Adelaide Protein Group Awards FEST 2022 Programme

SAHMRI Auditorium, North Terrace, Adelaide SA 5000

Thursday

3rd November

12:00 – 12:30	Registration
12:30 – 12:40	Opening
12:40 – 13:30	**Keynote lecture:** ***Prof. Leann Tilley***
13:30 – 13:45	Afternoon tea
13:45 – 15:00	**Student talks**
15:00 – 16:00	**Poster session**
16:00 – 17:00	**ECR talks**
17:00 – 17:45	Refreshments and drinks
17:45 – 18:00	**Closing and Award Presentations** Including: • ASBMB ECR Award • BMG Student award • Abcam People's Choice award • Poster prizes

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Contents

APG Committee 2022

Chair	Blagojce Jovcevski, *The University of Adelaide*
Treasurer and Sponsorship officer	Kimberley Taylor McLean, *The University of Adelaide*
Secretary	Bethiney Chantel Vandborg, *The University of Adelaide*
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	Emma Yu Xin Mao, *The University of Adelaide*
Events coordinator	Ellen Kate Potoczky, *The University of South Australia*
	Yu Chinn Joshua Chey, *The University of Adelaide*
General committee members	Emma Parkinson-Laurence, *The University of South Australia*
	Kate Wegener, *The University of Adelaide*
	Erin Brazel, *The University of Adelaide*
	Juan Miguel Balbin, *Animate Your Science*

APG AGM

APG Annual General Meeting Minutes Meeting called by Secretary Beth Vandborg	Committee Member Owner
Committee 2023 Nominations ***Chair*** *Bethiney Vandborg* ***Treasurer/ Sponsorship officer*** *Kimberley McLean* ***Secretary*** *Alix Harlington* ***ASBMB liaison officer*** *Daniel McDougal* ***Webmaster*** *Michael Roach* ***Events coordinator*** *Josh Chey* ***Promotions and Social Media officer*** *Tace Conlin* ***Promotions and Social Media officer*** *Emma Mao* ***General committee member*** *Erin Brazel*	
1. Present	
2. Apologies	
3. Treasurers/Sponsorship Report	Kim

Open Actions

Item	Action	Owner
1		

COVID-19 Statement

The Organising Committee is committed to taking all reasonable steps to provide the safest possible environment for participation at the APG Awards FEST 2022.

All participants are required to be aware of and comply with any relevant COVID Safety Guidelines and protocols in place at the time of the meeting and these will be communicated in advance.

Social distancing where possible will be encouraged; hand sanitiser will be available onsite (and at each exhibition booth) and face masks will be provided and expected where practical. We also encourage self-testing by RAT as a pre-caution.

Importantly, at the time of the event, if you are not feeling well and are displaying COVID-19 symptoms, we ask that you do not attend. We request you test accordingly for COVID-19 and notify us by email (apg.asbmb@gmail.com) immediately to advise you will not be in attendance.

Keynote Lecture

12:40 – 13:30

Chair: Ms. Bethiney Vandborg, University of Adelaide, SA

Location: AHMS 1059

Hijacking proteostasis for the development of anti-infectives

Professor Leann Tilley,

The University of Melbourne

Leann Tilley is Professor of Biochemistry and Pharmacology, working in the Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne. She was an Australian Research Council Georgina Sweet Australian Laureate Fellow (2015-2020).

Professor Tilley's group undertakes research in the areas of cell biology and drug development related to the malaria parasite, Plasmodium falciparum. Her lab investigates the mechanisms of action of and resistance to the antimalarial drug, artemisinin, and is working to target proteostasis for the development of new antimalarial drugs. She is interested in the unusual protein trafficking pathways that the malaria parasite uses to display virulence proteins at the erythrocyte surface, and in understanding the molecular basis for the remarkable transformation that allows the malaria parasite to be transmitted from a human host to a mosquito vector.

