Speaker Abstracts

Name: **Joel Mackay**

Theme: Gene Regulation

Title: **How does the chromatin remodelling enzyme CHD4 shape chromatin?**

Authors*: Joel mackay*

Any biological process involving the genome – be it transcription, replication or repair – requires the mechanical remodelling of histone-DNA interactions. This remodelling is achieved by sophisticated ATP-dependent DNA translocase enzymes. We have set out to determine the mechanism by which one prominent remodeller that is conserved across multicellular organisms – CHD4 – can move histones relative to DNA. I will describe our results to date and the mechanistic and functional insights that they have provided.

Speaker Abstracts

Speaker Abstracts

Name: **Irina Voineagu**

Theme: Gene Regulation

Title: **CRISPRi screening of enhancers in human primary astrocytes identifies regulatory circuitry disrupted in Alzheimer's disease**

Authors*: Nicole Green, Gavin J Sutton, Samuel Bagot, Hannah Danon, Juli Wang, Irina Voineagu,*
Affiliation - UNSW for all authors

Transcriptional control by enhancer elements play an important role in brain development and function, and many variants associated with neurodevelopmental and psychiatric disorders are located in predicted enhancers. However, there is little functional evidence of enhancer activity and limited identification of enhancer's target genes in brain-derived cells. Here we assessed the function of 979 putative enhancers in primary human astrocytes through CRISPRi screening, combining epigenetic silencing by dCAs9-KRAB with scRNA-seq. This screen identified over a hundred active enhancers and uncovered their target genes. We found that enhancer activity is associated with enhancer RNA (eRNA) expression, often doesn't involve the nearest gene, and most enhancers regulate genes within 200kb. The genes regulated by these enhancers were overrepresented among genes associated with Alzheimer's disease. Furthermore, 90% of the regulatory interactions captured by CRISPRi screening were not captured by eQTL data, suggesting that functional characterisation of enhancers allows for a greater understanding of the regulatory landscape of brain cells, with implications for understanding the molecular mechanisms underlying brain disorders.

Speaker Abstracts

Speaker Abstracts

Name: **Steven Zuryn**

Theme: Gene Regulation

Title: **Regulation of the state of the mitochondrial genome**

Authors*: Steven Zuryn*

With life expectancies increasing around the world, neurodegenerative disorders and other late-onset inflictions represent an enormous disease burden. A cellular hallmark of these diseases is a loss of mitochondrial function. Mitochondria harbour their own genome (mtDNA), which is essential for mitochondrial assembly and function but prone to mutation and molecular damage. A gradual accumulation of mtDNA damage over time has been proposed to contribute to the progressive nature of late-onset diseases and ageing itself. The transcription and repair of mtDNA requires separate enzymatic activities that can sterically compete, suggesting a life-long trade-off between these two processes. In Caenorhabditis elegans, we find that the bZIP transcription factor ATFS-1/Atf5 regulates this balance in favour of mtDNA repair by localizing to mitochondria and interfering with the assembly of the mitochondrial pre-initiation transcription complex between HMG-5/TFAM and RPOM-1/mtRNAP. ATFS-1-mediated transcriptional inhibition decreases age-dependent mtDNA molecular damage through the DNA glycosylase NTH-1/NTH1, as well as the helicase TWNK-1/TWNK, resulting in an enhancement in the functional longevity of cells and protection against decline in animal behaviour caused by targeted and severe mtDNA damage. Together, our findings reveal that ATFS-1 acts as a molecular focal point for the control of balance between genome expression and maintenance in the mitochondria.

