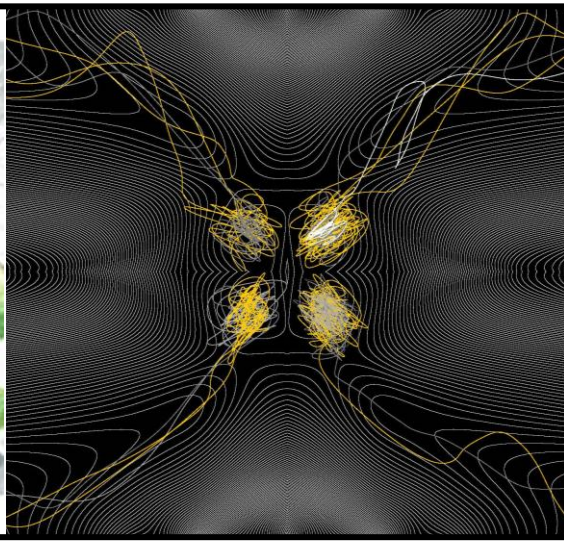
Friday 18th November 2022

18th Annual SCMB

Research Students Symposium

Program Booklet





Images courtesy of Cheyenne Dobbs and Ariel Jones

Cryptococcus neoformans being engulfed by macrophages (left)
Quasiclassical molecular dynamics trajectories on a potential energy surface (right)

Acknowledgements

The 2022 SCMB Research Student Advisory Group would like to thank all the attendees of this year's symposium. In addition, we would like to give special thanks to:

Our Plenary Speakers:
Professor Majid Ebrahimi Warkiani and Dr Natalee Newton
Our student speakers
Our generous sponsors
Staff who volunteered as judges
HDR & Honours students who submitted abstracts

Staff Representatives of the Research Student Advisory Group:

- Professor Luke Guddat
- Pelin Durali
- ECR Representative: Dr Natalee Newton

Sincerely,

2022 SCMB Research Student Advisory Group

Alicia Kirk, Shahla Asgharzadeh Kangachar, Connor Scott, Tom Gen Li, Athyah Alenzi, Summa Bibby, Dylan Bowman, Samuel Davis, Timothy Muusse, Dean O'Brien, Apoorva Prabhu, Shannon Hoe Min Quah, Forhard Karim Saikot, Sanjana Tule and Khristine Valdellon

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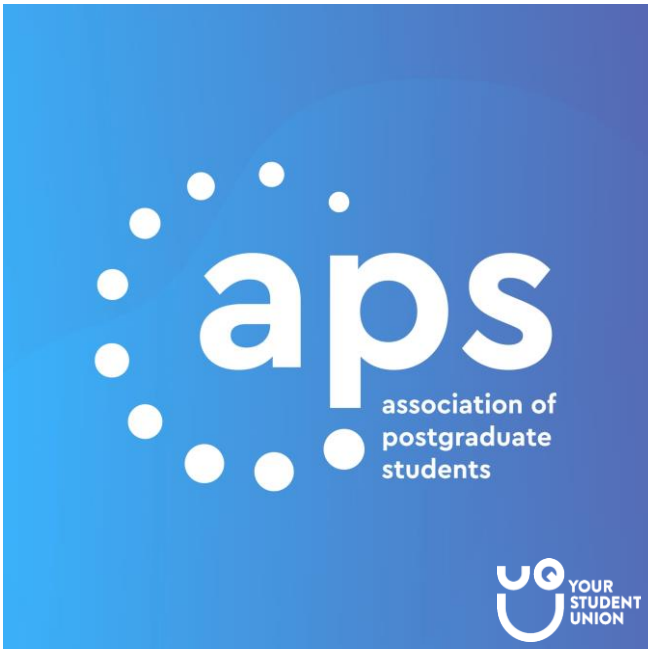

The voice of postgraduate students.

The UQ Association of Postgraduate Students (APS) is a community organisation, run by postgraduate students supporting over 14,000 postgraduate students at The University of Queensland.

APS aims to create a platform for all postgraduate students to engage socially and professionally with students from other schools, faculties, nationalities, cultures and general interests.

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Timetable

8.30 AM	**Registration & Poster Setup** Engineering Learning Centre (50-C207)	
9.00 AM	**Welcome address** Lecture Theatre (50-T203)	
9.10 AM	**Plenary Lecture 1 - Professor Warkiani** Lecture Theatre (50-T203)	
10.00 AM - 10.30 AM	**Poster Session 1** (odd # abstracts) Engineering Learning Centre (50-C207)	
	Student Oral Presentations 1	
	Session 1 (50-T203)	Session 2 (50-N201)
10.35 AM	**1. Korawich Uthayopas** PRIMITI: a computational approach for accurate prediction of miRNA-target mRNA interaction	**6. Jingyi Fan** Polyglutamic amino acids for delivery of self-adjuvanting peptide-based subunit vaccines against Group A *Streptococcus*
10.50 AM	**2. Brad Balderson** *Cytocipher* detects significantly different populations of cells in single cell RNA-seq data	**7. Lachlan Miller** Molecular dynamics simulations of water flow in graphene-based desalination membranes
11.05 AM	**3. Yibi Chen** Genome-wide transcriptome analysis reveals the diversity and role of non-coding RNAs in dinoflagellates	**8. Xin Lin** Structural insights into the bifunctional mechanism of class I ketol-acid reductoisomerase
11.20 AM	**4. Yi Peng** Probing the plastic degradation potential of marine microbes	**9. Matthew Jackman** Structural characterisation of the nucleosome remodelling and deacetylase (NuRD) Complex using integrative modelling, single particle analysis, and AlphaFold prediction
11.35 AM	**5. Lauren Carpenter** Understanding how industry conceptualizes a valuable science graduate.	**10. Mitchell Sorbello** Characterisation of plant immune TIR domains bifunctional enzymatic activity
11.50 AM - 12.20 PM	**Poster Session 2** (even # abstracts) Engineering Learning Centre (50-C207)	

Timetable

12.00 PM - 1.30 PM	**Lunch** Collaborative Learning Space (50-S201)	
	Student Oral Presentations 2	
	Session 3 (50-T203)	Session 4 (50-N201)
1.30 PM	**11. Morgan Freney** Japanese Encephalitis Virus: Near-Atomic Structures of Emerging Genotypes Reveal Epitope & Antigenic Differences	**16. Chyden Chang** Cross-regulatory control of antibiotic resistance and virulence in uropathogenic *Escherichia coli*
1.45 PM	**12. Summa Bibby** Envelope protein domain III is a key determinate of Yellow Fever Virus structure	**17. Qifeng Zhong** The DmsABC dimethyl sulfoxide reductase – an enzyme mediating anaerobic energy generation or a virulence determinant in pathogenic bacteria?
2.00 PM	**13. Mikaela Bell** A new recombinant alphavirus platform based on the insect-specific Yada Yada virus	**18. Jennifer Hosmer** Access to highly specialized growth substrates and production of epithelial immunomodulatory metabolites determine survival of *Haemophilus influenzae* in human airway epithelial cells
2.15 PM	**14. Jiawei Ma** Characterisation of MCMV genes involving in developing salivary gland persistence	**19. Yvette Ong** Dissecting the role of RfaH in uropathogenic *Escherichia coli* virulence
2.30 PM	**15. Sulin Li** Structural and Functional Characterization of Bacterial TIR Proteins	**20. Marium Khaleque** Differential N-Glycosylation Site Occupancy Depends on Distinct Amino Acid Sequence Features
2.50 PM	**Double Blind Challenge** Lecture Theatre (50-T203)	
3.20 PM	**Plenary Lecture 2 - Dr Newton** Lecture Theatre (50-T203)	
4.10 PM	**Closing Address & Awards** Lecture Theatre (50-T203)	
4.30 PM	**Speed Networking & SCMB Social Mixer** Join us on the podium! (68-level 3)	

Plenary Speakers

Professor Majid Ebrahimi Warkiani

Dr Warkiani is a Professor and CINSW Fellow in the School of Biomedical Engineering, UTS, Australia. He received his PhD in Mechanical Engineering from Nanyang Technological University (NTU, Singapore), and undertook postdoctoral training at Massachusetts Institute of Technology (MIT, USA). Dr Warkiani is co-director of the Australia-China Joint Research Centre for Point of Care Testing and also a core-member of Institute for Biomedical Materials & Devices (IBMD) and Center for Health Technologies (CHT) at UTS. Dr Warkiani's current research activities focus on three key areas of (i) **Microfluidics** involving the design and development of novel microfluidic systems for particle and cell sorting (e.g., circulating tumor cells, fetal cells & stem cells) for diagnostic and therapeutic applications, (ii) **Organ-on-a-chips** involving the fabrication and characterization of novel 3D lab-on-a-chip systems (e.g., Lung-on-a-chip, Tumour-on-a-chip) to model physiological functions of tissues and organs, and (iii) **3D micro-printing** involving the design and development of novel miniaturized systems (e.g., micromixers, micro-cyclones) for basic and applied research.

Group webpage: www.WarkianiLab.com

Plenary Speakers

Plenary title: *Microengineered Systems for Cell Biology and Translation into Practice*

Micro/nano-fluidics, a technology characterized by the engineered manipulation of fluids at the micro/nano-scale, has shown considerable promise in point-of-care diagnostics and clinical research. Micro/nano-fluidic platforms are creating powerful tools for cell biologists to control the complete cellular microenvironment, leading to new questions and new discoveries. By simply miniaturizing macroscopic systems and taking advantage of the possibility of massive parallel processing, some micro/nano-fluidic chips enable high-throughput biological experiments such as cell sorting, single cell analysis, PCR, ELISA and chromatography. Over the past 10 years, my group has developed several microfluidic systems, which are translated into practice. In this seminar, I will describe our recent efforts in development of new microfluidic systems using 3D microprinting and microfabrication for various applications such as rare cell sorting, sperm analysis and drug screening.

Plenary Speakers

Dr Natalee Newton

Dr Natalee Newton is an NHMRC Emerging Leadership Fellow in SCMB. She completed her PhD in 2020 at SCMB, which focused on understanding flavivirus maturation and architecture as well as developing a chimeric system to safely study the structure of highly pathogenic tick- and mosquito-borne flaviviruses. She continued her research on arboviruses as a Postdoctoral Researcher in A/Prof Daniel Watterson's laboratory and was awarded an NHMRC Emerging Leadership Fellowship in 2021.

Her main research focus is using cryo-electron microscopy to provide insights into flavivirus structure, with implications for viral evolution, rational vaccine design and therapeutic antibody development. This is facilitated by a host-restricted chimeric system, that produces safe authentic virions of pathogenic flaviviruses for high-resolution structural analysis, vaccines and diagnostic applications. Her current work tests the limits of this chimeric platform by safely "resurrecting" the evolutionary lineages of pathogenic flaviviruses using ancestral sequence reconstruction of the structural proteins. These intact ancestral virions provide a way to explore and understand the structural evolution of flaviviruses in the hope of designing broad-spectrum vaccines and improving outbreak preparedness.

Plenary title: *How to resurrect an ancestral flavivirus.*

Profile: scmb.uq.edu.au/profile/3549/natalee-newton

Poster Listing

P01	**Aaron Kovacs**	Computing Spatial Mutation Intolerance to Assess Selective Pressure Across the Human Proteome
P02	**Adam Serghini**	Prediction of oncogenic mutations in VHL disease
P03	**Anneka Pereira Schmidt**	Investigating mechanisms of synergy between amphotericin B and lactoferrin-derived peptides
P04	**Anthony Bengochea**	The Origin of Vertebrate Steroids and Steroidogenic P450 Enzymes
P05	**Ariel Jones**	Are ultrafast rebound rates explained by radical clock (mis)-calibration?
P06	**Bryan Lim**	Functional characterisation of plant TIR domains and their role in plant innate immunity
P07	**Chavali Harshita**	Characterising the membrane-binding properties of peptides that potentiate the effect of the anti-fungal drugs amphotericin
P08	**Chuyi Su**	Towards Azabioisostere Design – seco-azahomocubanes
P09	**Daniel Ellis**	Investigating Wild Yeast Metabolomes to Make Better Beer
P10	**Dylan Bowman**	Investigation of RNAi-inhibitor activity in the insect-specific flavivirus, Binjari virus
P11	**Elouise Comber**	Characteristics of patients receiving long-term intrathecal opioid infusions for management of chronic pain: A comparison with normative data derived from pain clinic populations in Australia and New Zealand
P12	**Gen (Tom) Li**	704: A novel ionophore antibiotic against Gram-positive multidrug-resistant bacterial infections
P13	**Hisatake Ishida**	Spatial genomic variation of Acropora holobionts across the Great Barrier Reef.
P14	**Hyoyoung Kim**	Structure-function analysis of TRAM/TRIF-dependent TLR4 signalling
P15	**Isabella Bernard**	Amaryllidaceae alkaloids isolated from Crinum arenarium

Poster Listing

P16	**Isabella Chantrill**	CRACing the Role of Flavivirus NS1: Effect of Mutations in the putative CRAC motif of Dengue NS1 in Membrane Association
P17	**Jiahui Zhang**	Investigation of Antigen Orientation on a Cyclic Lipopeptide Vaccine Delivery System
P18	**Jiayu Li**	The Ebola virus delta peptide (E40red) is a cation selective viroporin
P19	**Joao Paulo Linhares Velloso**	Evaluating disease-associated mutations in GPCRs
P20	**Joshua Dawurung**	Development and optimization of bioprocesses for scale-up of chimeric viral antigens for flavivirus vaccines and diagnostics.
P21	**Kyle Macauslane**	The Dynamic Host Cell Proteome and Glycoproteome in H1N1 and H3N2 Influenza Infection
P22	**Loic Bourdon**	Co-encapsulation of Ancestral Cytochrome P450 with their redox partner into VLP (P22)
P23	**Nadezhda Aleksandrova**	The molecular mechanisms of cellular recognition by ABC toxins
P24	**Nicholas See**	Mechanistic Insights into the Glycosylations of L-Idose Thioglycosides
P25	**Oliver Hughes**	Modelling Inter-Replicate Regional Bias in the Integration of ChIP-seq experiments
P26	**Pallav Joshi**	The role and impact of N- and O-glycosylation on cellular lipids, metabolites, and proteins of S. cerevisiae
P27	**Qisheng Pan**	Characterisation on the pathogenic effect of the missense mutations of p53 via machine learning
P28	**Ryan Johnston**	The Integration of innovative platform technologies for the diagnosis of flaviviral infections
P29	**Sam Davis**	Probabilistic inference of biochemical and biophysical properties for ancestral proteins
P30	**Satakshi Gupta**	Investigating the role of fluorinated cations in boosting the performance of lead halide perovskite-based solar cells

Poster Listing

P31	**Saurab Kishore Munshi**	Do growth substrates matter? - Metabolic adaptation of Haemophilus influenzae to the host
P32	**Sean Riek**	SERoM: Reengineering Mycobacterial Antigens for Improved TB Vaccination Strategy
P33	**Shannon Jewell**	Activation of NLRX1 for the Therapeutic Resolution of Inflammation in Parkinson's Disease
P34	**Shulei Liu**	Role of site-specific N-glycosylation on protein stability
P35	**Solace Roche**	Toxin complexes: uncovering the mechanisms of pore-formation
P36	**Sophie Leech**	Defining the relationship between infant and maternal gut microbiota, and early infant growth in a recent Queensland birth cohort pilot
P37	**Tanika Duivenvoorden**	A Computational Study on Cathode Structure in Lithium-Sulfur Batteries Using Molecular Dynamics Simulations
P38	**Timothy Muusse**	Characterisation of TIR domain interactions in the TLR4 signalosome
P39	**Vivian Shang**	Synthesis of M_4L_6 Metal Organic Cages via a Chiral Auxiliary Strategy
P40	**Vladimir Morozov**	Predicting toxicity of a protein from its primary sequence
P41	**Wanyi Wang**	Development of Highly Immunogenic Nanovaccine Delivery Systems
P42	**Xiaoqi Qian**	Structural and Functional analysis of the TIR-domain in Toll-like receptors 7, 8 and 9 signaling and the Interactions with adaptor proteins
P43	**Xiaowen Xu**	An efficient protocol for the synthesis of glycosyl fluorides
P44	**Yan Cheng**	Structural basis of resistance to herbicides that target acetohydroxyacid synthase
P45	**Yanhua gao**	Investigations of Mycobacterium tuberculosis Acetohydroxyacid synthases as therapeutic drug targets

Poster Listing

P46	**Yichi Zhang**	Nano-bioreactors for sustainable biopolymer production
P47	**Yizhou Liu**	Coumarin-derived vitamin K antagonists
P48	**Yumeng Hao**	Lactoferrin-derived peptides synergistically increase the membrane-disrupting activity of antifungal drug amphotericin B.
P49	**Yunzhuo Zhou**	DDMut: predicting mutation effects on protein stability using deep learning
P50	**Zannati Ferdous Zaoti**	Structural and functional characterisation of dsDNA binding by the MyD88 adaptor protein
P51	**Lachlan Miller**	Molecular dynamics simulations of water flow in graphene-based desalination membranes
P52	**Forhad Karim Saikot**	Structural studies of the active state assembly and functional diversity of SARM1.
P53	**Vishal Pandey**	Biochemical and structural basis of nanobodies' interaction with SARM1 (sterile alpha and TIR motif containing 1)
P54	**Joan Zapiter**	Nitrite Reduction by Electrode-Immobilised Human Molybdoenzymes
P55	**Sarah Shah**	The genomes of the free-living Symbiodiniaceae Effrenium voratum highlight the differences between symbionts adapted to ancient versus modern coral reefs
P56	**Helen McGuinness**	Characterisation of Axundead, a novel downstream regulator of Wallerian degeneration

Abstracts

1.