Leann obtained her BSc(Hons) in Biochemistry from the University of Melbourne working with Bill Sawyer, and her PhD in Biochemistry from the University of Sydney, supervised by Greg Ralston. After postdoctoral fellowships at Utrecht University in the Netherlands, the College de France in Paris, and at the University of Melbourne, she joined the Biochemistry Department at La Trobe University. She was awarded an ARC Australian Professorial Fellowship. In the middle of 2011 she joined the Department of Biochemistry and Molecular Biology at the University of Melbourne.

Leann is a scientist who embraces a large range of technologies to further her understanding of her chosen biological questions, from drug and protein chemistry, to molecular cell biology, to novel imaging technologies. She is assisted in this by fantastic collaborations with experts from other disciplines, ranging from molecular parasitologists to organic chemists and optical physicists.

She served as Director of the ARC Centre of Excellence for Coherent X-ray Science (CXS) (2013-2014), which brought together physicists, chemists and biologists together to develop fundamentally new approaches to probing biological structures and processes. CXS received international acclaim for its cross-disciplinary and cross-institution work and for its contributions to the development of novel imaging techniques. As part of this collaboration Leann's laboratory has helped to develop and implement a number of new imaging modalities and to apply them in pioneering applications. These include 3D Electron Tomography, CryoEM and Structured Illumination Microscopy. She believes that answering the major medical and biotechnology questions of the 21st century will require a convergence of the Life and Physical sciences. She believes that development of new antimalarial drugs requires collaborations of academia and industry. She would like to be part of the exciting developments in this area.

Student Presentations

13:45 – 15:00

Chair: Ms. Bethiney Vandborg, University of Adelaide, SA

Location: AHMS 1059

1. Investigation into Shigella flexneri surface polysaccharides and their role in disease.
   Alice Ascari

2. CRISPR/Cas9 allele-specific targeting of autosomal dominant Retinitis Pigmentosa disease variants.
   Ashleigh B. Geiger

3. Pregnancy zone protein; more than just a protease inhibitor?
   Demi K. Georgiou

4. Towards the development of novel herbicides targeting lysine biosynthesis.
   Emily Mackie

ECR Presentations

16:00 – 17:00

Chair: Ms. Bethiney Vandborg, University of Adelaide, SA

Location: AHMS 1059

1. Improving CRISPR gene editing efficiency through increasing the expression of the gRNA transcripts.
   Dr. Fatwa Adikusuma

2. Hypochlorite – a novel inhibitor of amyloid beta aggregation and toxicity.
   Dr. Noralyn B. Mañucat-Tan

3. Post-translationally sulfated proteins from the saliva of blood feeding organisms as novel anticoagulants.
   Dr. Emma E. Watson

Student Abstracts

Investigation into *Shigella flexneri* surface polysaccharides and their role in disease

Alice Ascari[1,2*], Elizabeth N. H. Tran[1], Bart A. Eijkelkamp[2], Renato Morona[1]

1. School of Biological Sciences, Department of Molecular and Biomedical Science, Research Centre for Infectious Diseases, University of Adelaide, Adelaide 5005, Australia.

2. Molecular Sciences and Technology, College of Science and Engineering, Flinders University, Adelaide 5042, South Australia, Australia.

Shigella flexneri is a significant cause of gastro-enteric disease in the developing world, predominantly devastating the paediatric age group. This bacterial pathogen synthesises a plethora of polysaccharide-based complexes on its cell surface that facilitate host cell adhesion, and influence key immunomodulatory responses. *S. flexneri* expresses two distinct variants of the O antigen polysaccharide (Oag); short-Oag (10-17 repeat units) and very long-Oag (>90 repeat units) through a multi-protein pathway. However, the protein interactions required for the synthesis of these Oag variants, and their relative roles during disease, remain poorly characterised. In this study, we implemented a combination of protein labelling, mutagenesis, and *in silico* analyses to decipher the molecular interactions between main protein candidates of the Oag biosynthetic pathway. Specifically, we identified a key disulphide bond within a critical protein-protein interaction domain. Furthermore, using a tissue infection model in combination with biochemical analyses we identified that the Oag length directly influences the ability of this pathogen to interact with key molecules within its infectious niche. This includes exogenous poly-unsaturated fatty acids that dramatically affect the cell's lipid homeostasis, morphology and viability. Collectively, this work has provided novel insights in the synthesis of Oag polysaccharides and has established a new role for these surface-presented macromolecules in *Shigella* lipid homeostasis. In addition to identifying putative druggable targets, our findings also lay a foundation for dietary lipid intervention strategies to protect susceptible children against this debilitating and potentially lethal pathogen.