Speaker Abstracts

Speaker Abstracts

Name: **Brian Morris**

Theme: Gene Regulation

Title: **Proteomic basis of the resilience to mortality that is mediated by FOXO3 longevity genotype**

Authors*: Brian J. Morris, 1-3 Timothy A. Donlon,1,4 Randi Chen,1 Eunjung Lim,5 Eric K. Morgen,6 Kristen Fortney,6 Naisha Shah,6 Kamal H. Masaki,1,2 Bradley J. Willcox1,2*

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FOXO3 is a ubiquitous transcription factor expressed in response to cellular stress caused by nutrient deprivation, inflammatory cytokines, reactive oxygen species, radiation, hypoxia and other factors. We showed previously that the well-established association of inherited FOXO3 variants with longevity was the result of partial protection against risk of mortality posed by aging-related life-long stressors, particularly cardiometabolic disease. We then referred to the longevity-associated genotypes as conferring “mortality resilience.” Serum proteins whose levels change with aging and are associated with mortality risk may be considered as “stress proteins.” They may serve as indirect measures of life-long stress. Our aims were to (1) identify stress proteins that increase with aging and are associated with an increased risk of mortality, and (2) to determine if FOXO3 longevity/resilience genotype mediates the expected increase in mortality. In the current study of 975 men aged 71–83 years, we quantified a total of 4,500 serum protein aptamers using the Somalogic SomaScan proteomics platform. Stress proteins that were associated with mortality were identified. We then used age-adjusted multivariable Cox models to investigate the interaction of stress protein with FOXO3 longevity-associated rs12212067 genotypes. For all the analyses, the p-values were corrected for multiple comparisons. This led to the identification of 44 stress proteins influencing the association of FOXO3 genotype with reduced mortality. Biological pathways were identified for these proteins. Our results suggest that the FOXO3 resilience genotype functions by reducing mortality in

pathways related to innate immunity, bone morphogenetic protein signaling, leukocyte migration, and growth factor response.

Speaker Abstracts

Speaker Abstracts

Name: **Gurveer Kaur Gaddu**

Theme: Gene Regulation

Title: **Characterising the role H2A.Z chromatin modifiers in zebrafish immunity and development.**

Authors*: Gurveer K Gaddu*

Amardeep S Dhillon

Faiza Basheer

DNA and proteins packaged in the cell nucleus are affected by histone acetylation, which affects chromatin organization and gene expression. The acetylation of core histones has been associated with chromatin opening and closing, gene transcription, DNA damage repair, and chromosome decondensation in mitosis and meiosis. Lysine residues are acetylated by tightly regulated histone lysine acetyltransferases (HATs: KATs) and deacetylases (HDACs). There is an increasing body of research indicating that dysregulation of KATs and aberrant lysine acetylation is linked to a spectrum of diseases including tumorigenesis, presenting an opportunity for finding new therapeutic targets in this area. Kat5 is a histone acetyltransferase family member and regulates DNA damage response by acetylating histones and chromatin remodelling.

My research is focused on investigating the effects of acute deletion of kat5 using CRISPR/Cas9 mediated deletion in zebrafish and its potential role in development and diseases. Zebrafish have been increasingly used as an animal model to better understand the genetics and biology of vertebrate development. I found that mutation of kat5 is lethal and associated with multiple developmental issues and diseases such as survival, lymphoma, and neurodegenerative disorders. Characterization of key mechanisms causing these defects and performing further studies will pave the way for a better understanding of the role of kat5 in zebrafish development and cancer studies.

Speaker Abstracts

Speaker Abstracts

Name: **Prof Andrea Yool**

Theme: Drug Discovery

Title: **Emerging Roles for Aquaporin Channels as Therapeutic Targets in Cancer and Neurodegenerative Disease**

Authors*: Andrea Yool*

Ongoing collaborative projects at the University of Adelaide in partnership with Flinders University, Griffiths University, and the University of Leipzig have discovered that unexpected classes of human aquaporins (AQPs) are expressed in the human brain. AQPs, originally thought to be simple membrane channels only for water, are being redefined as complex regulated multifunctional proteins. Human AQP subtypes now known to be permeable to redox signaling molecules such as hydrogen peroxide are rapidly upregulated in model cell lines, conferring a native protective mechanism for neurons and glia against oxidative stress associated with neurodegenerative disease. Another class of aquaporin which our group showed is permeable to both water and ions is upregulated in rapidly migrating cells; under pathological conditions these dual water-and-ion channels act to accelerate rates of spread of glioblastoma brain cancer, endometrial cancer, and colon cancer. Pharmacological modulators being identified for aquaporins show promise as novel anti-cancer therapeutics, by controlling cell invasiveness at non-toxic concentrations in vitro and ex vivo.