PRIMITI: a computational approach for accurate prediction of miRNA-target mRNA interaction

Korawich Uthayopas[1,2,3], Alex G. C. de Sá[1,2,3,4], Azadeh Alavi[5], Douglas E. V. Pires[1,2,3,6,*], David B. Ascher[1,2,3,4*]

[1] *School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, 4072, Australia*
[2] *Systems and Computational Biology, Bio21 Institute, University of Melbourne, Parkville, Victoria, 3052, Australia*
[3] *Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria, 3004, Australia*
[4] *Baker Department of Cardiometabolic Health, University of Melbourne, Parkville, Victoria, 3010, Australia*
[5] *School of Computational Technology, RMIT University, Melbourne, VIC 3000, Australia*
[6] *School of Computing and Information Systems, University of Melbourne, Parkville, Victoria, 3052, Australia*

Recent studies have highlighted the important roles microRNAs (miRNAs) play across a broad range of biological processes, with their dysregulation associated with multiple diseases. miRNA acts through the complementary binding with messenger RNAs (mRNAs), causing the degradation. Understanding the miRNA-mRNA interaction is therefore essential for developing effective diagnostic and therapeutic strategies, but have proven challenging for large-scale experimental screening. Several computational models aiming to predict miRNA-mRNA interactions have been proposed, albeit with limited predictive capabilities and usability. To overcome these drawbacks, we have developed PRIMITI, a novel machine learning model built using CLIP-seq and expression data, to accurately predict canonical binding sites and miRNA-mRNA repression activities. In PRIMITI, reliable negative sample selection was coupled with novel RNA characterisation approaches to derive great predictive performance for identifying both miRNA-target site bindings and miRNA-target mRNA repression activities, achieving Matthew's correlation coefficients up to 0.75 on internal and external validations. Additionally, PRIMITI succeeded in predicting repression in unseen microarray data, correctly identifying the largest number of validated miRNA-target repressions accurately predicted as repressed, when compared to state-of-the-art methods, demonstrating PRIMITI's utility for preliminary screening. PRIMITI was made publicly available on a user-friendly web server at https://biosig.lab.uq.edu.au/primiti.

Abstracts

2.

***Cytocipher* detects significantly different populations of cells in single cell RNA-seq data**

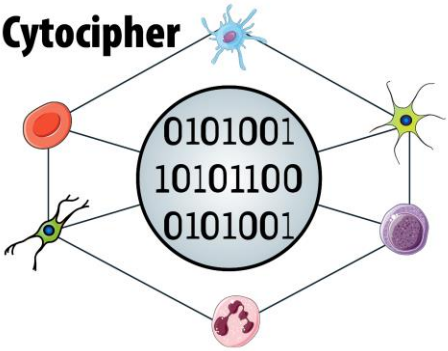
Brad Balderson[1], Michael Piper[2], Stefan Thor[2], Mikael Boden[1]

[1] School of Chemistry & Molecular Biosciences, The University of Queensland, Australia.
[2] School of Biomedical Sciences, The University of Queensland, Australia.

Identification of known or novel cell types with single cell RNA-seq (scRNA-seq) is revolutionising the study of multicellular organisms. Typical scRNA-seq analysis often results in clusters of single cells that may not display distinct gene expression. To mitigate this, post hoc manual curation is used to align clusters with transcriptionally distinct cell populations. Manual cluster editing is however time-consuming, error-prone, and irreproducible.

Here, we present *Cytocipher*, a bioinformatics method and software package that statistically ensures cell clusters are transcriptionally distinct. Application to normal tissue, development, disease, and large scale atlas data reveals the broad applicability and power of *Cytocipher* to generate biological insights in numerous contexts. This included the identification of cell types not previously described in the datasets analyzed, such as CD8+ T cell subtypes in human peripheral blood mononuclear cells; cell lineage intermediate states during mouse pancreas development; and subpopulations of luminal epithelial cells over-represented in prostate cancer. *Cytocipher* can also scale to large datasets with high test performance, as shown by application to the tabula sapiens atlas representing >480,000 cells.

Cytocipher is a novel and generalisable method that ensures transcriptionally distinct and programmatically reproducible single cell clusters. *Cytocipher* is available at https://github.com/BradBalderson/Cytocipher.



Abstracts

3.

Genome-wide transcriptome analysis reveals the diversity and role of non-coding RNAs in dinoflagellates

Yibi Chen[1], Katherine Dougan[1], Debashish Bhattacharya[2], and Cheong Xin Chan[1]

[1] The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Centre for Ecogenomics, Brisbane, QLD, 4072, Australia
[2] Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ 08901, United States

Dinoflagellates are protists of great economical and ecological importance. Increasing genome and transcriptome data from dinoflagellates reveal extensive genomic divergence even among closely related taxa, and lineage-specific innovation of gene functions. However, most studies thus far focused on protein-coding regions in the genomes; non-coding RNAs (ncRNAs), known to play key roles in regulating gene expression in eukaryotes, remain little known in dinoflagellates. Here, we developed an analytic workflow to identify, classify, and compare polyadenylated ncRNAs using RNA-Seq and genome data from 22 dinoflagellate taxa. We found up to 50% of the RNA-Seq reads in each transcriptome dataset mapped to putative ncRNAs in the corresponding genome. Using these data, we assessed the diversity of ncRNAs among the distinct lineages and identified the associated gene targets. We assessed the differential expression of ncRNAs relative to distinct growth conditions (*e.g.* heat stress or growth phase); for Family Symbiodiniaceae that comprise largely symbiotic species, we also assessed differential expression relative to free-living (in culture) and symbiotic (*in hospite*) stages. Our results reveal how ncRNAs contribute to regulation of gene expression in dinoflagellates, and provide novel insights into the molecular regulatory mechanisms that underpin diversification of these taxa.

Abstracts

4.

Probing the plastic degradation potential of marine microbes

Yi Peng[1], Tania Toapanta[2], Kevin V. Thomas[2], Christian Rinke[1]

[1] Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, 4072, Australia
[2] Queensland Alliance for Environmental Health Sciences, The University of Queensland, Brisbane, QLD, 4102, Australia

The durability of plastics is desirable for many applications. However, it also causes improperly disposed plastics to persist in nature and accumulate in many environments and organisms. In recent years, only 20% of global plastic waste has been recycled, and the majority has been discarded in landfills or the natural environment. This calls for the exploration of new ways to recycle plastic waste. Utilizing plastic degrading microbes is a promising approach as several microbial taxa and enzymes have been associated with plastic degradation.

In this study, we aimed to identify differentially abundant microbes on virgin and weathered polyethylene, polypropylene and polystyrene and their encoded enzymes with plastic degrading potential. To achieve our goals, we used a combination of microscopic and spectroscopic analyses and a culture independent microbial exploration via metagenomics.

Scanning electron microscopy images confirmed microbial settlement on the plastics, while Fourier-transform infrared spectroscopy indicated microbial plastic degradation. Metagenomic analysis revealed diverse microbial communities growing on plastics, with specific lineages like Thalassospiraceae showing significantly differentially abundances for certain plastic types. We have also identified enzymes that may participate in the initial breakdown of long chain polymer. These enzymes can be validated in subsequent studies, and potentially incorporated into advanced bio-recycling.

Abstracts

5.

Understanding how industry conceptualizes a valuable science graduate

Lauren J Carpenter[1], Deanne Gannaway[2] and Susan Rowland[1, 3]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, 4072, Australia.
[2] Institute of Teaching and Learning Innovation (ITaLI), The University of Queensland, Brisbane, Queensland, 4072, Australia.
[3] Associate Dean Academic and Deputy Executive Dean, Faculty of Science, The University of Queensland, Brisbane, Queensland, 4072, Australia.

The Australian Government consistently incentivises increasing the STEM capabilities of our workforce, with the recent Job-Ready Graduates Package illustrating this. However, science graduates struggle to find employment post-graduation, while industry struggles to recruit sufficiently skilled science graduates. It appears universities may be insufficiently preparing science graduates for the workforce. In this study, we explore the idea that miscommunication between universities and industry may contribute to the "problem" of science graduate "job-readiness". Does industry truly understand what they want from science graduates? Can they clearly articulate it? This study asks science industry employers about their conceptions of graduates who thrive in their workplace. We ask them to describe this graduate's qualities and capabilities and explain how they demonstrate value to employers. The results reveal surprising conceptions of "job-ready" and "industry-valuable" graduates, across a range of science industry contexts. The results also suggest ways universities can better prepare graduates to demonstrate their value to employers. In this interactive session, we will examine how our conceptions compare with the results from industry perceptions. We will discuss the current terminology of "job-readiness" and consider ways we can improve industry-university collaboration around "industry-valuable" graduates.

Abstracts

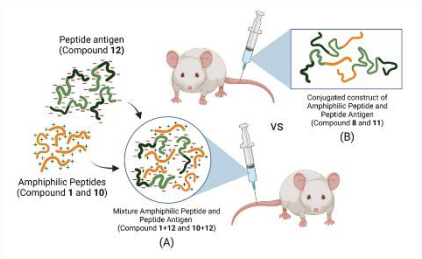
6.

Polyglutamic amino acids for delivery of self-adjuvanting peptide-based subunit vaccines against Group A *Streptococcus*

Farrhana Ziana Firdaus,[1] **Jingyi Fan**,[1] Jieru Yang,[1] Mariusz Skwarczynski,[1] Istvan Toth [1,2*]

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD 4072, Australia*
[2] *School of Pharmacy, The University of Queensland, Woolloongabba, QLD 4102, Australia*

Group A *Streptococcus* (GAS), a gram-positive bacterium, causes a broad spectrum of infections with high morbidity worldwide. Antibiotics are the main primary of intervention with GAS infections, but unfortunately antibiotics are unable to treat fatal GAS complications. Currently, no commercial vaccines are approved to treat GAS infection. Immunization with attenuated/inactivated whole pathogens can induce the adaptive immune response protection against infections; however, they are not fully safe. Peptide-based subunit vaccines, consisting of microbial components from pathogenic proteins, are safe, simple production, and can eliminate undesirable immune responses based on their defined formulations, while poor immunogenicity of peptide-based subunit vaccines requires potent adjuvants/delivery system to improve the adaptive immune response.
In our research, we applied negatively charged polyglutamic amino acids conjugated with multiepitopes (B-cell epitope **J8** derived from GAS M protein and T-helper epitope, **P25**), then physically mixing with lipoamino acids, forming vaccine complexes with self-adjuvanting delivery system. Vaccine candidates were designed to determine whether charge of vaccine candidates can affect the immune response and whether a simple physical mixture of self-adjuvanting vaccines is sufficient to induce the stronger humoral immune response compared to conjugation (**Figure 1**). All vaccine candidates were synthesized by Fmoc-solid-phase peptide synthesis (SPPS) method and tested their secondary structure and characterizes. Vaccination was performed subcutaneously in Swiss mice and antibody results were analyzed via ELISA. All vaccine conjugates elicited significant IgG titers following immunization, while physical mixture is unable to enhance the stronger humoral immune response compared to conjugation.



Abstracts

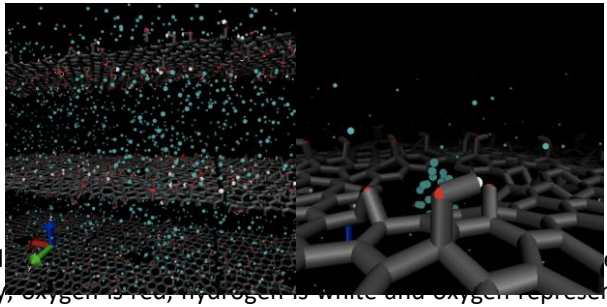
7.

Molecular dynamics simulations of water flow in graphene-based desalination membranes

Lachlan Miller[1,2], Debra J. Searles (Bernhardt)[1,2], Amy Geddes[1]

[1] Australian Institute for Biotechnology and Nanoengineering
[2] School of Chemistry and Molecular Biosciences, The University of Queensland

Reverse osmosis membranes are anticipated to be the future of desalination for increased freshwater needs worldwide. Graphene, a 2D carbon sheet, allows selective permeation of water and exclusion of salt ions through tunable size nanopores in an atomic-thick sheet. Nano-channels in the experimentally more stable multilayer graphene membranes can also be used.
Since graphene is highly hydrophobic, water subject to a force slips on the surface and flows through nanoconfined channels with a much higher velocity than would be predicted using the Navier-Stokes no-slip boundary condition. This enables high permeability and reduces the driving force needed for high water flux. Graphene oxide (graphene decorated with hydroxyl and epoxide functional groups) is used to synthesize these membranes and tune interlayer spacing, but will affect slip velocity.
Since these channels are 1-3 water molecules wide, atomic-scale molecular dynamics simulations are ideal for study of these membranes. In this work selective water and salt ion exclusion is demonstrated with varying pore sizes. In addition, with varied degrees of oxidation, there were changes to the flow profile of water through the channel, decreased slip velocity measured at non-equilibrium and an increased friction coefficient of the water on the channel wall measured at equilibrium.



Atomic model of graphene oxide membrane (carbon is grey, oxygen is red, hydrogen is white and oxygen representing fixed waters is blue)

Abstracts

8.

Structural insights into the bifunctional mechanism of class I ketol-acid reductoisomerase

Xin Lin, Thierry Lonhienne, Shan Zhen, Julia Kurz, You Lv, Gary Schenk and Luke Guddat.

School of Chemistry and Molecular Biosciences, University of Queensland

Ketol-acid reductoisomerase is an important enzyme for herbicide and drug discovery and also for use in fermentation pathways to produce high value chemicals such as isobutanol. Whilst extensive mechanistic studies have been performed on class II KARIs, a comprehensive analysis of class I KARIs is yet to be undertaken. Here, detailed kinetic and structural analysis have been performed that show the mechanism of the class I KARI from *Camphylobacter jejuni* (*Cj*) is not random, as has been shown previously for *E. coli* KARI, a class II KARI. Kinetic data suggested that 2-acetolactate and NADPH enter active site by an ordered mechanism that can lead to a dead-end complex. Our structures show that in each catalytical cycle, the enzyme active site switches between two conformations to enable the binding of the two substrates. The structure in complex with substrate, 2-acetolactate alone, and in complex with 2-acetolactate, $NADP^+$, and NADPH reveals structural changes take place during the catalytic cycle. The structures show that the binding of 2-acetolactate to the magnesium bound complex triggers the opening of the active site, in a conformation suitable for the subsequent binding of NADPH. Based on these data a novel catalytic mechanism has been proposed based on the crystal structures.

Abstracts

9.

Structural characterisation of the nucleosome remodelling and deacetylase (NuRD) Complex using integrative modelling, single particle analysis, and AlphaFold prediction

Matthew J Jackman[1], Jason Low [2] , Sarah R Webb [1] , Ana PG Silva [1] , Lou Brillault [2] , Shruthi Viswanath [3] , Joel P Mackay [3] , Michael Landsberg [2]

[1] The University of Queensland, Woolloongabba, QUEENSLAND, Australia
[2] School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia
[3] National Centre for Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India

The nucleosome remodelling and deacetylase (NuRD) complex regulates processes important to the epigenetic state of metazoan organisms, ranging from DNA repair to cell differentiation. There is little structural information about the intact complex, however, with most information limited to subcomplexes and structures of the functional domains of its subunits. This project attempts to determine a structure of the intact NuRD complex using single particle electron microscopy, a process that involves optimisation of sample preparation and imaging conditions prior to single particle analysis. Currently maps representing negatively-stained models of NuRD, NuDe (lacking the CHD4 subunit), and MHR (the core deacetylase module of NuRD) from HEK293 and MEL cells, or reconstituted from recombinant components - have been obtained using single particle analysis. Combining these with additional information obtained from crystal structures, chemical-crosslinking mass spectrometry and integrative modelling has allowed for the development of ensemble models that describe the overall architecture of the NuRD complex. Future efforts are now focused on validating these models by optimising approaches to cryo-electron microscopy analysis of these complexes; preliminary efforts in this regard will also be presented.

Abstracts

10.

Characterisation of plant immune TIR domains bifunctional enzymatic activity

Mitchell Sorbello, Bryan Ying Jie Lim, Dalton Heng Yong Ngu, Natsumi Maruta, Bostjan Kobe

School of Chemistry and Molecular Biosciences and Australian Infection Diseases Research Centre, University of Queensland, QLD 4072

Plant nucleotide-binding leucine-rich repeat immune receptors (NLRs) are activated by virulence factors (effector proteins) secreted inside cells by pathogens. NLRs consist of two regulatory domains which mediate effector recognition and NLR oligomerisation and a varying third N-terminal signalling domain. Toll-interleukin-1 receptor (TIR) domain containing NLRs (TNLs) posses' enzymatic activity to produce a variety of compounds. TIR domains cleave NAD^+ to produce adenosine diphosphate ribose (ADPR), which may also be cyclised (cADPR), where varying cyclic linkages are possible. These cADPR compounds have no characterised role in the cell. NAD^+ cleavage also led to production of four novel metabolites when ATP was present, these compounds activated the downstream signalling protein EDS1. A second activity of TIR domains was recently discovered where they formed filaments by binding dsDNA/RNA which led to production of, 2'3'-cyclic nucleotide monophosphates (2'3'-cNMPs). We aimed to characterise these activities, by investigating potential substrates, catalytic mechanisms, and products. We investigated catalytic activity by utilising ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) and nuclear magnetic resonance (NMR) and implemented negative stain electron microscopy to investigate filament formation. Our results indicate that plant TIRs vary in the products they produce which may highlight alternate catalytic mechanisms or signalling pathways within the cell. We show that dsDNA binding may be conserved in plant TIRs, however filament formation is not. We also show that dsDNA is likely not a substrate for 2'3'-cNMP production, contradicting that of previous studies. Together, this research will help develop an understanding of how TNLs mediate signalling in response to pathogen infections.

Abstracts

11.

Japanese Encephalitis Virus: Near-Atomic Structures of Emerging Genotypes Reveal Epitope & Antigenic Differences

Morgan Freney[1], Yu Shang Low[1], Alberto A Amarilla[1], Connor Scott[1], Summa Bibby[1], Benjamin Liang[1], Jessica Harrison[1], Mahali Morgan[1], Roy Hall[1,2], Jody Hobson-Peters[1,2], Naphak Modhiran[1], Daniel Watterson[1,2].

[1] School of Chemistry and Molecular Bioscience, University of Queensland, St Lucia, QLD
[2] Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD

Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in South-East Asia. Molecular genetic analysis clusters JEV into five genotypes (G1–G5), with each differing in their geographical distribution. Earlier this year, JEV (G4) was detected for the first time on mainland Australia, resulting in an unprecedented outbreak. Here, we employed an innovative chimeric flavivirus platform technology based on the Australian insect specific Binjari virus to generate structurally authentic viruses comprising each of the 5 genotypes. Cryo-electron microscopy revealed the first near-atomic structures of emerging JEV genotypes with atomic modelling of the envelope and membrane proteins enabling detailed structural comparisons. Utilising monoclonal antibodies (mAbs) known to recognise cross-reactive and JEV specific epitopes, we found unique antigenic differences between genotypes. We then tested human sera from vaccinated (G3) individuals and report substantial reduction of antibody neutralisation against emerging JEV genotypes; G4 and G5. In conclusion, we have performed the most extensive antigenic and structural characterisation of JEV to date. JEV genotypes appear to be more diverse than previously thought, with key epitope differences contributing to resistance against JEV G3 vaccine derived antibodies. These results have important implications for vaccine design and responding to future JEV outbreaks.