CRISPR/Cas9 allele-specific targeting of autosomal dominant Retinitis Pigmentosa disease variants

Ashleigh B. Geiger[1,2,3*], Fatwa Adikusuma[1,2,3], Laurence OW Wilson[3], Gelshan I. Godahewa[2,3], Robert J Casson[1], Paul Q Thomas[1,2]

1. School of Biomedicine, Faculty of Health and Medical Sciences, The University of Adelaide.

2. Genome Editing Program, Lifelong Health theme, South Australian Health and Medical Research Institute (SAHMRI).

3. CSIRO

Autosomal dominant Retinitis Pigmentosa (adRP) is an important cause of progressive, irreversible blindness. Clinically and genetically heterogeneous, adRP is known to be caused by a myriad of heterozygous variants in at least 29 genes. Where adRP is caused by point mutations in haplosufficient genes with dominant-negative and/or toxic gain-of-function disease mechanisms, selective ablation of the mutant allele using CRISPR/Cas9 technology represents a tantalising prospect for treatment. The Pro23His mutation in Rhodopsin (*RHO*) and the Gly56Arg mutation in Nuclear Receptor Subfamily 2 Group E Member 3 (*NR2E3)* are particularly attractive targets, representing the two most common causes of non-syndromic adRP, respectively. However, guide RNA design is heavily restricted due to minimal availability of – or in the case of *RHO* Pro23His, the absence of – canonical SpCas9 5'-NGG-3' PAM sequences in these regions.

In this study, we have explored the application of alternative naturally occurring or engineered CRISPR/Cas9 platforms to target both the *RHO* Pro23His and *NR2E3* Gly56Arg mutations. To assess disease-allele targeting activity, we generated clonal cell models of both mutations using a novel PRIME Editing construct. Analysis of gene editing outcomes by deep sequencing identified several candidate gRNAs for both heterozygous variants which display highly efficient and selective targeting of the mutant allele, with a prevalence of frame-shifting indels or larger deletions observed. These data provide evidence that our approach has the potential to mediate specific gene editing events that will ablate the mutant allele whilst sparing the wild-type allele, positing these strategies as therapeutic candidates for adRP.

Pregnancy zone protein; more than just a protease inhibitor?

Demi K Georgiou[1,*], Noralyn Manucat-Tan[1], Arezou Ghahghaei[1], Jordan Cater[2], Tanja Jankovic-Karasoulos[1], Claire T Roberts[1] , Amy R Wyatt[1]