Speaker Abstracts

Speaker Abstracts

Name: **David Craik**

Theme: Drug Discovery

Title: **Macrocyclic peptide scaffolds as tools in drug design**

Authors*: David J Craik*

Naturally occurring peptides offer great potential as leads for drug design. Our work focuses on a class of cyclic peptides known as cyclotides, which are topologically unique in that they have a head-to-tail cyclised peptide backbone and a cystine knotted arrangement of disulfide bonds. This makes cyclotides exceptionally stable to heat or enzymatic treatments and, indeed, they are amongst nature's most stable proteins. Their stability and compact structure favours them as attractive protein frameworks onto which bioactive peptide epitopes can be grafted to stabilise them. More than two dozen examples have now been reported where biologically active epitopes have been grafted onto cyclic peptide frameworks to produce lead molecules with potential in the treatment of cancer, cardiovascular disease, infectious disease, autoimmune disease (multiple sclerosis) and pain. This presentation will describe our efforts to improve the drug like properties of cyclotides and other cyclic peptides and in particular to improve their permeability to facilitate oral bioavailability and/or cell permeability.

Speaker Abstracts

Speaker Abstracts

Name: **Martin Scanlon**

Theme: Drug Discovery

Title: **REFiL - A systematic approach to the rapid elaboration of fragments into leads**

Authors*: Martin Scanlon*

Fragment-based drug design (FBDD) has been widely adopted to identify small molecules that bind to biomolecular targets. However, the hit rates observed in fragment screens can be relatively high, and it is common for fragment hits to bind very weakly to their target. In practice, many fragments bind with affinities that cannot be measured accurately – which makes ranking them challenging. Therefore, selecting the “best” fragments to advance into further development is not always trivial. In this presentation I will describe a strategy that we have implemented to provide a more robust and systematic approach to this early phase of FBDD. This workflow employs a combination of chemoinformatic design and biophysical screening approaches to validate and rank fragment hits, and to identify suitable vectors for chemical elaboration. Subsequently, it employs microscale parallel synthesis of targeted libraries that are designed to sample the chemical space around elaboration vectors.

Speaker Abstracts

Speaker Abstracts

Name: **Lani Davies**

Theme: Drug Discovery

Title: **Fragment-Based Drug Design of Heparanase Inhibitors: A Multi-Faceted Approach**

Authors*: Lani J. Davies, Colin J. Jackson, Christoph Nitsche*

Human heparanase is an enzyme that has received considerable attention as drug target in recent years. Heparanase is the only mammalian enzyme that catalyses the hydrolysis of heparan sulfate in the extracellular matrix and its activity is an essential part of inflammation, angiogenesis and modification of the cell's environment. It is therefore a promising drug target for the treatment of cancer, diabetes, inflammatory diseases, as well as COVID-19, where heparanase activity has been shown to contribute to pathogenesis. However, no drugs or therapeutic treatment designed to specifically inhibit heparanase have successfully passed phase III clinical trials yet. The majority of effective heparanase inhibitors have off-target effects and undesired high anticoagulant activity, such as oligo- and polysaccharide heparan sulfate mimetics. Similarly, small molecule inhibitors often fail clinical trials due to toxicity. In our research, we aim to use a combination of structural biology, high-throughput crystallography, computational methods and synthetic chemistry to screen fragment libraries against heparanase and elaborate hits into promising lead compounds. We have screened a small library of commercially available fragments, which has led to the discovery of 20 candidates that bind both at the active site cleft, and in shallow surface pockets. Of these 20 candidates, four were found to inhibit heparanase at the micromolar range. Moreover, five allosteric binding sites of heparanase were determined through in silico modelling, leading to an additional extensive high throughput virtual screening campaign. Four of the 20 fragments of interest selected from this study were found to bind heparanase, three of which were observed to inhibit at the micromolar range. Fragments that were found to bind and inhibit heparanase were then grown through information gained by using computational tools, leading to higher potency inhibitors. Altogether, this work has identified several fragments that have good affinity and inhibition of heparanase with the potential to be explored as potent small molecule inhibitors.