Abstracts

12.

Envelope protein domain III is a key determinate of Yellow Fever Virus structure

Summa Bibby[1], Alberto Amarilla Ortiz[1], Yu Shang[1], Natalee Newton[1], James Jung[1], Roy Hall[1], Jody Hobson-Peters[1], Paul Young[1], Naphak Modhiran[1], Daniel Watterson[1].

[1] School of Chemistry & Molecular Biosciences, The University of Queensland, St Lucia, Australia

Yellow fever virus (YFV) is a mosquito-borne flavivirus that causes severe hepatic disease and mortality in humans. Despite the development of the highly efficacious YFV_{17D} vaccine in 1937, very little is known regarding the molecular mechanisms underlying the 17D strains attenuation. This study aimed to investigate the structural differences between virulent and vaccine strains of YFV using cryogenic-electron microscopy (cryo-EM). Chimeric viruses consisting of the prME structural genes of three YFV strains (17D, Asibi and ES504) and the genomic backbone of Binjari virus (BinJV), a lineage II insect-specific flavivirus, were generated using an optimised circular polymerase extension reaction methodology. These BinJV/YFV-prME (bYFV) chimeric viruses retain their insect-specific nature and grow to higher titres than their parental YFVs, making them ideal for cryo-EM analysis. Following the generation of these bYFVs, they were purified and imaged via cryo-EM. Single particle analysis (SPA) was performed on the resulting data sets using Relion 3.1.3. Icosahedral reconstructions of $bYFV_{17D}$ and $bYFV_{ES504}$ revealed significant structural differences between the two strains, including increased structural heterogeneity for $bYFV_{ES504}$ relative to $bYFV_{17D}$. To further investigate the source of these structural differences, a panel of bYFV domain chimeras were generated and screened for structural anomalies via cryo-EM. All domain chimeras in the $bYFV_{ES504}$ backbone retained their parental ES504 phenotype, except for $bYFV_{ES504}/DIII_{17D}$ which resembled a 17D-like phenotype. Subsequent SPA of $bYFV_{ES504}/DIII_{17D}$ resulted in a 6.5 Å reconstruction. Similarly, all the bYFV domain chimeras in the $bYFV_{17D}$ backbone retained their parental 17D phenotype, except for $bYFV_{17D}/DIII_{ES504}$ which resembled a ES504-like phenotype. Furthermore, $bYFV_{17D}/DIII_{Asibi}$ and $bYFV_{Asibi}/DIII_{17D}$ also displayed a swapped phenotype; and subsequent SPA of $bYFV_{Asibi}/DIII_{17D}$ resulted in a of 5.4 Å reconstruction. These results define E-DIII as a key determinant of YFV's structure and suggests a possible mechanism underlying YFV_{17D}'s attenuation.

Abstracts

13.

A new recombinant alphavirus platform based on the insect-specific Yada Yada virus

Mikaela Bell[1], Jessica Harrison[1], Rhys Parry[1], Naphak Modhiran[1], Jana Batovska[2], Stacey Lynch[2], Daniel Watterson[1], Roy Hall[1], and Jody Hobson-Peters[1]

[1] *School of Chemistry and Molecular Bioscience, The University of Queensland*
[2] *Agriculture Victoria Research, AgriBio Centre for AgriBioscience, Victoria, Australia*

Mosquito-borne alphaviruses can cause disease of significant human and veterinary concern. Important vertebrate-infecting alphaviruses (VIAs) include chikungunya (CHIKV) and Ross River viruses (RRV), both of which can cause chronic, debilitating effects, with no current treatments or vaccines available. Insect-specific alphaviruses (ISAs) infect only mosquitoes and cannot replicate in vertebrates. Several were recently discovered, including the first Australian ISA; Yada Yada virus (YYV). Viable clones of many ISAs have never been isolated, including YYV. In this project, ISAs were utilised to construct recombinant viruses for application as potential vaccine candidates and to further alphavirus host restriction studies. An infectious clone of YYV and chimeric alphaviruses with the genetic backbone of YYV and the structural genes of various ISAs or VIAs were constructed using circular polymerase extension reaction (CPER). These viruses successfully replicated in mosquito cells and did not infect vertebrate cells. Antigenic analysis and electron microscopy confirmed structural and antigenic authenticity to parental pathogenic viruses. Purified virions were used to generate the first ISA-reactive monoclonal antibodies, which will become valuable tools for ISA research. ISA chimeras represent a versatile platform for alphavirus research, including application to diagnostics, assessment in mouse models as potential vaccine candidates, and structural analysis of other alphaviruses.

Abstracts

14.

Characterisation of MCMV genes involving in developing salivary gland persistence

Jiawei Ma[1], Kimberly Bruce[1], Nicholas Davis-Poynter[2], Philip Stevenson[1], Helen Farrell[1]

[1] *School of Chemistry and Molecular Biosciences, University of Queensland*
[2] *UQ Child Health Research Centre*

Congenital human cytomegalovirus (HCMV) infection has a 0.6–0.9% prevalence worldwide causing multiple health sequelae in newborns. There is no licensed vaccine. As maternal HCMV infection is asymptomatic and therefore difficult to capture, we use infection of mice with mouse CMV (MCMV) as a model for understanding how HCMV spreads systemically following mucosal entry and how it subsequently establishes a persistent salivary gland infection. Dendritic cells (DC) provide the main vehicle for MCMV spread and their trafficking is thus subject to regulation by engagement with host chemokines. MCMV encodes homologues of chemokine/chemokine receptors (designated m131/129 and M33 respectively) which are conserved with HCMV. In this presentation I will summarize results of my PhD investigations which aimed to evaluate how MCMV M33 and m131/129 modulates DC-dependent spread and salivary gland tropism. Firstly, I will describe how distinct components of the M33 G protein-coupled signalling repertoire enables novel DC systemic spread to the salivary glands. Secondly, I will demonstrate that MCMV m131/129 is an essential conduit for MCMV transfer from infected DC to salivary gland epithelial cells, a process necessary for establishing MCMV persistence. Taken together, these studies demonstrate how CMV-encoded chemokine and chemokine receptors coordinate key features of in vivo infection. Targeting their action may provide novel strategies in preventing virus spread or establishment of virus persistence.

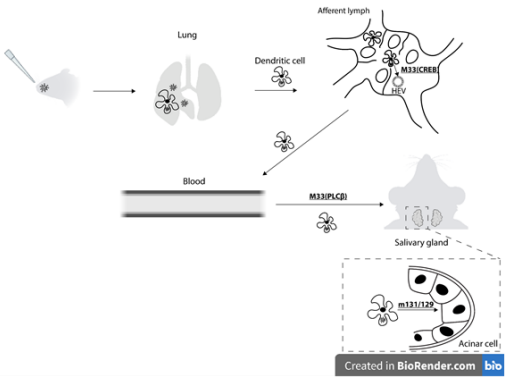


Figure1 The genes involved in MCMV systemic dissemination and salivary gland persistence. Following intranasal infection, 1) MCMV infect lung dendritic cells (DC). 2) Infected DC migrate via afferent lymph to draining LNs. 3) M33 Gq/11-dependent CREB signalling drives infected DCs enter high endothelial venules (HEVs) 4) M33Gi/o PLC-b signalling drives DC extravasation to the salivary glands. 5) In the salivary glands, MCMV m131/129 is essential for DC-dependent infection of salivary gland acinar cells.

Abstracts

1.5

Structural and Functional Characterization of Bacterial TIR Proteins

Sulin Li [1], Mohammad Kawsar Manik [1] , Thomas Ve [2] , Jeffrey D Nanson [1] , Bostjan Kobe [1]

[1] School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience, and Australian Infectious Diseases Research Centre, University of Queensland, St Lucia, QLD, Australia
[2] Institute for Glycomics, Griffith University, Southport, QLD, Australia

Bacterial Toll/interleukin-1 receptor (TIR) domain-containing proteins have been shown to mediate to the pathogenicity and anti-viral activity of bacteria. During bacterial infection, TIR domain-containing proteins may act as virulence factors to inhibit immune responses by interfering with Toll-like receptor signalling. Additionally, some bacterial TIR domain-containing proteins possess NAD^+ nucleosidase activity, which is not only also related to the virulence of pathogenic bacteria, but also plays an important role in bacterial anti-viral defence. Here we report our studies on two bacterial TIR domain-containing proteins: PumA and AbTIR. *Acinetobacter baumannii* TIR domain-containing (AbTir) is one of the few bacterial proteins that has been reported to produce v-cADPR after NAD^+ nucleosidase. We determined the crystal structure of AbTir TIR domain and the structure of v2-cADPR.–Furthermore, we found that addition of an inhibitor of AbTir NAD^+ nucleosidase activity (3AD) induces the formation of AbTir TIR domain filaments, and appears to trap the protein in an active conformation. Using cryoEM, we successfully determined the AbTir filament structure. Recent studies showed that PumA, which is from multi-drug resistant pathogen *Pseudomonas aeruginosa* PA7, is essential for PA7 strain virulence. We determined that PumA also has NAD^+ nucleosidase activity. We also saw observed AbTir TIR domain filaments upon incubation with 3AD. Further structural and functional studies will determine the structure of these filaments and investigate the role these assemblies play in bacterial virulence and anti-viral defence.

Abstracts

16.

Cross-regulatory control of antibiotic resistance and virulence in uropathogenic *Escherichia coli*

Chyden Chang[1,2], Minh-Duy Phan[1,2], Dalton H.Y. Ngu[1,2], Steven J Hancock[1,2], Nguyen Nhu[1,2], Kate M. Peters[1,2], Bostjan Kobe[1,2], and Mark A. Schembri[1]

[1]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia
[2]Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Queensland, Australia

Uropathogenic *Escherichia coli* possess transcriptional regulators that facilitate its capacity to adapt to different environments and stresses. One such regulator belonging to the widespread MarR family, MprA, controls capsule and flagella expression, as well as expression of the EmrAB-TolC drug efflux pump. While MprA provides an intriguing link between the coordinated expression of virulence and antibiotic resistance, its regulon and mechanisms of transcriptional control remain to be elucidated. Here, we employed transcriptome analysis (RNA-Seq) in conjunction with complementary protein-DNA mapping (ChIP-Seq) to define the MprA regulon. Transcriptome analysis identified 66 differentially expressed target genes of MprA, including 35 novel genes. Of these 66 target genes, ChIP-Seq determined that 37 were directly regulated by MprA via interaction with nine promoter elements. The novel MprA-regulated targets included a gene encoding a common lipoprotein as well as genes involved in sialic acid catabolism, which were confirmed at the protein and phenotypic levels, respectively. We revealed a new role for MprA as a central activator of sialic acid scavenging, catabolism and utilisation. Taken together, we have elucidated the regulon and protein-DNA interactions of MprA, a global regulator that coordinates the expression of both virulence and antibiotic resistance in a clinically important antibiotic resistant pathogen.

Abstracts

17.

The DmsABC dimethyl sulfoxide reductase – an enzyme mediating anaerobic energy generation or a virulence determinant in pathogenic bacteria?

Qifeng Zhong[1], Marufa Nasreen[1], Alastair McEwan[1], Bostjan Kobe[1,2], Ulrike Kappler[1]

[1] Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD 4072, Australia
[2] Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia

The dimethyl sulfoxide (DMSO) reductase from *Escherichia coli,* DmsABC (EcDmsABC), is an S- and N-oxide reductase with a proposed role in anaerobic respiration with DMSO, a compound minimally present in the human body. Interestingly, DmsABC complexes in *Actinobacillus pleuropneumoniae* and *Haemophilus influenzae,* which can support bacterial virulence, are highly similar to the *E. coli* enzyme. Here, we revealed that in uropathogenic *E. coli* (UPEC), the expression of S/N-oxide reductases, including *dmsA*, can be induced upon hypochlorite exposure. We also showed that EcDmsABC can reduce nicotinamide N-oxide and pyrimidine N-oxide that, unlike DMSO, occur naturally in *E. coli*'s niche. A *ΔdmsA* strain constructed in UPEC strain EC958 exhibited a 70% decrease in growth and a 75% decrease in DMSO reductase activity, compared to the WT strain. Interestingly, the hypochlorite resistance of the Δ*dmsA* strain was 7 times higher than the WT strain. However, the loss of DmsABC did not affect adherence to or invasion of the T24 human bladder cell line. In summary, the HOCl-dependent induction of EcDmsABC, which has been reported *H. influenzae,* supports a role in *E. coli* host interactions, but additional phenotypes may be masked by functional redundancy, as *E. coli* genomes encode a related enzyme complex.

Abstracts

18.

Access to highly specialized growth substrates and production of epithelial immunomodulatory metabolites determine survival of *Haemophilus influenzae* in human airway epithelial cells

Jennifer Hosmer [1] , Marufa Nasreen [1] , Rabeb Dhouib [1] , Ama-Tawiah Essilfie [2] , Horst Schirra [3] , Anna Henningham [4] , Emmanuelle Fantino [4] , Peter Sly [4] , Alastair McEwan [1] , Ulrike Kappler [1]

[1] *School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, Australia*
[2] *QIMR Berghofer Medical Research Institute, Herston, QLD, Australia*
[3] *Centre for Advanced Imaging, University of Queensland, St Lucia, QLD, Australia*
[4] *Child Health Research Centre, South Brisbane, QLD, Australia*

Haemophilus influenzae (Hi) infections are associated with recurring acute exacerbations of chronic respiratory diseases in children and adults. Here, we show that persistence and recurrence of Hi infections are closely linked to metabolic properties, where preferred growth substrates are aligned to the metabolome of human airway epithelial surfaces and include lactate, pentoses, and nucleosides. Enzymatic and physiological investigations revealed that utilization of lactate required the LldD L-lactate dehydrogenase (conservation: 98.8% of strains), but not the redox-balancing D-lactate dehydrogenases Dld and LdhA. Utilization of preferred substrates was directly linked to Hi infection and persistence. When unable to utilize L-lactate or forced to rely on salvaged guanine, Hi showed reduced persistence in a murine model of lung infection and in primary normal human nasal epithelia, with up to 3000-fold attenuation observed in competitive infections. Interestingly, acetate, the major Hi metabolic end-product, had anti-inflammatory effects on cultured human tissue cells in the presence of live but not heat-killed Hi, suggesting that metabolic end-products also influence Hi-host interactions. Our work provides significant new insights into the critical role of metabolism for Hi persistence in contact with host cells and reveals for the first time the immunomodulatory potential of Hi metabolites.

Abstracts

19.

Dissecting the role of RfaH in uropathogenic *Escherichia coli* virulence

Yvette Ong[1], Nhu Nguyen[1], Duy Phan[1], Mark Schembri[1]

[1] University of Queensland, Schembri Lab

Uropathogenic *Escherichia coli* (UPEC) are a major cause of urinary tract and bloodstream infections. UPEC possess key virulence determinants such as capsule and lipopolysaccharides (LPS) that confer protection from host immune factors while advancing disease progression. Many virulence determinants are regulated by the transcriptional regulator RfaH, which binds to a specific motif (JUMPstart sequence) in the leader sequence of target virulence genes to enhance RNA polymerase activity.

We investigated the RfaH regulon in UPEC using a combined bioinformatic and molecular (RNAseq) approach. First, a HMM motif matrix built using characterized JUMPstart sequences was used to predict JUMPstart sequences in 1378 completely sequenced *E. coli* genomes. Next, the RfaH regulon was mapped using RNAseq. Overall, the matrix successfully identified all known JUMPstart sequences and detected a novel JUMPstart sequence belonging to an uncharacterised operon. The RNA-seq analysis and subsequent qRT-PCR validation were congruent with the bioinformatic analysis, demonstrating that capsule, LPS and O-antigen transcription requires RfaH.

Overall, our study demonstrates that RfaH is a key regulator of multiple UPEC virulence determinants. Deciphering RfaH function will improve our understanding of UPEC pathogenesis and may enable the development of urgently needed anti-virulence therapeutics to address the enormous problem of increasing antibiotic resistance.

Abstracts

20.

Differential N-Glycosylation Site Occupancy Depends on Distinct Amino Acid Sequence Features

Marium Khaleque[1], Amanda Nowens[1], Benjamin Schulz[1]

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia QLD 4072, Australia*

N-glycosylation is an essential co/post-translational modification of proteins in eukaryotes, which involves the transfer of glycan from lipid-linked oligosaccharide (LLO) to asparagine side chains in Asn-Xaa-Thr/Ser (Xaa≠Pro) sequons of newly synthesized polypeptides in the lumen of endoplasmic reticulum. N- glycosylation directly affects protein folding and plays important roles in protein function, stability, solubility, secretion, half-life, and resistance to proteases and temperature. Interestingly, the importance of N-glycosylation is highly variable between different glycosylation sites. However, the factors that determine the efficiency of site-specific N-glycosylation are not well understood due to the lack of tools to quantify site-specific glycosylation occupancy. Here, we aimed to understand how control of LLO biosynthesis affected site-specific N-glycosylation occupancy. We developed and optimised a targeted DIA LC-MS/MS MRM-HR method for quantifying site-specific occupancy at diverse N-glycosylation sequons in yeast cell wall glycoproteins, and used this method to compare global site-specific glycosylation under two LLO stress conditions: deficiency of Alg6p or Alg7p, enzymes which catalyse distinct key steps in LLO biosynthesis. We found that a subset of N-glycosylation sites were differentially occupied in different LLO stress conditions, consistent with active regulation of site-specific N-glycosylation depending on distinct amino acid sequence features surrounding the sequons. Our results are consistent with a model in which cells under glycosylation stress maintain efficient glycosylation at critical sites through regulated recognition of specific extended N-glycosylation sequons.

Abstracts

Poster 1

Computing Spatial Mutation Intolerance to Assess Selective Pressure Across the Human Proteome

Aaron Kovacs[1], Michael Silk[2,3], Carlos Rodrigues[1,4], Stephanie Portelli[1,3,4], David Ascher[1,3,4]

SCMB, The University of Queensland, Saint Lucia Campus, Saint Lucia, Queensland, Australia
Centre for Population Genomics, Murdoch Children's Research Institute, Parkville, Victoria, Australia
Systems and Computational Biology, Bio21 Institute, The University of Melbourne, Parkville, Victoria, Australia
Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia

Advances in genomic sequencing, despite initially showing promise, have yet to successfully catalyze routine personalised treatment approaches. Current state-of-the-art computational methods rely on evolutionary conservation measures, which, although capable of effectively identifying important functional and interaction loci, remain weak predictors of pathogenicity.
To address this, we have instead leveraged genomic sequencing information to develop the missense tolerance ratio (MTR), which identifies genomic regions in humans that are under selective pressure. These regions have been found to be greatly enriched in pathogenic variants.