1. College of Medicine and Public Health, Flinders University & Flinders Health and Medical Research Institute.

2. School of Chemistry and Molecular Biosciences, University of Wollongong.

Pregnancy zone protein (PZP) is typically found in low concentrations throughout the body, however, during pregnancy PZP production is significantly upregulated, becoming one of the most abundant plasma proteins by the third trimester. PZP is also dramatically upregulated in pregnancy-independent inflammatory states (e.g. arthritis, gingivitis) and in response to infection (e.g. HIV, COVID-19). Despite being a quantitatively significant component of biological fluids (e.g. blood plasma, synovial fluid, interstitial fluid) during these scenarios, the activities and corresponding importance of PZP remains elusive. Compared to the constitutively abundant alpha-2-macroglobulin (α_2M), a closely-related protein that is best known as a broad-spectrum protease inhibitor, few studies have attempted to characterize the functions of PZP. PZP has traditionally been considered a relatively selective and ineffective protease inhibitor compared to α_2M. Recent research, however, supports the idea that PZP is a highly multifunctional protein, and certainly far more than just a protease inhibitor. Our unpublished data suggests that low concentrations of PZP in maternal plasma is associated with preeclampsia, a major cause of perinatal morbidity and mortality involving systemic accumulation of misfolded proteins, and inflammatory pathology. My ongoing research will demonstrate how the recently identified holdase-type chaperone activity, and immunomodulatory functions of PZP are important to healthy pregnancy. This has the potential to contribute to the framework for the development of novel prognostic and therapeutic strategies for preeclampsia. Considering that the accumulation of extracellular misfolded proteins is also implicated in many other human pathologies (e.g. Alzheimer's disease, arthritis, macular degeneration) this research may have far-reaching importance.

Towards the development of novel herbicides targeting lysine biosynthesis

Emily Mackie[1,2*], Andrew Barrow[2], Marie-Claire Giel[2], Santosh Panjikar[3], Anthony Gendall[4], Tatiana Soares da Costa[1,2]

1. School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, Waite Campus, SA 5064, Australia.

2. Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, La Trobe University, VIC 3086, Australia.

3. Australian Synchrotron, ANSTO, 800 Blackburn Road, Clayton, VIC 3168, Australia.

4. Department of Animal, Plant and Soil Sciences, AgriBio, La Trobe University, VIC 3086, Australia.

Herbicide resistance is one of the major threats to our natural environment and agricultural industry. The emergence of herbicide-resistant weeds has been driven by repeated application of the same herbicides, combined with a lack of new herbicides entering the market in the past 30 years. It is evident that herbicides with new modes of action are urgently required. Although several commercial herbicides inhibit amino acid production in plants, lysine biosynthesis is yet to be exploited as a herbicide target. We have developed the first herbicidal inhibitors of lysine biosynthesis, which target the first enzyme in the pathway, DHDPS. We have shown that these compounds exhibit potent *in vitro* and *in planta* activity using enzyme kinetic and herbicidal activity assays, whilst lacking off-target effects as demonstrated by cytotoxicity and antibacterial assays. Using X-ray crystallography, we have revealed that these inhibitors bind in a novel allosteric site in DHDPS which is highly conserved across plant species. We subsequently employed enzyme kinetic assays and *in silico* docking to demonstrate that this inhibitor has an additional mode of action through inhibition of the second enzyme in the lysine biosynthesis pathway, DHDPR. This inhibitor represents the first example of a dual-target herbicidal compound, which may have a reduced susceptibility to the generation of resistance. Additionally, this is the first time that an inhibitor with allosteric and orthosteric activity at two different enzymes has been reported. This work has the potential to contribute novel herbicide modes of action to help combat the global herbicide resistance crisis.

ECR Abstracts

Improving CRISPR gene editing efficiency through increasing the expression of the gRNA transcripts

Fatwa Adikusuma[1,2,*], Yu CJ Chey[1,2], Paul Thomas[1,2]

1. School of Biomedicine, Faculty of Health and Medical Sciences, The University of Adelaide.

2. Genome Editing Program, Lifelong Health theme, South Australian Health and Medical Research Institute (SAHMRI).

Achieving high editing efficiency is desirable when performing genome editing using CRISPR-Cas9 technology. Here, we showed that CRISPR-SpCas9 could produce consistently near-perfect (close to 100%) editing efficiencies in cultured cells when optimized protocol of plasmid delivery was used. In several cases of lower apparent gRNA activity, we found that editing was limited by inefficient U6-driven gRNA transcription rather than intrinsic gRNA activity. Increasing gRNA transcript levels with a simple modification within the gRNA scaffold can improve the editing efficiencies of these gRNAs. This modification is also compatible with high-fidelity SpCas9 variants SpCas9-HF1 and eSpCas9(1.1), without reducing the targeting specificity of these high-fidelity SpCas9 variants. When this modification was applied in SaCas9 CRISPR system as well as prime editing, the increase of the editing efficiencies was striking. This modification should be applied whenever genome editing is performed for optimal and improved editing efficiencies.