Speaker Abstracts

Speaker Abstracts

Name: **Ashleigh Paparella**

Theme: Drug Discovery

Title: **Inhibition of Clostridioides difficile TcdA and TcdB toxins with transition state analogues**

Authors*: Ashleigh S Paparella, Briana L Aboulache, Rajesh K Harijan, Kathryn S Potts, William Ferreira, Hong Huynh, Simon Cutting, Peter C Tyler and Vern L Schramm*

Clostridioides difficile is a bacterial pathogen that causes serious and potentially fatal inflammatory disease of the colon. Anti-virulence strategies to treat C. difficile infection are attractive options to antibiotic therapy because the human gastrointestinal (GI) microbiome is spared and damage to the host tissue is minimized. TcdA and TcdB are the major virulence factors produced by C. difficile and mediate host cytotoxicity by glycosylating and inactivating Rho GTPases using UDP-glucose as a glycosyl donor, leading to cell rounding, cell death and loss of intestinal integrity. We used kinetic isotope effects (KIEs) and transition state theory to solve the transition state structure of the glycosyltransferase domain of TcdA and TcdB. This permits the design of transition state analogues which are powerful enzyme inhibitors. KIE analysis supported the formation of a dissociative glucocation transition state where positive charge develops on the anomeric carbon. Iminosugars mimic glucocationic transition states as they contain cationic nitrogen groups at or near the anomeric carbon. We identified iminosugars, isofagomine and noeuromycin as transition state analogue inhibitors of TcdA and TcdB and we characterized them by kinetic, thermodynamic and structural analysis. Both iminosugars exhibit nM inhibition constants and inhibit TcdA and TcdB by forming ternary complexes with UDP, a by-product of the glycosyltransferase reaction. X-Ray crystal structures revealed that isofagomine and noeuromycin interact with UDP in the TcdB active site by forming an ion pair interaction between the cationic nitrogen and the β-phosphate of UDP. Isofagomine and noeuromycin also prevented TcdA and TcdB induced cytotoxicity of mammalian cells by preventing glycosylation of Rho GTPases. We next investigated the efficacy of isofagomine in a mouse model of C. difficile infection. Treatment of C. difficile infection with isofagomine tartrate provided significant protection from C. difficile induced mortality with an 84% survival rate in mice treated with 15 mg/kg isofagomine tartrate. Finally, microbiome analysis of mice fecal samples, revealed that isofagomine treatment allowed recovery of microbial diversity within the GI microbiome which is a critical barrier to C. difficile re-infection. In summary, isofagomine and noeuromycin inhibit TcdA and TcdB by mimicking the transition state of the TcdA/B glycosylation reaction and show potential for use as therapeutics against C. difficile pathology.

Speaker Abstracts

Speaker Abstracts

Name: **Prof Oliver Rackham**

Theme: RNA Biology

Title: **Engineering improved gene editing systems to target DNA and RNA**

Authors*: Oliver Rackham*

The ability to alter the genomes and transcriptomes of cells is key to understanding how genes influence the functions of organisms and will be critical to modify living systems for useful purposes. However, this promise has long been limited by the technical challenges involved in genetic engineering. Recent advances in gene editing have bypassed some of these challenges but they are still far from ideal. To target DNA, we have used computational approaches to create Cas9 enzymes with improved editing activities. We used genetic circuits linked to cell survival in yeast to screen and quantify Cas9 activity and discover enzymes with desirable properties. These Cas9 variants have improved efficiency in mammalian cells and provide tools to enhance and expand the possible applications of CRISPR-based gene editing. To target RNA, we have hijacked RNA-binding repeat proteins that play key roles in mammalian gene expression. Because of their modular structure, repeat domain proteins are particularly well suited for manipulating diverse RNAs. We have expanded the RNA recognition code of naturally occurring PUF RNA-binding proteins and showed they can be programed with new specificities. Furthermore, we have created synthetic proteins from another family of RNA-binding repeat domains: PPR proteins. These artificial proteins have revealed the mode for RNA binding by natural PPR domains and provide unique tools for manipulating cellular RNAs, as well as single-stranded DNA. In recent work we extended this concept further and used computational evolution to create unnatural RNA-binding proteins. The design of proteins that can bind any RNA or DNA sequence of interest and modulate its function will be important to elucidate the mechanisms by which genes are controlled and for building programmable therapeutics in medicine.