Applying these principles within the 3-dimensional protein structure also proved discriminative, however, the resulting spatial measure of intolerance (MTR3D) was initially limited to experimental protein structures. Given recent advances in protein folding prediction, we have now calculated spatial mutational tolerance across the entire human proteome, greatly expanding the predictive scope of the MTR3D score.

We are now exploring the intersection between conservation within a population (MTR) and across evolution (traditional measures), specifically as the latter method lacks sufficient sampling of protein functional space, suggesting that MTR would be a more sensitive approach.

When combined, our optimised MTR scores offer a deeper insight into the evolutionary forces driving protein structure-function-phenotype relationships, which are crucial next steps towards personalised medicine.

Abstracts

Poster 2

Prediction of oncogenic mutations in VHL disease

Adam Serghini[1], Guillaume Troadec[2], Douglas E.V. Pires[3,4], Stephanie Portelli[1,2,4], David B. Ascher[1,2,4,]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Saint Lucia, Queensland, Australia
[2] Systems and Computational Biology, Bio21 Institute, The University of Melbourne, Parkville, Victoria, Australia
[3] School of Computing and Information Systems, University of Melbourne, Melbourne, Victoria
[4] Australia Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria

The Von Hippel-Lindau (VHL) gene is a tumor suppressor gene, which plays an important role in the hypoxia response. Mutations within the VHL gene are known to cause VHL disease, which is characterized by the formation of cysts and tumors in multiple organs of the body, including the central nervous system, retina, and kidneys. The most common type of tumor to develop as a result of this disease is clear cell renal carcinoma (ccRCC).A major challenge in clinical practice is determining the probability that a tumor will form from a given mutation in the VHL gene. To overcome this limited performance, we characterized the effects of mutations within VHL using in silico biophysical tools describing changes in protein stability, dynamics and affinity to binding partners to provide insights into the structure-phenotype relationship. Preliminary results show that our model can identify ccRCC-causing missense mutations with an accuracy up to 0.79, and MCC up to 0.51 on 10-fold cross validation. Our predictive model achieved comparable performance across two separate independent test sets, out performing previous methods. This work offers a promising initial performance for an ultimately clinically-applicable tool.

Abstracts

Poster 3

Investigating mechanisms of synergy between amphotericin B and lactoferrin-derived peptides

Anneka Pereira Schmidt, Chandra Harshita Chavali, Yumeng Hao, Evelyne Deplazes

School of Chemistry and Molecular Biosciences, The University of Queensland

Invasive fungal infections (IFIs) are characterised as systemic, severe, and life-threatening infections caused by pathogenic fungi. IFIs require systemic antifungal therapy, for which there are four major antifungal drug classes: polyenes, flucytosine, azoles, and echinocandins. Amphotericin B (AmB) is a polyene antifungal, which, first licensed in 1959, is considered one of the most valuable antifungal drugs of the last 60 years. However, its clinical usefulness is limited by severe, dose-limiting side effects. Recent research has focussed on identifying synergistic partners that could potentiate the effect of AmB, reducing the required dose and thus toxicity. It was recently discovered that peptides derived from lactoferrin, a multifunctional glycoprotein present in high concentrations in human colostrum and breast milk, reduced the minimum inhibitory concentration of AmB up to 4- to 8-fold against selected *Candida* and *Cryptococcus* species. Understanding the mechanism of synergy between AmB and LFG is required for developing this combination therapy for clinical use. This project will employ biophysical techniques such as isothermal titration calorimetry, nuclear magnetic resonance spectroscopy, and mass spectrometry to probe the mechanism of synergy between AmB and lactoferrin-derived peptides.

Abstracts

Poster 4

The Origin of Vertebrate Steroids and Steroidogenic P450 Enzymes

Anthony Bengochea, Gabriel Foley, Elizabeth Gillam

School of Chemistry and Molecular Biosciences, University of Queensland

Steroid molecules are known perform to many conserved roles in the physiology of vertebrate animals and probably also perform important but as yet uncharacterized roles in invertebrates. Steroids found in vertebrates are considered distinct due to the lack of the alkyl side chain that is removed via side-chain cleavage by, CYP11A1, a cytochrome P450 enzyme. Several other P450s are conserved in the biosynthesis of vertebrate steroids, such as CYP17A1 and CYP19A1, but clear orthologs to these enzymes have not been identified outside the chordate lineage. Identification of orthologs to vertebrate steroidogenic enzymes would help elucidate the origin of vertebrate steroids and how invertebrate animals are biosynthesizing and utilizing steroids. To identify putative orthologs, protein and transcriptome databases were searched using BLAST and query sequences from *Danio rerio* and *Branchiostoma spp.*. The search results were scored using HMMER, then used to produce a phylogenetic tree. The sequences which aligned well, scored highly, and consistently branched with vertebrate P450 enzymes will be included in sequence datasets used for the ancestral sequence reconstruction of vertebrate P450 enzyme families. Several putative orthologs of CYP19, a P450 family which has only been identified in chordates previously, have been identified in Cnidaria, Echinodermata, and Protostoma.

Abstracts

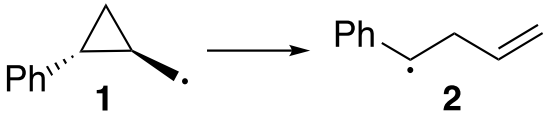
Poster 5

Are ultrafast rebound rates explained by radical clock (mis)-calibration?

Ariel Jones, Elizabeth Krenske

School of Chemistry and Molecular Biosciences, The University of Queensland

The trans-(2-phenylcyclopropyl)carbinyl radical (1) is a short-lived reactive intermediate, which undergoes ring-opening with a 3□1011 s-1 rate constant.1 1 has been employed in competition kinetics studies to time Cytochrome P450 (CYP) catalysed hydroxylation reactions.2, 3 The ring-opening rate constant for 1 may depend on the method by which 1 is generated; if so, the experimentally-derived CYP hydroxylation rates may be unfounded.



This research aimed to determine whether the rearrangement rate constant for 1 depends on the method by which 1 is formed. Combined Density Functional calculations and Molecular Dynamics smulations were used to determine rearrangement rate constants for 1 when generated from different chemical precursors. The calculated rearrangement rate constants for 1 were: 1.07□1012 s-1 when generated from a thiohydroxamate ester precursor; and 9.50□1011 s-1 when generated by hydrogen atom abstraction of (2-methylcyclopropyl)benzene. While these rate constants differ with statistical significance, they cannot entirely account for the extent of experimental variation in measured CYP hydroxylation rates.

References

1. Newcomb, M.; Johnson, C. C.; Manek, M. B.; Varick, T. R., Picosecond radical kinetics. Ring openings of phenyl-substituted cyclopropylcarbinyl radicals. J. Am. Chem. Soc. 1992, 114 (27), 10915-10921.
2. Atkinson, J. K.; Ingold, K. U., Cytochrome P450 hydroxylation of hydrocarbons: Variation in the rate of oxygen rebound using cyclopropyl radical clocks including two new ultrafast probes. Biochemistry 1993, 32 (35), 9209-9214.
3. Toy, P. H.; Newcomb, M.; Hollenberg, P. F., Hypersensitive Mechanistic Probe Studies of Cytochrome P450-Catalyzed Hydroxylation Reactions. Implications for the Cationic Pathway. J. Am. Chem. Soc. 1998, 120 (31), 7719-7729.

Abstracts

Poster 6

Functional characterisation of plant TIR domains and their role in plant innate immunity

Bryan Ying Jie Lim[1], Natsumi Maruta[1], Mitchell Sorbello[1], Dalton Heng Yong Ngu[1], Boštjan Kobe[1]

[1] School of Chemistry and Molecular Biosciences and Australian Infection Diseases Research Centre, University of Queensland, QLD 4072

In plant innate immunity, the superfamily of nucleotide-binding leucine-rich repeat receptors (NLRs) are responsible for the recognition of pathogen effectors. Recent studies have shown that TIR domains of TIR-NLRs (TNLs) or TN/TIR-only proteins are able to cleave nicotinamide adenine dinucleotide (NAD^+) into a large variety of nucleotide containing products. These products were observed to be essential for the activation of downstream signalling eventually leading to localised cell death. However, the exact mechanism for the formation of each product and its associated substrates are still poorly understood. Additionally, TIR domains has also been found to form a filament along double stranded DNA and cleave DNA/RNA to produce 2',3'-cAMP/cGMP. My project aims to structurally and functionally characterise four different plant TIR domains (TX10, $TN11^{TIR}$, $RUN1^{TIR}$, and $RPS4^{TIR}$). In support of the current hypothesis of TNLs roles in innate immunity, $RUN1^{TIR}$ and $RPS4^{TIR}$ has been found to produce the downstream signalling molecules using liquid chromatography-mass spectrometry (LC-MS). Additionally, while electrophoretic mobility shift assays (EMSA) on TIR domains demonstrate DNA binding activity, these TIR domains were unable to produce 2',3'-cAMP/cGMP. $RUN1^{TIR}$ has also been found to produce filamentous structures upon incubation with DNA using negative staining electron microscopy. Future work will utilise different substrates and mutants in order to further current understanding of the conversion of NAD^+ to nucleotides as well as structural characterisation of the DNA-TIR filament.

Abstracts

Poster 7

Characterising the membrane-binding properties of peptides that potentiate the effect of the anti-fungal drugs amphotericin

Chandra Harshita Chavali[1], *Kenya Fernandes*[2], *Sameer Kulkarni*[2], *Richard Payne*[2], *Dee Carter*[2], *Evelyne Deplazes*[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia
[2] School of Life and Environmental Sciences and the Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, NSW, Australia.
ED: e.deplazes@uq.edu.au

Invasive fungal infections cause severe clinical outcomes and have high mortality rates. Amphotericin B is one of the most effective antifungal drugs and is used to treat life-threatening fungal infections despite its dose-limiting toxicity causing severe side effects including vomiting, fever and chills, pain at the injection site, and chronic kidney damage. Nevertheless, AmB is still used because it remains effective against a wide range of pathogens, and resistance is rare. AmB interacts with ergosterol in the fungal cell membrane to form pores and sequester the sterol from the membrane. Cell death is induced by pores leading to leakage of ions, thus disrupting ion homeostasis and the lack of ergosterol that interferes with various cell-internal processes.

Lactoferrin (LF) is a protein derived from milk that has a synergistic effect with AmB. Lactofungin (LFG) is a peptide obtained by pepsin digestion of LF, that when used with AmB was found to increase the efficacy of AmB by a 4-16-fold in many clinically relevant fungal species such as *Candida albicans*, *Candida neoformans* and *Candida glabrata*.

Recent experiments with model membranes show that the synergy is lipid-dependent and specific to ergosterol (unpublished). It is hypothesized that the LFG binds AMB in solution or inside membranes and that the LFG-AMB complex then further interacts with ergosterol on fungal membranes to form pores that are more stable than AMB pores.

To address this hypothesis, we study the binding of LFG to AmB and AMB in ergosterol-containing membranes using Isothermal Titration Calorimetry (ITC). We found that when AmB is titrated into solutions of LFG, heat is released, indicating that LFG binds to AmB. Analysis shows that the binding is dominated by enthalpy, with a small entropic component.

Abstracts

Poster 8

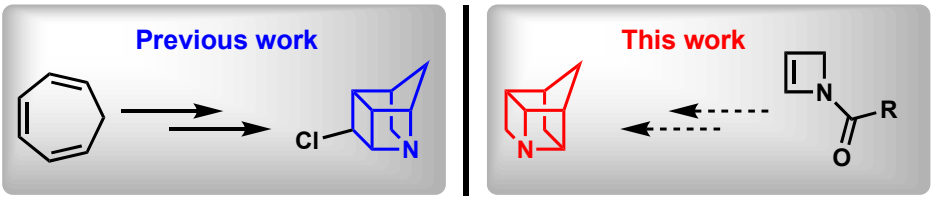
Towards Azabioisostere Design – seco-azahomocubanes

Chuyi Su,[1] Madeleine A. Dallaston,[1] Tyler Fahrenhorst-Jones,[1] Renée D. Watson,[1] G. Paul Savage,[2] Craig M. Williams[1]

[1] *School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, Australia.*
[2] *CSIRO Manufacturing, Ian Wark Laboratory, Melbourne, VIC, Australia*.

Recent investigations into the potential and efficacy of the bioisosteric replacement of aromatic rings with hydrocarbon cages have been carried out to provide novel and more efficient structures for drug development and optimization. For example, cubane has been validated by the Williams group as a benzene bioisostere in some drug and agrochemical templates. Tertiary nitrogen-containing caged hydrocarbons are less common, but have potential to act as heterocyclic isosteres in a similar way to that of cubane or bicyclo[1.1.1]pentane for benzene.

A range of *N*-substituted 2-azetines have been explored to further develop an understanding of the nature of this highly reactive system. Efforts in the deployment of azetines towards seco-azahomocubanes will be presented.



Abstracts

Poster 9

Investigating Wild Yeast Metabolomes to Make Better Beer

Daniel J. Ellis[1] , Edward D. Kerr[1] , & Benjamin L. Schulz[1]

School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD 4072, Australia

The production and consumption of fermented beverages has been carried out for millennia by numerous cultures, and today represents a vast global industry of diverse products. *Saccharomyces* yeasts have traditionally dominated this industry, however, consumer demands for new products with varying sensory profiles and actual or perceived health benefits is driving the use of non-*Saccharomyces* yeasts in fermented beverage production. The influence of non-*Saccharomyces* yeasts on fermented beverage sensory characteristic diversity is, in large part, due to the diverse metabolites they produce. The use of metabolomic analyses allows for the exploration of the impact non-*Saccharomyces* yeasts have on fermented beverage sensory qualities. Here we have used a suite of untargeted DIA-LC-MS/MS and HS-GCMS approaches to identify and quantify the different metabolites produced by yeasts from our library of native non-*Saccharomyces* yeasts. We identified significant differences in metabolite production between our native wild yeasts and when compared to US05, a commercial *S. cerevisiae* brewing yeast.

Abstracts

Poster 10

Investigation of RNAi-inhibitor activity in the insect-specific flavivirus, Binjari virus

Dylan Bowman, Henry de Malmanche, Jody Peters and Roy Hall

School of Chemistry and Molecular Biosciences, The University of Queensland

Binjari virus (BinJV) is a lineage-II insect specific flavivirus with qualities that make it suitable for biotechnology. BinJV has a malleable genome, making it tolerant to genome swapping events while maintaining a high replication efficiency. Deliberate swapping of structural genes of BinJV with those of pathogenic flaviviruses has facilitated the development of chimeric vaccines and diagnostic antigens. As the BinJV chimera's development has progressed so has the need for understanding the interaction of BinJV and the BinJV chimeras with the production cell line, and in mosquitoes. The interactions between BinJV's non-structural proteins and RNAi, a form of anti-viral immunity prevalent in insects is of particular interest. Other groups have reported that proteins encoded in flavivirus genomes (such as dengue virus), namely NS2A and NS4B act as viral suppressors of RNAi (VSRs). This connection is what spurred the current research project. Several reporter assay set ups were developed with the end goal of testing if proteins encoded by BinJV also exhibit VSR activity. To this end, insect cell lines Sf9, S2 and RML-12 cells were all tested for their ability to express enhanced green fluorescence protein, suppress it via RNAi and have that suppression quantitatively measured via flow cytometry.

Abstracts

Poster 11

Characteristics of patients receiving long-term intrathecal opioid infusions for management of chronic pain: A comparison with normative data derived from pain clinic populations in Australia and New Zealand

Elouise Comber[1], Em Prof Maree Smith[1], Em Prof Jenny Strong[1], Assoc Prof Brendan Moore[1,2], Assoc Prof Asad Khan[1]

[1] The University of Queensland
[2] Axxon Pain Medicine, Brisbane Private Hospital

Chronic pain is defined as pain that persists for over three months. Chronic pain encompasses a complex tridirectional interaction between biological, psychological and social factors. This complex interplay of factors often contributes to the ineffectiveness of the exclusive use of pharmacological treatments for pain management. Chronic pain medications dosed via the oral route often lack efficacy and/or cause dose-limiting side-effects. An alternative option is an intrathecal (IT) pump, which administers opioids alone or in combination with analgesic adjuvant drugs (e.g. local anaesthetic, clonidine) close to spinal cord receptors/ion channels, increasing analgesia duration and decreasing systemic side effects. This study used standardised assessment tools to evaluate the pain severity and quality of life of 49 patients with chronic pain, managed via IT opioids (with and without adjuvant agents). Data was collected via questionnaire-based interviews either face-to-face or via telephone call. Data collected was compared against a normative dataset published by Nicholas et al. (2019) for patients not receiving intrathecal therapy for chronic pain. Comparison of this study's research data with the dataset published by Nicholas et al. (2019) suggests that IT opioid therapy is valuable in decreasing pain severity and increasing quality of life for individuals suffering from chronic pain.

Abstracts

Poster 12

704: A novel ionophore antibiotic against Gram-positive multidrug-resistant bacterial infections

Gen (Tom) Li, David De Oliveira, Mark J Walker

School of Chemistry and Molecular Biosciences and Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, Qld 4072, Australia.

Antimicrobial resistance (AMR) is a critical threat to human health. In recent decades, the Gram-positive bacteria *Enterococcus faecium* and *Staphylococcus aureus* have acquired broad-spectrum resistance to several major classes of antibiotics. With the rise of AMR concurrent with a reduction in novel drug discovery, new treatments are urgently needed. One promising source of new antimicrobials are the ion transporter compounds, termed ionophores, which have demonstrated broad-spectrum antimicrobial activity. Here, we sought to identify novel ionophores and characterize their antimicrobial properties. One compound, designated "704," exhibited antimicrobial activity across a panel of multidrug-resistant (MDR) strains of *E. faecium* and *S. aureus* with activity in the low (1 – 4) μg/ml range with dose-dependent bactericidal activity against selected strains. In addition, 704 was found to be non-toxic to mammalian cells *in vitro.* Bacterial resistance was not detected following 30 consecutive days of serial passage of bacterial growth. Finally, 704 demonstrated efficacy in reducing the burden of MDR *S. aureus* in a murine wound infection model and effectively perturbed metal ion homeostasis in treated bacteria. Taken together, 704 is a promising candidate for further investigation to treat Gram-positive MDR pathogens.

Abstracts

Poster 13

Spatial genomic variation of *Acropora* holobionts across the Great Barrier Reef.