Hypochlorite – a novel inhibitor of amyloid beta aggregation and toxicity

Mañucat-Tan, N.B.[1*], Abdallah R.F. [1], Chowdhury, A[2]., Kumita, J.[2] and Wyatt, A.R.[1]

1. Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University, Bedford Park, South Australia 5042.

2. Molecular Horizons and the School of Chemistry and Molecular Bioscience, University of Wollongong, Northfields Avenue, Wollongong, Australia; Illawarra Health and Medical Research Institute, Northfields Avenue, Wollongong, Australia.

3. Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, CB2 1EW Cambridge, United Kingdom.

Hypochlorite (OCl^-), a chemical generated by the enzyme myeloperoxidase (MPO), is an oxidant that is produced abundantly during inflammation. The generation of hypochlorite is a mechanism designed to kill invading microbes; however, the non-specific action of hypochlorite also results in the modification of host proteins during inflammation. It is well established that proteins can acquire cytotoxic and/or proinflammatory properties following modification by hypochlorite and this process is implicated in disease (e.g., atherosclerosis, kidney disease). However, recent studies demonstrate that hypochlorite-induced protein modification is not exclusively deleterious. Our unpublished data show that hypochlorite-induced modification of Alzheimer's disease-associated amyloid beta peptide (1-42; $A\beta_{1-42}$), promotes the formation of high molecular weight $A\beta_{1-42}$ assemblies that are not on the amyloid forming pathway. Additionally, our data show that hypochlorite-induced modification of $A\beta_{1-42}$ influences its binding to neuron-like and microglia-like cells and preferentially directs $A\beta_{1-42}$ to bind to the neurotrophin receptor p75 and scavenger receptors in vitro. Strikingly, hypochlorite-induced modification potently abolishes the toxicity of $A\beta_{1-42}$ via at two distinct mechanisms (i) preventing the formation of toxic $A\beta_{1-42}$ assemblies and (ii) neutralizing pre-formed toxic $A\beta_{1-42}$ assemblies. A greater understanding of the diverse biological roles of hypochlorite could help us design novel ways to treat chronic inflammatory pathologies.

Post-translationally sulfated proteins from the saliva of blood feeding organisms as novel anticoagulants

Emma E. Watson[1*], Richard J. Payne[2]

1. School of Physical Sciences, The University of Adelaide.

2. School of Chemistry, The University of Sydney.

Blood-feeding arthropods (such as ticks, mosquitoes and leeches) produce potent anticoagulant proteins in their saliva to facilitate access to their blood meal. These compounds interfere with the coagulation cascade - a series of enzymes which regulate the process of blood clotting - particularly the central protease thrombin. Undesired blood clotting is implicated in several serious human diseases, including deep vein thrombosis (DVT) and stroke. Stroke is the fifth leading cause of death and single leading cause of permanent disability in developed countries such as Australia and DVT is becoming more prevalent with an ageing population.[1] However, very few treatment options exist for stroke and other diseases that involve unwanted formation of blood clots, and those that are approved show poor efficacy and serious side-effects.

We sought to evaluate anticoagulant proteins produced by blood-feeding organisms as potential treatment options for thrombotic diseases. Through initial bioinformatic analysis, novel anticoagulant proteins could be identified through their sequence homology to known thrombin inhibitors and, additionally, potential sites of post-translational modifications known to modulate anticoagulant properties were identified. Access to such proteins identified in a variety of tick, fly and mosquito[2] species could then be achieved through total chemical protein synthesis, to enable evaluation of these potential antithrombotics. Additionally, the modular synthetic strategy employed allows for combinatorial synthesis and access to non-native protein architectures with enhanced anti-coagulant activity.