Speaker Abstracts

Speaker Abstracts

Name: **Jennifer Zenker**

Theme: RNA Biology

Title: **MICROTUBULES DIRECT EARLY EMBRYONIC CELL FATE DECISIONS BY DIFFERENTIAL RNA LOCALISATION AND TRANSLATION**

Authors*: Azelle Hawdon, Jessica Greaney, Yi Louise Li, Oliver Anderson, Sebastian Palacios Martinez, Gemma Stathatos, Xiaodong Liu, Niall D Geoghegan, Anja Elsenhans, Charles Ferguson, Rob Parton, Harald Janovjak, José Polo and Jennifer Zenker*

How equipotent cells develop into complex tissues containing many diverse cell types is one of the most fundamental questions in biology. The organisation of a cell's interior, the cytoskeleton and organelles, is pivotal for every cell's functionality. However, unlike most differentiated cells, our knowledge about the contribution of the sub-cellular architecture to pluripotency remains scarce.

Using cutting-edge live imaging technologies, we uncovered polarised CAMSAP3-dependent non-centrosomal microtubules as central player for spatiotemporal RNA heterogeneities during early mammalian embryogenesis, determining differential translation capacities unevenly inherited by outer and inner daughter cells as they adopt different cell fates. Outer cells, marked by transcription factor Cdx2, undergoing differentiation into extraembryonic tissue, require an elevated translation efficiency shown by a higher mRNA, tRNA, endoplasmic reticulum and ribosome content, and live visualisation of translation events. Contrary, Sox2-positive inner cells, giving rise to the pluripotent inner cell mass are characterised by a higher rRNA content compared to outer cells, and thus less translationally active. To enable spatio-temporal regulation of cytoplasmic RNA localisation, we have successfully engineered an optogenetical-controllable CAMSAP3-Halo (= Opto-CAMSAP3-Halo) cassette to translocate the origin of microtubule growth in pluripotent stem cells. Such light-inducible tools can overcome the current limitations of typical microtubule drugs to non-invasively and with spatial-temporal precision manipulate the organisation of the microtubule network and its direct link to the potency of stem cells in vivo and in vitro. Dissecting how intrinsic cellular regulation contributes to pluripotency, complementing the genetic and epigenetic regulation, may lead a revolutionary era of regenerative and reproductive medicine.

Speaker Abstracts

Speaker Abstracts

Name: **Cecile King**

Theme: RNA Biology

Title: **Retrotransposon control of the immune response to virus infection**

Authors*: Cecile King*

The mammalian genome has an abundance of transposable elements but their contribution to complex biological systems remains poorly understood. Here, we report the CRISPR/Cas9 deletion of a single retrotransposon (Lx9c11) in mice and its effect on the immune response to viral infection. The deletion of Lx9c11 altered the splicing and function of novel lncRNA transcripts and led to an exaggerated and lethal immune response to viral infection. Gene expression analyses revealed a negative regulatory role for Lx9c11, with marked over-expression of virus response immune genes in Lx9c11-/- mice. Lx9c11-mediated regulation of the immune response to virus could be replicated by replacing Lx9c11 RNA. These findings provide evidence that transposable elements can function to neutralise the deleterious effects of duplicated genes in the immune system, favouring host survival during virus infection.