Hisatake Ishida[1,2], Iva Popovic[3], Jia Zhang[4], Ira Cooke[4], Cynthia Riginos[3], Cheong Xin Chan[1,2]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia
[2] Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia
[3] School of Biological Sciences, The University of Queensland, St Lucia, QLD, Australia
[4] Centre for Tropical Bioinformatics and Molecular Biology, James Cook University, Townsville, QLD, Australia

Dinoflagellates of family Symbiodiniaceae and the bacterial genus of *Endozoicomonas* are dominant microbial associates of reef-building corals. Despite the recent establishment of their functional contributions to holobiont health and survival, spatial genomic variation of these microbial partners remains poorly understood. To simultaneously assess the spatial genomic differentiation of host coral and its microbial symbionts, we analyzed low-coverage whole genome sequencing data of two reef-building corals *Acropora tenuis* and *Acropora hyacinthus* across the Great Barrier Reef. These genomic data were generated from coral holobionts and therefore are dominated by host coral genomic sequences. Yet, these data also provide the first genome-wide data from *in hospite* microbial symbionts. The spatial distribution of these microbial symbionts was assessed with respect to host genetic identity and environmental gradients using *k*-mer based alignment-free approach and *de novo* metagenomic binning, allowing us to capture phylogenomic signals regardless of genomic locus. We identified distinct symbiont composition in these data, including near-complete recovery of metagenome-assembled genomes of *Endozoicomonas* spp. Our results suggest that the microbial genetic diversity was strongly associated with the environmental gradients (but not host genetic identity) for both *Acropora* holobionts, supporting the role of microbial shifting/switching as an adaptive response of these coral holobionts.

Abstracts

Poster 14

Structure-function analysis of TRAM/TRIF-dependent TLR4 signalling

Hyoyoung Kim[1], Timothy W. Muusse[1], Parimala R. Vajjhala[1], Mengqi Pan[1], Andrew Hedger[1], Morris Y.L Lee[1], Jeff D. Nanson[1], Sara J. Thygesen[1], Bostjan Kobe[1], Katryn J. Stacey[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland

Toll-like receptor 4 (TLR4) senses the invasion of Gram-negative bacteria by recognising lipopolysaccharide (LPS), and promoting innate immune responses. A better understanding of TLR4 interactions with its signalling adapters will aid anti-inflammatory drug development.

LPS binding leads to the dimerisation of the cytosolic TIR (Toll-interleukin 1 receptor) domain of TLR4 that then recruits adaptor proteins through TIR-TIR interactions. LPS-bound TLR4 homodimer at the cell membrane recruits MAL and MyD8, leading to NF-κB activation to induce proinflammatory cytokines. Subsequently, TLR4 dimer is internalised, and interaction with TRAM and TRIF on the endosome activates interferon regulatory factors to produce type Ⅰ interferon, and also activates NF-κB. The overall aim of this project is to determine the structure formed by TLR4 interaction with TRAM. We have solved cryo-EM structures of spontaneous *in vitro* TRAM filaments, and TLR4-MAL cofilaments. We are investigating whether TLR4 interacts with TRAM and MAL through similar interfaces, and validating TRAM-TRAM interaction interfaces using novel flow cytometric cell signalling assays in HEK293 cells.

We generated a TLR4-expressing MyD88 KO cell line with a NF-κB-driven mScarlet-I (a red fluorophore) reporter which permits analysis of TRAM/TRIF activation of NF-κB by flow cytometry. Expression of TRAM constructs tagged with mEGFP allows analysis of signalling function in cells with defined levels of TRAM. Based on the structure of TRAM filaments, we conducted site-directed mutagenesis to alter thirteen interface residues and are testing them for spontaneous and LPS-induced signalling. Also, to examine the TLR4-TRAM interaction interfaces, we utilise another MyD88 KO cell line without TLR4 expression, and test mEGFP-tagged TLR4 mutants. This enables us to compare TLR4 mutants for their effect on TRAM signalling with our prior data on MAL. Through this flow cytometric analysis, we are able to validate *in vitro* structures in the cellular environment and refine a model of the TLR4 signalosome.

Abstracts

Poster 15

Amaryllidaceae alkaloids isolated from *Crinum arenarium*

Isabella Bernard, James De Voss, Joanne Blanchfield

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Qld, Australia

Plant-based traditional medicines have been used globally for millennia, and they remain widespread today. Phytochemical analysis of these medicinal plants is important, as a chemical knowledge of the extracts enables safe regulation, and the identified biologically active compounds are regularly employed for drug development.

Crinum arenarium is a member of the Amaryllidaceae family native to northern Australia, where it has been used medicinally by the Indigenous peoples for thousands of years. During preliminary testing it was shown to contain alkaloids and saponins, but this has never been pursued further. Thus, this work aimed to begin a phytochemical characterisation of *C. arenarium*, with a focus on the alkaloids.

The Amaryllidaceae alkaloids lycorine (**1**), ungeremine (**2**) and vittatine (**3**) were isolated from an extract of the bulb (Figure 1).

Lycorine was difficult to work with and confirm the structure of due to the intwined issues of solubility and pH, and the *N*-oxide (**4**) was also observed as an isolation artefact. Nevertheless, it was positively identified as lycorine, and a crystal structure was obtained (Figure 2). Although the absolute configuration of dihydrolycorine hydrobromide has been determined, this is the first crystal structure to show the absolute configuration of lycorine itself.

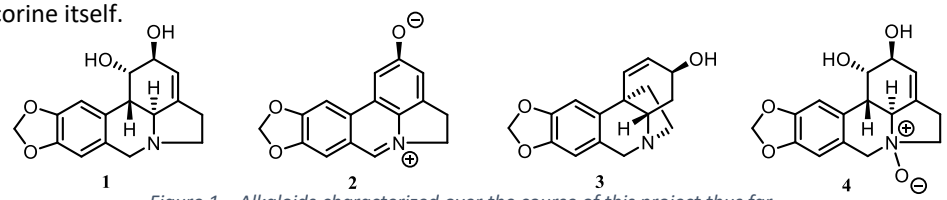


Figure 1 – Alkaloids characterized over the course of this project thus far

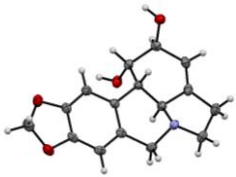


Figure 2 – Crystal structure of lycorine, showing the absolute configuration

Abstracts

Poster 16

CRACing the Role of Flavivirus NS1: Effect of Mutations in the putative CRAC motif of Dengue NS1 in Membrane Association

Isabella F. Chantrill[1], Christopher L.D. McMillan[1,2], Paul R. Young[3], David A. Muller[1,2]

1. School of Chemistry and Molecular Biosciences
2. Australian Infectious Diseases Research Centre, Global Virus Network Centre of Excellence
3. Institute for Molecular Bioscience

Dengue virus is one of the leading causes of death from arthropod-borne infections worldwide, and with one limited vaccine and no antivirals able to treat dengue it is a disease of growing concern. Of interest as a potential antiviral target is nonstructural glycoprotein one (NS1) due to its role in flavivirus replication and in disease severity. NS1 associates with cellular membranes but has no traditional transmembrane domain, instead within the flexible loop of the protein previous work has identified a putative cholesterol recognition amino acid consensus sequence (CRAC) motif. To investigate the role of this motif in dengue NS1 function, a series of mutants were made with key hydrophobic residues mutated into alanine. Utilizing immunofluorescent confocal microscopy, cells were infected with mutant virus and transfected with NS1 mutant plasmids to examine changes in colocalization of cholesterol and NS1 within cells. From this, insight was gained into the importance of the motif for membrane associations over the course of dengue infection. Moreover, these results support dengue virus NS1 as a potential target for antivirals and vaccine development.

Abstracts

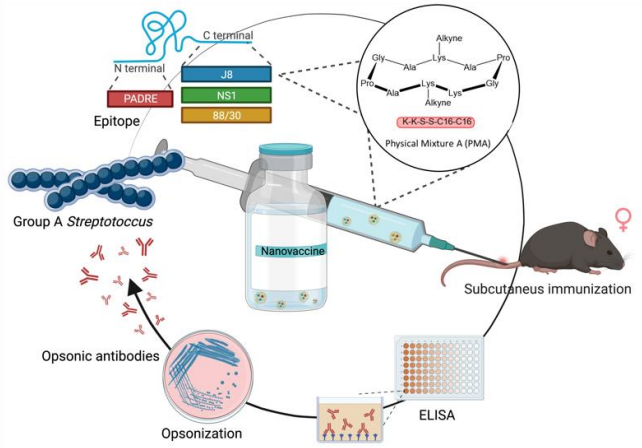
Poster 17

Investigation of Antigen Orientation on a Cyclic Lipopeptide Vaccine Delivery System

Jiahui Zhang[1], Wenbin Huang[1], Harrison Y. R. Madge[2], Waleed M. Hussein[2], Zeinab G. Khalil[2], Prashamsa Koirala[1], Robert J. Capon[2], Istvan Toth[1,2,3] and Rachel J. Stephenson[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane 4072, Australia
[2] Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland 4072, Australia
[3] School of Pharmacy, The University of Queensland, Brisbane 4072, Australia

Peptide-based vaccines universally suffer from a lack of immunogenicity unless they are delivered with adjuvants or delivery systems. We investigated the promoting cyclic lipopeptide delivery system as a universal vehicle for presenting different peptide antigens against group A *Streptococcus* (GAS). In this study, we assessed the structure immunogenicity relationship of a universal T helper epitope conjugated at the C- or N-terminus to three different GAS B cell epitopes in a mouse model and observed the importance of antigen orientation in vaccine design. All vaccine candidates stimulated high antigen-specific systemic IgG titers and high opsonic potential compared with the antigen co-administered with a commercial adjuvant (complete Freund's adjuvant). Interestingly, the vaccines containing the GAS B-cell epitope exposed at the C-terminus of the T helper elicited stronger immunogenicity, and this corresponded to the alpha helix levels in the peptide secondary structure verified by the physicochemical assay. This study revealed the importance of the free B cell epitope at the C-terminus of the T helper epitope, and the impact secondary structure plays on the immunogenicity of peptide-based vaccines.



Abstracts

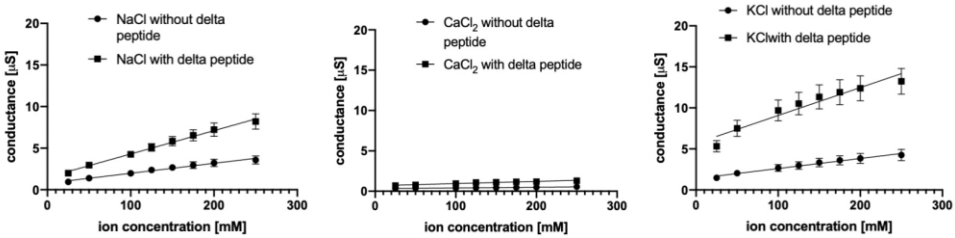
Poster 18

The Ebola virus delta peptide (E40red) is a cation selective viroporin

Jiayu Li [1], *Anneka Pereira Schmidt* [2], *Evelyne Deplazes* [1,2]

[1] *School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia;*
[2] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia;*

Ebola virus disease is a rare but severe infectious disease with an average mortality rate of 50% (WHO, 2020), and it lacks effective countermeasures and targeted treatment. E40red is the Ebola virus delta peptide without disulfide bond. It is a partially conserved and non-structural protein encoded in the viral genome. The E40red functions as a viroporin. These pore-forming peptides are found in most viruses and form ion channels in membranes to disrupt ion homeostasis and related physiological processes in the host cells. Like the inhibitors developed for the M2 viroporin in the influenza virus, the delta peptide viroporin could be a novel target for developing anti-viral treatments. To facilitate this, more information on the structure and function of the delta peptide pore is required. This study aims to characterize the ion selectivity of the E40red using tethered bilayer lipid membranes (tBLMs) and electrochemical impedance spectroscopy (EIS). We form E40red pores in tBLMs composed of the neutral phospholipid POPC and the negatively charged lipids POPG (50:50 mol%). Pores are titrated with increasing concentrations of different solutions. To control for background ion permeation, bilayers without pores are also tested. As a result, E40red is a cation-selective peptide.



Abstracts

Poster 19

Evaluating disease-associated mutations in GPCRs

Joao P. L. Velloso[1,2,3], Stephanie Portelli[1,2,3], Douglas E. V. Pires[1,2,3,4], David B. Ascher[1,2,3,5]

[1] School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, 4072, Australia
[2] Systems and Computational Biology, Bio21 Institute, University of Melbourne, Parkville, Victoria, 3052, Australia
[3] Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria, 3004, Australia
[4] School of Computing and Information Systems, University of Melbourne, Parkville, Victoria, 3052, Australia
[5] Baker Department of Cardiometabolic Health, University of Melbourne, Parkville, Victoria, 3010, Australia

Mutations in G proteins are associated with predispositions to diseases and discrepancy in drug response. They have been linked to more than 66 different human monogenic diseases. While a variety of algorithms for predicting pathogenicity are in clinical use, their applicability to studying GPCRs has not been evaluated. We therefore manually curated 2313 mutations with clinical data across 73 different Class A GPCRs. Evaluating the performance of commonly used predictive tools, they were observed to have limited predictive utility, with a maximum Matthew's correlation coefficients observed of 0.04. Using information describing protein geometry, physicochemical properties and interatomic interactions, we developed a supervised machine learning model to identify pathogenic mutations in GPCR proteins. Our initial models achieved a Matthew's correlation coefficient of 0.2 when evaluated against a non-redundant test set, limited by the unbalanced training data. Pushing forward, I am working to increase the reliability of the model through collection of more neutral mutations, and exploring alternative approaches for handling imbalance data.

Abstracts

Poster 20

Development and optimization of bioprocesses for scale-up of chimeric viral antigens for flavivirus vaccines and diagnostics.

Joshua S. Dawurung , Henry De Malmanche, Jessica Harrison, Jody Hobson-Peters, Roy A. Hall

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

Our Lab at the University of Queensland recently pioneered a novel and safe recombinant vaccine platform using the genome of a benign insect-specific flavivirus, Binjari virus (BinJV), to create a chimeric virus, displaying the structural proteins of pathogenic vertebrate-infecting flaviviruses (VIFs). These BinJV/VIF chimeras have shown great promise as next generation diagnostic antigens and vaccines for flaviviruses diseases. In the current study, we developed a bioprocess of growing C6/36 cells in suspension using serum-free media (SFM) to upscale the production of these chimeric viruses. Adherent C6/36 cells traditionally passaged in media containing fetal bovine serum (FBS) were successfully adapted to SFM in suspension culture growing at 28^{o}C and shaken at 250 rotations per minute (rpm). Our results show that these cultures can be continuously passaged for at least 4 months and can be cryo-preserved and successfully thawed directly into suspension culture. Infection studies of suspension cultures infected with a BinJV chimera (Binj/WNV) showed the chimera grew poorly in a SFM with low pH, but grew to a much higher titre upon switching to chemically defined SFM of higher pH. These findings will allow the development of commercial and scalable mass production of vaccines and diagnostics using serum-free suspension culture conditions.

Abstracts

Poster 21

The Dynamic Host Cell Proteome and Glycoproteome in H1N1 and H3N2 Influenza Infection

Kyle Macauslane, Cassandra L. Pegg, Kirsty R. Short, Benjamin L. Schulz

School of Chemistry and Molecular Biosciences, The University of Queensland

Influenza is a severe respiratory disease, particularly amongst the elderly, infants, and pregnant women. Influenza A virus infection is one of the predominant causes of Influenza. Improving our understanding of viral-host protein interactions during infection is critical for developing new therapeutics.

Identifying and quantifying the dynamic changes in both the host cell proteome and the glycoproteome, specifically at the subcellular level, during infection could provide new insight into viral-host interactions. We developed a LC-MS/MS based protocol using tandem mass tag labelling to identify and quantify these changes in protein abundance, localisation, and glycosylation throughout infection with two Influenza A virus strains, representative of the two subtypes that predominate infections in humans (H1N1 and H3N2). We characterised these changes in the human A549 cell line in four protein fractions relevant to Influenza A virus infection: the nuclear, cytosolic and organellar subcellular fractions and the secreted protein fraction.

Here we demonstrate the perturbations of the host subcellular proteome and glycoproteome in Influenza A virus infection and how these changes differ with two Influenza A virus strains from different subtypes.

Abstracts

Poster 22

Co-encapsulation of Ancestral Cytochrome P450 with their redox partner into VLP (P22)

Loïc Bourdon[1], Frank Sainsbury[2], Elizabeth Gillam[1]

[1] School of Chemical and Molecular Biosciences, The University of Queensland
[2] School of Environment and Science – Bioscience, Griffith University

The superfamily of enzymes, cytochrome P450 monooxygenases catalyse a large diversity of reactions making them highly interesting for diverse biotechnological industry. The application of this enzyme at the industrial scale has been affected by various working factors. Those factors include stability (thermo-related, solvent-related), catalytic efficiency, and their reliance on a co-partner protein (Cytochrome P450 reductases (CPR)) as electron donor partner. This reliance on the CPR is a bottleneck in the P450 research field.

In the cell P450 and CPR are enclaved in the membrane giving the possibility for the electron transfer from CPR to P450. One solution for the industrial scale is the encapsulation of biocatalyst in Virus-Like-particle (VLP). VLP encapsulation constitute a technological way to increase the encapsulated biocatalyst thermostability, reusability and give proximity between the different encapsulated proteins.
The aim of my PhD research project aims to develop a controlled systems (*in vitro* / *in vivo*) to co-encapsulate resurrected ancestral P450 and CPR into the bacteriophage VLP, P22.

The final VLP construct will then be tested against a range of compounds of interest used in the industry and compared to P450s expressed in recombinant bacterial membranes.

Abstracts

Poster 23

The molecular mechanisms of cellular recognition by ABC toxins

Aleksandrova N.A.[1], Gu W.[1], Low Y.S.[1], Roche S.G.[1], Piper S.J.[2] Stehbens S.[3] and Landsberg M.J.[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, 4072, QLD, Australia.
[2] Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, 3052, VIC, Australia.
[3] Institute for Molecular Bioscience, The University of Queensland, St Lucia, 4072, QLD, Australia.