Speaker Abstracts

Speaker Abstracts

Name: **Natalia Pinello**

Theme: RNA Biology

Title: **Site-specific METTL3-dependent m6A RNA methylation regulates gene expression of the NLRP3 inflammasome sensor**

Authors*: Natalia Pinello, Quintin Lee, Renhua Song, Xi Yang, Majid Mehravar, Jessica Tieng, Chinh Ngo, Xing Huang, Zhouli Chen, Mark Larance, Fei Lan, Ben Roediger, Jocelyn Widagdo, Victor Anggono, Si Ming Man, Dan Ye, Xiangjian Zheng & Justin J. -L Wong.*

Inflammasomes are signalling complexes that are activated in response to infection, cellular stress and other cues to initiate an inflammatory response. The NLRP3 inflammasome is a cytosolic sensor that mediates caspase-1-dependent maturation and release of the pro-inflammatory cytokines IL-1β and IL-18, and Gasdermin D-induced pyroptotic cell death. While a broad range of pathogens can activate the NLRP3 inflammasome, in most cases, it appears to be dispensable to fight infection. However, NLRP3 activation has been identified as a driver of many inflammatory disorders, including autoinflammatory, cardiovascular and neurodegenerative diseases. Therefore, big efforts have been invested in understanding the molecular mechanisms driving the NLRP3 pathway. N6-methyladenosine (m6A) deposition by METTL3 on mRNA has emerged as a key regulator of the immune response. However, the specific role of METTL3 and RNA m6A methylation during inflammasome activation remains unclear. Using genetic manipulation and pharmacological inhibition, we demonstrate that METTL3 is essential for activation of the NLRP3 inflammasome in vitro and in vivo. METTL3 depletion impaired inflammasome activation in cultured mouse and human primary cells. We show that pharmacological inhibition of METTL3 attenuates NLRP3 hyperactivation in peripheral blood mononuclear cells isolated from Cryopyrin Associated Periodic Syndrome (CAPS) patients. Furthermore, METTL3 ablation in the myeloid compartment rendered mice relatively more resistant to NLRP3-mediated endotoxic shock. Combining transcriptome-wide m6A mapping and programmable CRISPR-based genomic and m6A editing techniques, we identified NLRP3 amongst METTL3 targets in macrophages as well as specific m6A sites controlling NLRP3 transcripts' fate. Mechanistically, we found that site-specific METTL3-mediated m6A methylation regulates NLRP3 gene expression at two levels: i) co-transcriptionally, promoting H3K9me2 demethylation nearby the NLRP3 promoter; and ii) promoting NLRP3 translation via the action of the m6A reader protein, YTHDF1. Overall, our study places m6A-mediated regulation of gene expression at the centre of immunity and brings forward METTL3 and YTHDF1 as potential targets for therapeutic intervention in inflammation-driven syndromes.

Speaker Abstracts

Speaker Abstracts

Name: **Qi Zhang**

Theme: RNA Biology

Title: **Inseparable RNA binding and chromatin modification activities of a nucleosome-interacting surface in PRC2**

Authors*: Qi Zhang 1, Evan Healy 1, Emma H. Gail 1, Sarena F. Flanigan 1, Natasha Jones 1, Xiao Han Ng 1, Michael Uckelmann 1, Vitalina Levina 1, Chen Davidovich 1,2*

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Various chromatin modifiers bind to RNA, but separating these RNA-binding activities from their other molecular functions can be challenging for mechanistic studies. One such modifier is the polycomb repressive complex 2 (PRC2), a histone methyltransferase crucial for maintaining the repressed state of developmentally expressed genes. PRC2 interacts with RNA in cells, but there is an ongoing debate on how RNA regulates its canonical function: chromatin modification. RNA-binding defective PRC2 mutants were previously used to study the potential role of RNA in PRC2 regulation [1,2] and one of these mutants resulted in defective cell differentiation [2]. Furthermore, RNase A treatment during chromatin immunoprecipitation (rChIP) led to the apparent depletion of PRC2 from chromatin [2]. These observations led to a proposed model suggesting that RNA is essential for PRC2 chromatin occupancy [2]. Yet, it remains unknown how RNA-binding defective mutations affect the canonical function of PRC2. It is also unclear to what extent the apparent loss of ChIP signals after RNase A treatment is indicative of real changes in PRC2 chromatin occupancy.