The ABC toxins are large (>1.7 MDa) pore-forming protein complexes- the main virulence factors of a variety of pathogenic bacteria. These toxins are of interest due to their insecticidal activity and potential involvement in human disease, with prospects for the development of novel bioinsecticides and targeted drug delivery systems. Protein complexes from the ABC toxins family are known to recognise their target cells with high specificity through interactions of their receptor-binding domains with cell-surface glycans. We are currently studying the structure and molecular mechanisms of YenTc- an ABC toxin secreted by the insect pathogen *Yersinia entomophaga*. Even though we have previously solved the cryo-EM structure of full-length YenTc, the mechanisms of how it recognises its target cells remain poorly understood. To tackle this, we want to investigate how the receptor-binding domains of YenTc, Chi1 and Chi2, recognise and bind to cell surface glycans. We have recombinantly expressed and purified Chi1 and solved its crystal structure, which uncovered functionally important cis-peptides in the catalytic cleft of the enzyme that were not reported previously. In addition, we are employing further structural biology, biophysical and cell-based approaches to better understand the overall mechanisms of YenTc glycan recognition and ABC toxin host cell binding.

Abstracts

Poster 24

Mechanistic Insights into the Glycosylations of L-Idose Thioglycosides

Nicholas W. See, Vito Ferro, Elizabeth H. Krenske

School of Chemistry and Molecular Biosciences, The University of Queensland

To construct a glycosidic bond with high stereoselectivity, the donor substrate (the glycosyl donor) must be thoughtfully designed. In parallel to this, the potential influence of all reaction conditions (promotor, solvent, temperature, and the alcohol acceptor) must be strongly considered. For glycosylations of D-hexoses, installing a participating (acyl) group at C-2 is a widely adopted strategy which enables the exclusive formation of the 1,2-*trans* glycoside. However, for the analogous reactions of L-hexose donors, incomplete stereoselectivity is often achieved with simple acceptors – even in the presence of a C-2 participating group. Given the profound biological and medicinal significance of L-idose glycosides and encouraged by recent advances in the modelling of glycosylation reactions, we sought to explain this unusual phenomenon with density functional theory (DFT) calculations. In what represents the first complete DFT study of an L-sugar glycosylation, the relationships between highly reactive intermediates along multiple reaction pathways are probed to unveil a valuable set of mechanistic insights.

Abstracts

Poster 25

Modelling Inter-Replicate Regional Bias in the Integration of ChIP-seq experiments

Oliver Hughes, Gabriel Foley, Mikael Boden

School of Chemistry and Molecular Biosciences, The University of Queensland

Databases such as ENCODE enable the integration of multiple datasets from ChIP-seq experiments. Integration of these datasets aims to mitigate noise and systematic factors influencing individual experiments. Unlike other technologies, there are no established strategies for the optimal integration of independent ChIP-seq datasets. Externally validated sites cannot be relied upon to be discovered across every replicate, and recurring false positive signals can be present across replicates. These issues lead to ambiguities in the ideal permissiveness of integrative methods. To address these issues, we use statistical evidence from a range of experiments to demonstrate systematic patterns of disagreement between ChIP-seq replicates (n=16 experiments). These patterns occur across all replicates, with strong inter-lab variability. By constructing a sequence of binding sites or “peaks”, we show that regional patterns be identified using Hidden Markov Models. Motif analysis of these regions reveal that they can be either regions of increased false positives, or reproducible regions worth salvaging. More data is needed for robust interpretation of these patterns, however their potential utility is clear. Overall, our findings indicate that systematic inter-replicate bias exists in a regional form, is readily modellable, and can be used to increase precision in the integration of multiple ChIP-seq replicates.

Abstracts

Poster 26

The role and impact of *N*- and *O*-glycosylation on cellular lipids, metabolites, and proteins of *S. cerevisiae*

Joshi, P.[1], Ellis, D.J.[1], Kerr, E.D.[1], Schulz, B.L.[1]

[1] School of Chemistry & Molecular Biosciences, The University of Queensland, Australia

N- and *O*-Glycosylation plays a variety of roles in the eukaryotic cells. The structure of proteins influences their functions, and macromolecules like glycans can significantly alter the structure of a protein. Glycosylation also plays a very important role in lipid metabolism, assisting in ER protein quality control, lipid homeostasis, and peroxisome biogenesis. While it is well known the role and impact *N*- and *O*-glycosylation has on the proteome and the effect glycosylation deficient has on the proteome, the exact effect of *N*- and *O*-glycosylation have on the lipidome, and metabolome is poorly understood. In this study, we compare the lipidome, metabolome, and proteome of BY4741 (wild type *Saccharomyces cerevisiae*) to glycosylation deficient *S. cerevisiae* strains, Δost3 and Δpmt1 and BY4741 grown with tunicamycin. Ost3 & Pmt1 genes encode for regions of different protein complexes that helps with *N*- and *O*-glycosylation respectively. Several significant deviations in the abundance of secreted proteins, surface-exposed proteins, metabolites, and cellular lipids have been observed. Finally, this study attempts to explain how changes in the glycosylation of proteins affect the lipidome and metabolome of the yeast cells.

Abstracts

Poster 27

Characterisation on the pathogenic effect of the missense mutations of p53 via machine learning

Qisheng Pan[1,2], Stephanie Portelli[1,2], Thanh Binh Nguyen[1,2], David B. Ascher[1,2]

[1] School of Chemistry and Molecular Bioscience, University of Queensland, Brisbane Queensland 4072, Australia
[2] Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne Victoria 3004, Australia

Dysfunctions caused by missense mutations in the tumour suppressor p53 have been extensively shown to be a leading driver of many cancers. Unfortunately, it is time-consuming and labour intensive to experimentally elucidate all the possible effects of all missense variants. To address this, we used computational biophysical tools to investigate the functional consequences of missense mutations in p53, informing a bias of deleterious mutations with destabilising effects. Combining these insights with experimental assays, we employed a machine learning analysis to identify deleterious mutations, and validated model robustness on clinical data, showing clinical translation potential.
Our final model was able to accurately predict benign and pathogenic mutations, achieving a Matthew's Correlation Coefficient of 0.88 across non-redundant test sets. This was comparable to performance on the clinical validation set (0.83), providing confidence in the generalisability of the model. Interpreting our models revealed that information on residual p53 activity, polar atom distances, and changes in p53 thermodynamic stability were instrumental in the decisions. Using structural information, our tools obtained comparable performance with state-of-the-art p53 specific methods, and outperformed other conventional variant effect predictors. Our predictors offer clinical diagnostic utility, which is crucial for patient monitoring, and the development of personalised cancer treatment.

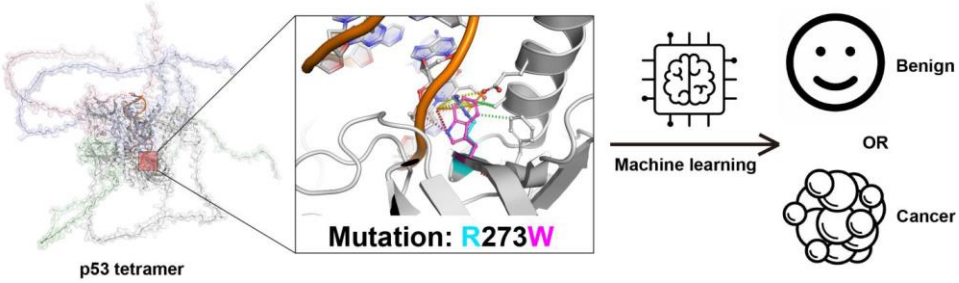


Figure. *in silico* mutation analysis pipeline of p53.

Abstracts

Poster 28

The Integration of innovative platform technologies for the diagnosis of flaviviral infections

Ryan A. Johnston[1], Gervais Habarugira[1,4], Helle Bielefeldt-Ohmann[1,2,4], Sally Isberg[3], Christopher B. Howard[5], Roy A. Hall[1,2], Jody Hobson-Peters[1,2].

[1] School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD, Australia 4072. S46766872@student.uq.edu.au, g.habarugira@uq.net.au, h.bielefeldtohmann1@uq.edu.au, roy.hall@uq.edu.au, j.peters2@uq.edu.au.
[2] Global Virus Network Centre of Excellence, Australian Infectious Diseases Research Centre, Brisbane, QLD, Australia 4072 and 4029. h.bielefeldtohmann1@uq.edu.au, roy.hall@uq.edu.au, j.peters2@uq.edu.au
[3] Centre for Crocodile Research, Noonamah, NT 0837, Australia; research@crocresearch.com.au.
[4] School of Veterinary Science, University of Queensland, Gatton, Qld, Australia; g.habarugira@uq.net.au, h.bielefeldtohmann1@uq.edu.au.
[5] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St. Lucia, QLD 4072. c.howard2@uq.edu.au.

Introduction: The past 50 years has seen global spread of flaviviruses such as dengue, Zika and West Nile (WNV) viruses resulting in over 4 billion people and countless numbers of livestock at risk of infection. Current detection methods employ expensive, time-consuming platforms requiring skilled operation. Point-of-care (POC) lateral flow assays (LFA) are cost efficient, rapid and simple alternatives to traditional lab-based diagnostics. The chimeric insect specific flavivirus platform based on Binjari virus (BinJV) offers a rapid system to efficiently produce diagnostic antigens for a range of pathogenic flaviviruses. As a test case for the application of BinJV-based antigens to LFA BinJV/WNV antigens were applied to a rapid screening device for the serological detection of WNV infection in crocodiles which results in skin lesions.
Methods: Anti-crocodile IgY was conjugated to 40nM gold nanoparticles through covalent bonding and used as a reporter molecule. Chimeric BinJV/WNV was applied to nitrocellulose membrane as a sample capture. Strips were assessed by imaging and quantifying with a colorimetric strip reader at 15 mins.
Results: Using a panel of 30 crocodile sera confirmed positive for WNV antibodies using both virus neutralisation test (VNT) and WNV-specific blocking ELISA, all samples were positive in LFA, while serum from animals not exposed to WNV returned a negative LFA result. Assessment of sera from experimentally infected crocodiles showed concordant detection of the anti-WNV immune response with that of VNT. Further proprietary innovations were applied to the LFA to eliminate false-positive LFA results caused by cross reactive antibodies developed to other flaviviruses.
Conclusion: We successfully applied chimeric BinJV/WNV to LFA to produce a sensitive and specific pen-side test for the detection of WNV in crocodiles. The assay design will form a blueprint for the optimisation of similar POC tests for flavivirus infection detection in both humans and other animals.

Abstracts

Poster 29

Probabilistic inference of biochemical and biophysical properties for ancestral proteins

Samuel Davis[1], Sebastian Porras[1], Gabriel Foley[1], Mikael Boden[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland

Testing evolutionary hypotheses in functionally diverse protein families involves reconstruction of ancestral proteins by inference of their sequence, followed by laboratory characterisation. Such investigations can inform the evolutionary trajectories which shaped the family, as well as sequence and structural determinants responsible for functional variation. The typically non-rigorous selection of ancestors for reconstruction is prone to producing proteins with unanticipated properties. This challenge may be mitigated by the ability to reliably predict an ancestor's properties prior to characterisation

Existing tools for ancestral state prediction predominantly incorporate continuous-state stochastic models. These are generally unsuitable for inferring biochemical and biophysical protein property values, which are often sensitive to discrete mutations. We developed a model for ancestral state inference to address these deficiencies, utilising discrete, latent states as proxies for evolution of continuous-valued properties along branches. Latent states emit property values via Gaussian features, the parameters of which can be learnt from known data.

The tool was evaluated for its ability to predict withheld data values from comprehensive ancestral reconstruction studies and curated datasets. It generally outperforms naïve baselines for a variety of property types. Utilisation of this tool in future reconstruction studies will likely facilitate a more targeted selection of candidate ancestors.

Abstracts

Poster 30

Investigating the role of fluorinated cations in boosting the performance of lead halide perovskite-based solar cells

S. Gupta[1,2], P.E. Shaw[1], S. Kumar[2], P.L. Burn[1]

[1] Centre for Organic Photonics and Electronics, School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, 4072
[2] Femtosecond Spectroscopy and Nonlinear Photonics Laboratory, Department of Physics, Indian Institute of Technology Delhi, New Delhi 110016, India

The unprecedented surge in power conversion efficiency achieved by solution-processed perovskite solar cells (PSC) has put them at the forefront as alternatives to Si-based solar cells. However, their application is limited by their poor stability in the ambient atmosphere. It is well established that adding large organic cations in lead halide-based systems can enhance their stability albeit at the cost of efficiency of these PSCs. Recent developments suggest that adding small amounts of fluorinated cations can not only improve the stability against temperature and humidity, but also the efficiency of the lead halide-based PSCs. However, the understanding of the photo-physics of these devices and the effect of fluorinated cations on the charge carrier dynamics is currently not well developed.
In this work, we aim to unravel these carrier dynamics using ultrafast spectroscopic techniques. Our preliminary investigation through Time-resolved Photoluminescence demonstrates that the presence of a fluorinated additive at a concentration of 0.3 mol % leads to a longer decay time as compared to the neat methylammonium iodide perovskite, thus indicating a reduced radiative recombination rate. Moreover, the devices fabricated in an inverted structure show a slight improvement in V_{oc} with the addition of these fluorinated cations. By and large, the outcomes of this work will aid in the development of design principles for high-performance photovoltaic devices.

Abstracts

Poster 31

Do growth substrates matter? - Metabolic adaptation of *Haemophilus influenzae* to the host

Saurab Kishore Munshi[1], Jennifer Hosmer[1], Marufa Nasreen[1], Alastair G McEwan[1], Ulrike Kappler[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, 4072, Queensland, Australia

Nontypeable *Haemophilus influenzae* (NTHi) causes mucosal infections including otitis media, pneumonia and exacerbates chronic respiratory diseases such as chronic obstructive pulmonary disease and asthma. Metabolic adaptation of pathogens is emerging as a key aspect of persistence, and virulence in the host. Our group previously demonstrated NTHi relies on organic acids such as lactate, nucleosides, and pentoses for growth, and that L-lactate utilization was required for Hi long-term persistence into the host cells. The current study aimed to evaluate key enzymes that could be a potential target for drug design. To better understand the significance of pentose utilization, Hi2019 strains with single gene mutation in ribose uptake (*rbsB*) and phosphorylation (*rbsK*) were constructed, and initial data showed their inability to grow on ribose and uridine containing medium. We are currently evaluating the effect of *rbsB* and *rbsK* mutations for NTHi virulence. Future work could include enzymatic characterization of these proteins, similar to work that is already under way for LldD. We have successfully overexpressed this enzyme in *Escherichia coli*. The full evaluation of NTHi energy generation and its role during host-pathogen interactions is an understudied aspect of NTHi biology and will significantly expand our knowledge on the pathogenesis of this bacterium.

Abstracts

Poster 32

SERoM: Reengineering Mycobacterial Antigens for Improved TB Vaccination Strategy

Sean N. Riek, Emily J. Strong, Nicholas P. West

School of Chemistry and Molecular Biosciences, The University of Queensland

Tuberculosis (TB) is among the leading causes of death from a single infectious agent i.e., *Mycobacterium tuberculosis*, resulting in 1.5 million deaths in 2020. The only currently licensed vaccine for TB, the live-attenuated *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) vaccine, fails to prevent *Mtb* infection or protect against pulmonary TB in adults. Consequently, there is a clear need for the development of new vaccines against TB.

The Mycobacterial membrane protein large (MmpL) protein family contains 13 member proteins responsible for the transport of essential *Mtb* substrates and supports the pathogenicity of *Mtb* and its capacity to persist within a host. MmpL proteins are characterised by their numerous transmembrane domains and presence of large extracellular or periplasmic loops. Here, three synthetic antigens were constructed comprising of the soluble regions of MmpL (SERoM)-1, MmpL3 and MmpL11 (SERoM-3 and 11 respectively) as potential vaccine candidates. Synthetic antigens were cloned into pET19b and expressed in *E. coli* BL21(DE3) prior to purification using an N-terminal polyhistidine-tag. The immunogenicity and protective efficacy of purified SERoM antigens will be examined *in vivo* in an adjuvanted subunit vaccine trial in combination with the licensed oil-in-water emulsion ASO3.

Abstracts

Poster 33

Activation of NLRX1 for the Therapeutic Resolution of Inflammation in Parkinson's Disease

Shannon Jewell [1], Kerry Roper[1], Katerina Hanton[1], Richard Gordon[1,2]

[1] Translational Neuroscience Laboratory, Centre for Clinical Research, The University of Queensland, Brisbane
[2] School of Biomedical Sciences, The University of Queensland, Brisbane

NLRX1 plays a broad role in natural immunity, acting as a negative regulator of NFκB. There is substantial loss of NLRX1 in Parkinson's disease (PD) patient samples compared to age matched healthy controls, suggesting that NLRX1 may establish a protective microenvironment within the brain through regulation of inflammation. The loss of protective NLRX1 results in an uncontrollable cycle of inflammasome activation that is known to occur in PD patients. Small molecule NX13 developed by Landos Pharmaceutical is an activator of NLRX1 for treatment of irritable bowel disease (IBD) that exhibits suppression of inflammatory mediators and is neuroprotective in cellular models of PD. However, NX13 is gut restricted, and has little systemic exposure and no brain penetration (CNS MPO = 1.6), suggesting that it may be inadequate for the treatment of PD. As such, the aim of this work is to identify novel CNS targeted therapeutics for NLRX1 for the resolution of PD. New potential leads show promising affinity for NLRX1, similar activity to NX13 and an improved CNS MPO value of 4.7, substantially better than that of NX13.

Abstracts

Poster 34

Role of site-specific N-glycosylation on protein stability

Shulei Liu, Benjamin Schulz

School of Chemistry and Molecular Biosciences, The University of Queensland.

Glycosylation is an important protein post-translational modification (PTM), involved in both physical and biological properties of proteins. Especially for N-glycosylation, which assists with protein folding in the Endoplasmic Reticulum (ER), and stabilization of mature glycoproteins. However, the effects of site-specific glycosylation on glycoprotein stability are not well understood. Our study pursued a novel strategy using genetic perturbation of glycosylation together with thermal proteome profiling (TPP) and quantitative multiple reaction monitoring mass spectrometry (MRM-MS) proteomics, to reveal the role of specific N-glycosylation sites on yeast glycoprotein thermal stability, which will be valuable for fundamental glycoprotein biology as well as in biopharmaceutical production and quality control.

Abstracts

Poster 35

Toxin complexes: uncovering the mechanisms of pore-formation

Solace Roche[1], Yu Shang Low[1], Nadezhda Aleksandrova[1], Jason KK Low[2], Joel P Mackay[2], Evelyne Deplazes[1] and Michael J Landsberg[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD 4072, Australia
[2] School of Life and Environmental Sciences, The University of Sydney, Camperdown, NSW 2006, Australia

Toxin complexes (Tc) are mega-Dalton bacterial pore-forming proteins that deliver cytotoxic cargo to target cells and are key virulence factors in some bacterial pathogens of animals. Tcs have the potential for use in biotechnology and pharmaceuticals, given the ease of cargo modification and the potential for engineering specificity. However, mechanisms of pore-formation and cellular recognition remain unknown and contested. Our investigations aim to functionally and structurally characterise the *Yersinia entomophaga* Tc, YenTc, and uncover the mechanisms of cellular localisation, internalisation, and the triggers of pore-formation. Insights gained from such investigations are anticipated to guide approaches for engineering the complex for therapeutic use. Here, we present the first complete structure of the YenTc pre-pore using cryo-EM, AlphaFold2, crosslinking mass-spectrometry and molecular dynamics simulations. Furthermore, recent high resolution cryo-EM structures of YenTc reveal a novel structural motif, and a possible pH-dependent structural intermediate in the pre-pore to pore transition.

Abstracts

Poster 36

Defining the relationship between infant and maternal gut microbiota, and early infant growth in a recent Queensland birth cohort pilot

Sophie Leech[1], Vicki Clifton[2], Danielle Borg[2] and Marloes Dekker Nitert[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, 4067.
[2] Pregnancy and Development, Mater Research Institute, The University of Queensland, South Brisbane, QLD, 4101

Previous research suggests the infant gut microbiota may be important for long-term alterations to metabolism, and is influenced by a variety of factors. The primary aims of this pilot study were to (1) characterise the relationship between the infant and maternal gut microbiota in sixteen mother-infant dyads with analysis of the effect of birth and feeding mode, and (2) investigate the relationship between the infant gut microbiota and early growth.

The profile of the infant (n = 16) and maternal (n = 16) gut microbiota at 6 weeks postpartum was assessed by shotgun metagenomic sequencing, with MetaPhlAn3 to identify taxonomy.

There was a significant difference in alpha and beta diversity between infant and maternal gut microbiotas. The gut microbiota of exclusively breast-fed infants did not differ from mixed-fed infants. Beta diversity was different by birth mode and/or maternal antibiotic use, which suggests much of the difference previously attributed to birth mode may instead be due to antibiotic exposure. Early infant growth was not associated with the infant gut microbiota, though one infant with rapid weight gain (Δweight-for-age z-score > 0.67) had a gut uniquely dominated by *Ruminococcus gnavus* and *Klebsiella pneumonia* which have been previously associated with obesity.

Abstracts

Poster 37

A Computational Study on Cathode Structure in Lithium-Sulfur Batteries Using Molecular Dynamics Simulations

Tanika Duivenvoorden[1,2], Stephen Sanderson[1], Debra J. Searles[1,2]

[1] Centre for Theoretical and Computational Molecular Science, Australian Institute for Bioengineering and Nanotechnology, the University of Queensland, Queensland 4072, Australia
[2] School of Chemistry and Molecular Biosciences, the University of Queensland, Queensland 4072, Australia

Designing and investigating new materials for energy storage devices is vital to securing a future of sustainable energy. Conventional Lithium-Ion Batteries (LIBs) revolutionised the energy industry but are now close to reaching their limits in energy density and are insufficient to support growing industrial demand for applications such as electric vehicles or house batteries. Lithium-Sulfur Batteries (LSBs) offer a promising alternative, with theoretical energy densities approximately six times higher than conventional LIBs. However, LSBs face their own challenges such as the insulating nature of sulfur, or the polysulfide shuttle effect; where polysulfide products at the cathode migrate across the cell and react at the anode to cause self-discharge. A better understanding of the chemical behavior and precise structure of these systems at the atomic level could lead to improved cathode design and battery performance. This presentation will discuss a method of computationally simulating LSB cathode materials using molecular dynamics with the reactive force field ReaxFF. A range of cathode structures have been successfully assembled, equilibrated, and characterized by calculating their X-ray diffraction pattern and radial distribution function. The simulated structures were consistent with experimental observations, and shed new light on the reactive behavior of sulfur in LSB cathodes.

Abstracts

Poster 38

Characterisation of TIR domain interactions in the TLR4 signalosome

Timothy Muusse[1], Jeff Nanson[1], Parimala Vajjhala[1], Morris Lee[1], Hyoyoung Kim[1], Bostjan Kobe[1] and Katryn J. Stacey[1].

[1] School of Chemistry and Molecular Biosciences and Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD 4072, Australia

Lipopolysaccharide (LPS) signalling via Toll-like receptor 4 (TLR4) is extensively studied as a major inflammatory pathway, yet there is no structural understanding of the recruitment of its proximal signalling adaptors. Upon LPS binding, dimerisation of TLR4 leads to recruitment of adaptor proteins MAL and MyD88 through toll-interleukin receptor (TIR) domain interactions, and ultimately to activation of transcription factors including NF-κB. We have shown that purified TIR domains can spontaneously form large filamentous assemblies *in vitro*. We have solved structures for a MAL filament, MAL-induced MyD88 microcrystals, and TLR4-MAL co-filaments. We hypothesise that the protein-protein interaction interfaces within these assemblies reflect the biological interactions. Establishing the biological relevance of the interaction interfaces required analysis of the signalling function of interface protein variants. NF-κB driven mScarlet-I fluorescent reporter HEK293 cell-lines, in conjunction with GFP-tagged TLR4 or MyD88 variants, allow the development of flow cytometry-based dual fluorophore assays for rapid analysis of both protein expression level and LPS-induced signalling activity. The robust signalling data we generated supports the interactions predicted by *in vitro* structures. Together these findings define TIR domain interactions that are vital to TLR4 signalosome formation and inflammatory signalling, provide interfaces for targeted drug development and generate an updated model of signalosome formation.

Abstracts

Poster 39

Synthesis of M4L6 Metal Organic Cages via a Chiral Auxiliary Strategy

Vivian G. Shang, Jack K. Clegg

School of Chemistry and Molecular Biosciences, The University of Queensland

Metal organic cages (MOCs) are a promising class of discrete molecular containers that exhibit highly selective guest encapsulation. Their structure consists of metal centres at the vertices of the cage, linked together by organic ligands as either the edges or faces of the polyhedron. MOCs exhibit highly selective encapsulation of guest molecules due to the well-defined size, shape and chemical environment within their internal cavities. Their high modularity enable MOCs to be an excellent system for the separation of racemic mixtures.

Enantiopure coordination cages have presented a long-standing challenge to the field, as racemic mixtures generally form upon self-assembly in the absence of enantiopure building units. The aim of this study is to synthesise chiral MOCs for the enantioselective discrimination of guest molecules. Here, we employ protected chiral amino acid motifs on 3,3'-bipyridine based ligands to impart chirality throughout the cage (Figure 1). This synthetic route establishes predetermined chirality in the cage structure, unaffected by self-assembly through relatively labile coordination bonds.

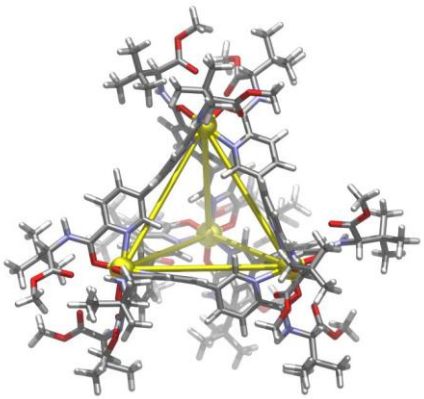


Figure 1: [ZnII4L6]8+ cage modelled on Spartan '14 (v1.1.8). The equilibrium geometry at ground state was calculated with Hartree-Fock theory methods, performed using 3-21G in a vacuum. The tetrahedron is outlined in yellow for clarity.

Abstracts

Poster 40

Predicting toxicity of a protein from its primary sequence

Vladimir Morozov[1,2], Carlos H. M. Rodrigues[1,2], David B. Ascher[1,2].

[1] School of Chemistry and Molecular Biosciences, University of Queensland, QLD, Australia
[2] Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria,
Australia

Biologics are one of the most rapidly expanding classes of therapetuics, but can be associated with a range of toxic properties. In small molecule drug development, early identification of potential toxicity led to a significant reduction in clinical trial failures, however we currently lack similar robust qualitative rules or predictive tools for peptide and protein based biologics.
To address this, we have manually curated the largest set of high quality experimental data on peptide and protein toxicities. Using the data not present in previously published databases, we found that existing approaches performed poorly. This may be due to errors in previous data curation attempts.
Harnessing this data, we developed a novel in-silico protein toxicity classifier which relies solely on the protein primary sequence. Protein sequence information was encoded using a deep learning natural languages model called BERT that was adapted to understand "biological" language, with residues as words and protein sequences as sentences. Our predictive models achieved robust and
generalisable predictive performance across multiple non-redundant blind tests. We are using interpretative approaches in order to better understand the biological basis for protein toxicity. This work will serve as a valuable platform to minimise potential toxicity in the biologic development pipeline.

Abstracts

Poster 41

Development of Highly Immunogenic Nanovaccine Delivery Systems

Wanyi Wang[1], A. O. Shalash[1], M. Skwarczynski[1], R. Stephenson[1], and I. Toth[1]

[1] University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, St. Lucia, 4072, Australia

Peptide-based vaccines are primarily composed of B-cell and T-cell antigens, and an adjuvant to stimulate the production of antibodies. In this study, a model B-cell epitope was selected as an example antigen sequence. The universal T-helper epitope (PADRE), a widely used epitope in the development of synthetic vaccines, promotes long-lived and robust immune responses via activating helper T-cells. Polyhydrophobic amino acids (pHAAs), which is a newly introduced class of adjuvants, were used to self-assemble the vaccine construct into immunogenic nanovaccine particles. In addition, the commercial Saponin adjuvant, Quil A was also physically mixed with self-assembled pHAA-adjuvanted nanovaccines. The peptides were synthesized using solid phase peptide synthesis and characterized using ESI-MS and RP-HPLC. We evaluated the immunogenicity of vaccine candidates after single-dose immunization using ELISA. The **PADRE-peptide + CFA** immunized group, generated the highest IgG level (Figure 1). The **Leu15-PADRE-peptide + Quil A** immunized group produced significantly higher level of IgG antibody titer than **peptide** or **peptide + Quil A**, after single-dose immunization on the 28th day. This suggest that polyhydrophobic amino acid works as a self-adjuvant in peptide-based vaccines by forming self-assembled nanoparticle. A simple, cost effective and single-dose delivery system for peptide vaccine development has been established.

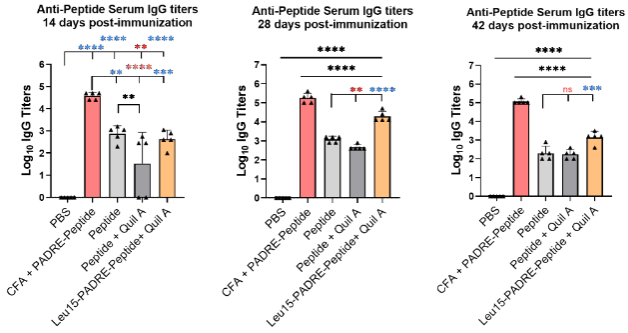


Figure 1. Antibody titer assessment following single immunization by subcutaneous injection. ((*) P < 0.05, (**) P < 0.01, (***) P < 0.001, (****) P < 0.0001)

Abstracts

Poster 42

Structural and Functional analysis of the TIR-domain in Toll-like receptors 7, 8 and 9 signaling and the Interactions with adaptor proteins

Xiaoqi Qian[*1], Jeffrey Nanson[1], Boštjan Kobe[1]

[1] *School of Chemistry and Molecular Biosciences, Faculty of Science, The University of Queensland, Australia.*

Toll-like receptors (TLR) recognize pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs) via their leucine rich repeat (LRR) domains and initiate innate immune system signaling in response to detected threats. TLRs contain a cytoplasmic TIR domain, upon PAMP/DAMP binding by the non-cytoplasmic TLR LRR domain. The cytoplasmic TIR domain recruits downstream adaptors and effector enzymes such as MAL (MyD88 adaptor-like) and MyD88 (myeloid differentiation primary response gene 88) to initiate immune system signaling.

Among ten identified TLRs, TLR7/8/9 are localized to endosomal membranes and sense nucleic acids. Once activated, TLR7/8/9 recruit MyD88 to the membrane of the endosome, initiating the downstream signaling. However, the underlying mechanism causing these diseases remains elusive. Given the similarity of TLR7/8/9, this project aims to 1) express and purify the protein TLR7/8/9 ^{TIR}; 2) identify the structural basis of human TLR7/8/9 ^{TIR}, 3) analyze the intermolecular interactions between human TLR7/8/9 ^{TIR} and their adaptor proteins MAL or MyD88. The hypotheses of the project are 1) TLR7/8/9^{TIR} dimerize and interact with adaptor proteins through TIR-TIR interactions, 2) TLR7/8/9^{TIR} directly interact with MAL or MyD88 to initiate immune system signaling, 3) TIR domains of TLR7/8/9 can induce formation of MAL or MyD88 assemblies.

Abstracts

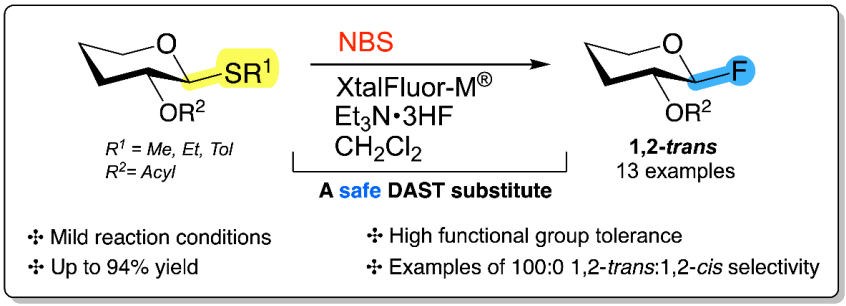
Poster 43

An efficient protocol for the synthesis of glycosyl fluorides

Nicholas W. See[1], **Xiaowen Xu[1]**, Vito Ferro[1]*

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland*

Glycosyl fluorides carry high utility as glycosyl donors, enzyme inhibitors and synthons for the construction of rare L-hexoses. However, the reagents currently available to prepare them are toxic, promote unwanted side reactions and can effect poor 1,2-trans stereoselectivity; these drawbacks are particularly pertinent to fluorinations with diethylaminosulfur trifluoride (DAST). Desiring a safer and more efficient avenue for the conversion of readily available thioglycosides to 1,2-trans glycosyl fluorides, we explored the efficacy of XtalFluor salts as alternatives to DAST. We reveal that a combination of XtalFluor-M®, N-bromosuccinimide (NBS) and $Et_3N{\cdot}3HF$ can mediate facile, high-yielding and stereoselective syntheses of 1,2-trans glycosyl fluorides. Optimisation and mechanistic studies suggest that a highly reactive XtalFluor-M®-bromide adduct plays an instrumental role in the fluorination process, and scoping studies show that routinely exploited protecting groups, including those which are acid-labile, are tolerant of the reaction conditions.



Abstracts

Poster 44

Structural basis of resistance to herbicides that target acetohydroxyacid synthase

Thierry Lonhienne[1#], **Yan Cheng[1#],** Mario Garcia[1,2#], Shu Hong Hu[1], Yu Shang Low[1], Gerhard Schenk[1], Craig M. Williams[1], Luke W Guddat[1*]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, 4072, Australia
[2] Faculty of Food Science, Engineering and Biotechnology, Technical University of Ambato, Tungurahua, Ambato, 180210, Ecuador
[#]Joint first authors

Acetohydroxyacid synthase (AHAS) is the target for more than 50 commercial herbicides, first applied to crops in the 1980s. Since then, 197 site-of-action resistance isolates have been identified, with mutations at P197 and W574 the most prevalent. Consequently, AHAS is at risk of not being a useful target for crop protection. Here, we show that these mutations can have two effects (i) to reduce binding affinity of the herbicides and (ii) to abolish time-dependent accumulative inhibition, critical to the exceptional effectiveness of this class of herbicide. In the two mutants, conformational changes occur resulting in a loss of accumulative inhibition by most herbicides. However, bispyribac, a bulky herbicide is able to counteract the detrimental effects of these mutations, explaining why no site-of-action resistance has yet been reported for this herbicide. Amidosulfuron is a member of the sulfonylurea herbicide family. We have determined the crystal structures of amidosulfuron in complex with wild-type and S653T mutant *At*AHAS. In both structures, ThDP has been modified to a peracetate adduct. The study further emphasizes that an AHAS inhibiting herbicide can have a relatively high K_i value but also be a potent herbicide, as long as accumulative inhibition occurs. Thus, the acceleration of the oxygenase side reaction of AHAS and the resultant production of ROS may strongly contribute to herbicidal activity.

Abstracts

Poster 45

Investigations of *Mycobacterium tuberculosis* Acetohydroxyacid synthases as therapeutic drug targets

Gao, Y.G., Wun, S.J., Lonhienne, T., Lin, X. and Luke, G.

School of Chemistry and Molecular Biosciences, The University of Queensland, Australia

Tuberculosis (TB) is one of the world's leading threats to human life. It is an infectious disease caused by *Mycobacterium tuberculosis* (MT) bacteria. Treatment for the TB patient is usually a combination of antibacterial medications. However, patients' adherence during treatment has become a problem due to the complicated combination of drugs and the long course of drug treatment. Also, the emergence of multidrug-resistant strains of MT has called for a search for the new drugs.

Acetohydroxyacid synthase (AHAS) is a flavin-dependent enzyme that catalyses the first common step in the biosynthesis pathway of branched-chain amino acids (BCAA; leucine, valine and isoleucine) in plants, algae, fungi and bacteria, but not in animals. Furthermore, BCAA auxotrophic strain of MT bacteria unable to proliferate due to their inability to use BCAA from their hosts. Therefore, by studying the inhibition of *Mycobacterium tuberculosis* acetohydroxyacid synthase (MTAHAS) can lead to the discovery of new anti-TB drugs.

In this study we have expressed, purified and characterized four different isoforms of the catalytic subunits of MTAHAS (ilvb1, ilvb2, ilvg and ilvx). In addition, several know inhibitors of plant AHAS were tested as inhibitors of these enzymes.

Abstracts

Poster 46

Nano-bioreactors for sustainable biopolymer production

Yichi Zhang, Elizabeth Gillam

School of Chemistry and Molecular Biosciences, The University of Queensland

More than 300 million tons of plastics are produced each year, and most of the plastics cannot be degraded easily. The main plastics synthesis pathway also consumes a massive amount of petrochemicals. To mitigate the demand for petrochemicals and the pollution from plastics, producing degradable plastics using alternative raw materials receives a lot of research attention. One potential pathway is the polymerization of ω-hydroxylated fatty acids, which forms bioplastics that perform similarly to conventional plastics. Catalyzing the ω-hydroxylation of fatty acids is the bottleneck step to produce bioplastics from fatty acids, which is hard to be accomplished by chemical catalysis. P450 are a superfamily of haem-thiolate monooxygenases with great potential to be engineered as biocatalysts to produce fine chemicals. In this study, we aim to apply different protein engineering techniques including ancestor sequence reconstruction (ASR), directed evolution, and virus-like particle (VLP) encapsulation to modify P450s into a thermostable biocatalyst that can catalyze the ω-hydroxylation of fatty acids with high regioselectivity and activity.

Abstracts

Poster 47

Coumarin-derived vitamin K antagonists

Yizhou Liu[1], Xuejie Chen[2], G. Paul Savage,[3] Jianke Tie[2], Craig M. Williams[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia;
[2] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;
[3] CSIRO Manufacturing, Ian Wark Laboratory, Melbourne, 3168, Victoria, Australia.

Coumarin-derived vitamin K antagonists (VKAs, e.g., warfarin), which target vitamin K epoxide reductase (VKOR), are clinically used for anticoagulation therapy. However, the dose requirement of VKAs is susceptible to the genetic variations of VKOR which lead to a limited therapeutic index. Recently, we reported that replacing a phenyl group with cyclooctatetraene (COT) in warfarin significantly increased its tolerance to naturally occurring VKOR mutations, but it still had a 25-fold difference in activity. To achieve the desirable anticoagulation effects with fixed doses of VAKs, COT-Vitamin K_3 was further explored. It was discovered that COT-Vitamin K_3 inhibited both VKOR and gamma-glutamyl carboxylase (GGCX) in the vitamin K redox cycle and it was tolerant of genetic variations of VKOR whose warfarin resistance varied over 400-fold. Interestingly, replacing the methylene group with carbonyl group at 3-positon of sidechain reversed the anticoagulation effect of COT-Vitamin K_3. This reverse resulted from the cleavage of sidechain and conversion to menaquinone-4 (MK-4) by the prenyltransferase UBIAD 1.

Abstracts

Poster 48

Lactoferrin-derived peptides synergistically increase the membrane-disrupting activity of antifungal drug amphotericin B.

Yumeng Hao[1], Bhavana Ramakrishnan[1], Amina Al Halawani[2], Kenya Fernandes[3], Sameer Kulkarni[3], Richard Payne[4], Dee Carter[3], Evelyne Deplazes[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia;
[2] School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia;
[3] School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia;
[4] School of Chemistry, University of Sydney, Sydney, NSW, Australia

Invasive fungal infections (IFIs) cause an estimated 1 million deaths per year globally and are challenging to treat. Despite its concentration-dependent toxicity, amphotericin B (AMB) remains a clinically important anti-fungal drug, particularly for treating multi-drug resistance IFIs. Developing an adjuvant drug to reduce the dose of AMB can potentially improve the mortality of IFIs and patient outcomes.
Lactofungin (LFG) is a 30-residue peptide derived from the milk protein lactoferrin. LFG is non-toxic to human and fungal cells but synergic with AMB. This study aims to investigate the sterol-dependent membrane-disrupting activity of AMB with LFG peptides that show a range of synergy profiles on different fungal pathogens. To achieve this, we use tethered lipid bilayer membranes with electrochemical impendence spectroscopy (tBLM/EIS), in which membrane disruption is reported as an increase in membrane conductance. tBLMs were composed of the neutral phospholipid POPC and increasing concentrations of cholesterol and ergosterol.

The LFG peptides show no membrane-disrupting activity on ergosterol- or cholesterol-containing membranes. However, when combined with AMB, LFG peptides significantly increase the membrane-disrupting activity of AMB on ergosterol-containing membranes. The synergy profiles of the different variants from tBLM/EIS experiments are consistent with cell-based data, suggesting the synergy is lipid dependent and ergosterol-specific.

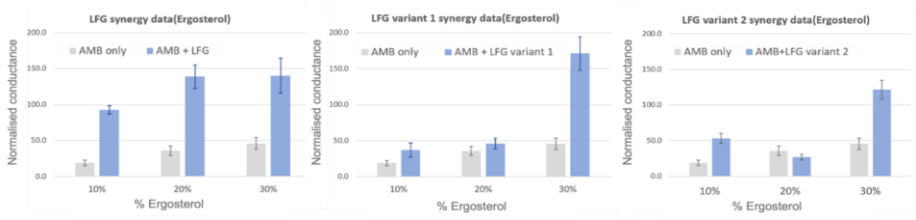


Figure 1 Normalised conductance from tBLM/EIS experiments representing the different effects of AMB only and AMB combined with LFG peptides on membranes composed of POPC with different percentages of ergosterol (10%, 20%, and 30%).

Abstracts

Poster 49

DDMut: predicting mutation effects on protein stability using deep learning

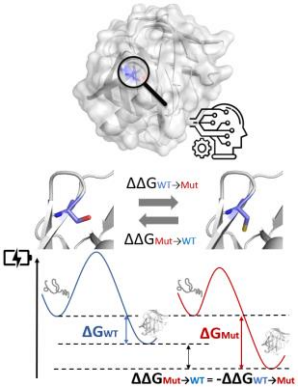
Yunzhuo Zhou[1,2], Carlos H.M. Rodrigues[1,2], Qisheng Pan[1,2], Douglas E.V. Pires[1,2,3] & David B. Ascher[1,2]

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia*

[2] *Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia*

[3] *School of Computing and Information Systems, University of Melbourne, Melbourne, Victoria, Australia*

Understanding the effects of mutations on protein stability is crucial for protein engineering, variant interpretation, and protein biophysics. While experimental measurements are the gold-standard, they can be time consuming, expensive and technically challenging. The data generated from these approaches, however, has driven an explosion in computational approaches to predict the effects of mutations on protein stability. Despite these efforts, community assessments of these tools have highlighted a range of limitations, including availability, high time costs, low predictive power, and biased predictions towards destabilizing mutations. To fill this gap, we developed DDMut, a fast and accurate Siamese network to predict changes in Gibbs Free Energy (ΔΔG) upon missense mutations. The deep learning model was built by integrating graph-based representations of the localised 3D environment, with convolutional layers and transformer encoders, which enabled capture of long-range interactions. DDMut achieved a pearson's correlation of 0.70 on cross-validation, and outcompeted most available methods on non-redundant blind test sets. Importantly, DDMut was highly scalable and demonstrated anti-symmetric performance on both destabilizing and stabilizing mutations. Our freely-available tool, DDMut (https://biosig.lab.uq.edu.au/ddmut), will be a useful platform to better understand the functional consequences of mutations, and guide rational protein engineering.



Abstracts

Poster 50

Structural and functional characterization of dsDNA binding by the MyD88 adaptor protein

Zannati Ferdous Zaoti*[1], Yan Li[1], Jeffrey D. Nanson[1], Bostjan Kobe[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, 4072, Australia

In response to pathogens and danger-associated molecules, the innate immune system initiates downstream signal transduction to produce pro-inflammatory cytokines. Allergies, atherosclerosis, auto-immune diseases, and several types of cancer result from signaling disorders of this immune response. Toll-like receptors (TLRs) dependent signaling is a well-known innate immune signaling process that can recognize pathogens and danger signals in a process called pattern recognition. The process goes through receptors and the adaptor's response. The cytosolic adaptor protein MyD88 (myeloid differentiation primary response gene 88) is used by all TLRs (1-10) except TLR3. MyD88 contains a TIR (Toll/interleukin-1 receptor/resistance protein) domain and a DD (death domain). There are several kinds of cytosolic receptor proteins that can recognize and bind to viral, bacterial and host DNA; these DNA sensor receptors (cGAS, AIM2) can catalyze the synthesis of cyclic nucleotides and activate the immune system. These receptors are good targets for some cancer and DNA vaccine studies. DNA vaccination studies show that overexpression of MyD88 adaptor protein improves DNA vaccine immunogenicity *in vitro*. Our studies suggest that the cytosolic TIR domain of MyD88 also interacts with DNA to form large assemblies. We show that dsDNA can induce the higher-order assembly of MyD88 TIR domains, which may represent a new cytoplasmic DNA-induced immune pathway. We aim to investigate the structural basis of MyD88 TIR domain binding to dsDNA and determine the role of cytosolic DNA in TLR-mediated immune system responses. This study could open a new research field of TLR-associated innate immunity.

Abstracts

Poster 51

Characterisation of the polysialic acid O-acetyltransferase gene *neuO* in *Escherichia coli*

Lachlan Walker[1,2], Minh-Duy Phan[1,2], Nhu Nguyen[1,2], Mark A. Schembri[1,2]

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia*
[2] *Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, Australia*

Escherichia coli strains of capsule type K1 are associated with severe disease such as pyelonephritis, neonatal meningitis and sepsis. O-acetylation of the K1 capsule has been implicated in immune evasion. The key regulator of O-acetylation in *E. coli* K1 is encoded by the *neuO* gene, which is phase variable due to a heptanucleotide tandem repeat (5' AAGACTC 3')$_n$ in the 5'-region of the coding sequence. In this study we screened a large genome dataset and showed the *neuO* gene is present in a restricted group of *E. coli* sequence types (STs), with highest prevalence in ST95 (90%), ST428 (67%) and ST62 (40%). We further showed that the *neuO* gene is present in 63.5% of K1-positive strains and absent in non-K1 strains. Examination of the phase status of *neuO* using PCR and sequencing revealed variable lengths of the heptanucleotide tandem repeat, reflecting both 'ON' and 'OFF' states. Two major allelic variants of NeuO were identified. To assess the impact of K1 O-acetylation, we employed a phage killing assay and showed that *neuO* phase 'ON' strains were protected from phage attack. Overall, this study provides the foundation for future research to understand the impact of K1 capsule O-acetylation on *E. coli* virulence.

Abstracts

Poster 52

Structural studies of the active state assembly and functional diversity of SARM1.

Forhad Karim Saikot[1], Vishal Pandey[1], Jeff Nanson[1], Thomas Ve[2], Bostjan Kobe[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, QLD 4072, Australia. [2] Institute for Glycomics, Griffith University, Southport, QLD 4222, Australia.

SARM1 is a pro-axon degenerative NADase protein. Injury or stress to CNS leads to an increase in the NMN/NAD^+ ratio, which is responsible for SARM1 activation. Upon activation, SARM1 rapidly depletes NAD^+ in the affected cells and thereby causes axon degeneration. Recently, the inactive structure of SARM1 was solved. Both NMN and NAD^+ can bind to the autoinhibitory ARM domain of SARM1; however, NMN activates and NAD^+ retains the inhibitory state of the protein. In stressed cells, these two molecules compete, and NMN replaces NAD^+ to activate the protein. In the active form, the enzymatic TIR domain of the protein binds and cleaves NAD^+. This NADase activity of the TIR domain is self-assembly dependent. Although the molecular mechanism of SARM1 dependent axon degeneration is unwinding, there are still few gaps. The interaction of TIR with NAD^+ is elusive. Another question is how TIR maintains the self-assembly during their elongated NADase mission. In this study, we are aiming to understand the assembly of the active form of SARM1 through cryo-electron microscopy. To achieve this, we are using adducts, a base-exchanged product of NAD^+. These adducts can retain the oligomeric state of the TIR domain and makes them imageable under cryo-EM. We are also using protein specific nanobodies to determine functional diversity of SARM1 in the abovementioned pro-neurodegenerative pathway.

Abstracts

Poster 53

Biochemical and structural basis of nanobodies' interaction with SARM1 (sterile alpha and TIR motif containing 1)

Vishal Pandey[1], Forhad Karim Saikot[1], Bostjan Kobe[1]

[1] School of Chemistry & Molecular Biosciences, The University of Queensland

Axon degeneration is the first indicator in numerous progressive neurological disorders. SARM1 is known to play a key role in axon degeneration. The activation of SARM1 is regulated by the NMN/NAD^+ ratio. The increase in NMN/NAD^+ ratio results in SARM1 activation, due to conformational changes in its autoregulatory ARM domain, resulting in the disruption of the ARM from catalytic TIR domain lock, enabling TIR self-association. The self-association of TIR is crucial for NADase catalytic activity. In the active form SARM1 cleaves NAD^+ into nicotinamide and ADPR/cADPR. Understanding the mechanisms of constitutive activation or deactivation of the SARM1 protein could further enable our understanding of SARM1 and potentially identify novel therapeutic targets to delay axon degeneration. In many cases, nanobodies are found to be capable of stabilizing specific conformations of dynamic proteins/domains for structure determination. Structure determination of nanobody activated SARM1 would help determine the underlying mechanism of action for ligand independent SARM1 protein activities. In this study, we are using nanobodies raised against SARM1 to understand how nanobodies interact and modify the target protein's functionality. After purification of target protein and nanobodies, co-elution and size exclusion chromatography were used to check complex formation. We are exploring further to test nanobody and target protein interaction.

Abstracts

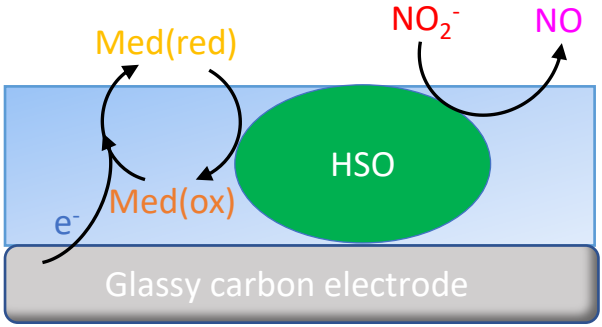
Poster 54

Nitrite Reduction by Electrode-Immobilised Human Molybdoenzymes

Joan Zapiter and Paul Bernhardt

School of Chemistry and Molecular Biosciences, The University of Queensland

The sulfite oxidase family of molybdoenzymes catalyzes oxygen transfer redox reactions involving their molybdenum cofactors. For example, human sulfite oxidase (HSO) oxidizes sulfite to sulfate in the final step of cysteine catabolism. The human mitochondrial amidoxime reducing component 1 and 2 (mARC1 and mARC2) catalyzes the reduction of *N*-hydroxylated prodrugs. Recently, these enzymes have also been identified to moonlight in the reduction of nitrite to nitric oxide, an important signaling molecule in several physiological processes. In this presentation, HSO and mARC enzymes immobilized on glassy carbon electrodes are shown to reduce nitrite in the presence of artificial electron mediators (see scheme below). These electrochemical systems provide a more simplified and greener approach to further understand hypoxic nitrite reduction in humans.



Scheme 1. Cartoon representation of the GC/HSO-glutaraldehyde enzyme electrode catalyzing the conversion of nitrite to nitric oxide in the presence of an artificial electron mediator (Med).

Abstracts

Poster 55

The genomes of the free-living Symbiodiniaceae *Effrenium voratum* highlight the differences between symbionts adapted to ancient versus modern coral reefs

Sarah Shah[1], Yibi Chen[1], Katherine Dougan[1], Rosalyn Lo[1], Gemma Laird[1], Subash Rai[2], Valentine Murigneux[2], Anthony J. Bellantuono[3], Mauricio Rodriguez-Lanetty[3], Debashish Bhattacharya[4], Cheong Xin Chan[1]

[1]The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Centre for Ecogenomics, Brisbane, 4072 QLD, Australia
[2]The University of Queensland, Genome Innovation Hub, Brisbane, 4072 QLD, Australia
[3]Florida International University, Department of Biological Sciences, Miami, FL 33099, USA
[4]Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ, 08901, USA

Dinoflagellates of Family Symbiodiniaceae are symbionts essential to corals and other reef organisms. They are highly diverse, encompassing a broad spectrum of symbiotic associations and host specificity. *Effrenium voratum* is the sole species of an exclusively free-living genus that diverged early in Symbiodiniaceae, branching between two groups of largely symbiotic lineages: the earlier diverging genus *Symbiodinium* and later-branching genera such as *Breviolum, Cladocopium, Durusdinium* and *Fugacium* ("S2"). To understand the impact of symbiosis on genome evolution of Symbiodiniaceae, we generated *de novo* genome and transcriptome from three isolates of *E. voratum* (genome size: 1.4 Gbp) and compared them against available Symbiodiniaceae genomes. We identified contrasting phylogenetic signals to traditional biomarkers in conserved non-coding regions of *E. voratum* genomes, and maintenance of ancestral intron sizes that are twice as large as those seen in symbiotic lineages. Conversely, genomes of *Symbiodinium* and the S2 group exhibit greater pseudogenization, gene-function innovation indicated by lineage-specific gene families related to cell signalling and trans-splicing, and potential convergent evolution. Our results demonstrate the interplay between Symbiodiniaceae diversification and coral evolution: a) *Symbiodinium* spp. arose as symbionts to ancient (Jurassic) uniserial corals due in part to their capacity to thrive in high/variable light intensities, b) the S2 group radiated as symbionts to modern (Cretaceous) multiserial corals, whereas c) *E. voratum* retained genomic features of the ancestral free-living Symbiodiniaceae.

Abstracts

Poster 56

Characterisation of Axundead, a novel downstream regulator of Wallerian degeneration

Helen Ying McGuinness[1], Arnau Llobert Rosell[2], Weixi Gu[1], Jeffrey Nanson[1], Thomas Ve[3], Bostjan Kobe[1]

[1] School of Chemistry and Molecular Biosciences, University of Queensland, QLD 4072, Australia
[2] Department of Fundamental Neurosciences, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne VD, Switzerland
[3] Institute for Glycomics, Griffith University, Southport, QLD 4222, Australia

Wallerian degeneration is a conserved program of injury-induced axon degeneration, and is analogous to the blockage of transport that characterises the early stages of many neurodegenerative diseases. In recent years, the TLR adaptor SARM1 has been well characterised as the initiator of Wallerian degeneration. SARM1 has intrinsic NADase activity through the dimerization of TIR domains, and this NADase activity is essential for Wallerian degeneration. However, details of the Wallerian degeneration pathway downstream of SARM1 are not well understood. Axundead (axed) is a regulator of Wallerian degeneration in *Drosophila*, with loss of function mutants providing near-total protection from SARM1-dependent axon degeneration. Axed is a novel member of a family of BTB-domain containing proteins, which play roles in a range of cellular functions, including cytoskeleton organisation, transcriptional regulation, and protein ubiquitination. Presented here is preliminary research that suggests axed may play a key role in the dysfunction of energy metabolism leading to the functional death of the axon. As a downstream converging point for axon death pathways, axed represents an ideal target of therapeutic intervention for axon degeneration in disease.