Using methyltransferase enzymatic assays and binding assays in vitro, we show that a portion of the RNA binding surface of PRC2 interacts with nucleosomal DNA. Importantly, this RNA-binding surface of PRC2 is required for its chromatin modification activity in vitro, in an RNA-independent manner. We next tested a panel of separation-of-function and loss-of-function PRC2 mutations in cells, in order to dissect the RNA binding activity of PRC2 from its methyltransferase activity. Using CUT&Tag and quantitative ChIP-seq (ChIP-Rx), we show that PRC2 mutants maintain normal chromatin modification and binding sites in cells if they are active in methyltransferase in vitro, irrespective of their RNA binding activity. RNA-seq confirmed that PRC2 mutants are active in repressing PRC2-target genes if they are active in methyltransferase in vitro, irrespective of their RNA binding activity. Accordingly, an RNA-binding defective PRC2 mutant, which is active in methyltransferase in vitro, phenocopies the wildtype PRC2 in cells. Additionally, we show that the apparent reduction in PRC2

ChIP signals after RNase A treatment is not indicative of real changes in PRC2 chromatin occupancy. Instead, this reduction is attributed to a global gain of non-targeted DNA during chromatin immunoprecipitation due to RNA depletion [3].

Our data rationalize previously-identified disease-associated mutations in nucleosome-binding surfaces of PRC2, externally to its catalytic centre. More broadly, we show that part of the RNA-binding surface of PRC2, rather than the RNA-binding activity per se, is required for chromatin modification in vitro and in cells.

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Speaker Abstracts

Speaker Abstracts

Name: **Haibo Yu**

Theme: Computation

Title: **Free energy calculations for mechanistic studies and molecular design**

Authors*: Haibo Yu*

Free energy calculations have traditionally posed significant computational challenges in molecular modelling. However, ongoing advancements in computational techniques and hardware have made them increasingly accessible for practical use. In this presentation, I will share our recent research focusing on applying free energy calculations to investigate mechanistic aspects of biomolecular systems and facilitate lead optimisation in drug discovery. I will begin by discussing our work on utilising bioluminescence to address a longstanding mechanistic inquiry in this field. Subsequently, I will present our collaborative efforts in drug discovery, specifically in optimising lead compounds for anticancer agents.

Speaker Abstracts

Speaker Abstracts

Name: **Sanjib Senapati**

Theme: Computation

Title: **Identifying crucial E-protein residues responsible for unusual stability of Zika virus envelope**

Authors*: Sanjib Senapati*

Outbreak of zika virus (ZIKV) infections in 2015-16 that caused microcephaly and other congenital abnormalities in newborns prompted intense research across the globe. These studies have suggested that ZIKV can sustain high temperatures and harsh physiological conditions, unlike the other flaviviruses such as dengue virus (DENV). In contrast, recent cryo-EM studies have shown very similar architecture of the ZIKV and DENV envelopes that constitute the primary level of viral protection. Encouraged by these findings, we attempted to identify the crucial protein residues that make the ZIKV envelope so robust by employing coarse-grained and all-atomic molecular dynamics simulations, and computational mutagenesis studies. In accordance with more recent cryo-EM findings, our simulation results exhibited stable ZIKV envelope protein shell both at 29o and 40oC, while the DENV2 shell loosened up significantly at 40°C. Subsequently, we simulated a series of ZIKV variants to identify the specific domain and residues involved in maintaining the structural integrity of the viral protein shell at high temperatures. Our results suggest that the DIII domain, more specifically, the CD- and FG-loop residues of the ZIKV protein shell play a crucial role in making the virus envelope thermostable by inducing strong raft-raft interactions. These findings can accelerate the rational design of ZIKV therapeutics.

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Speaker Abstracts

Speaker Abstracts

Name: **Elaine Tao**

Theme: Computation

Title: **A binding site for phosphoinositide modulation of voltage gated sodium channels described by multiscale simulations**

Authors*: Elaine Tao*, Yiechang Lin*, James P. Champion, Ben Corry*

Voltage gated sodium (Nav) channels are membrane proteins which open to facilitate the influx of sodium ions into excitable cells. They are fundamental for action potential generation in the brain and peripheral nerves, as well as skeletal and cardiac muscle. In response to changes in membrane potential, Nav channels undergo a transition from the resting closed state, to an open activated state which allows ion conduction, before rapidly inactivating. Mutations that disrupt this functional cycle often lead to severe diseases, including epilepsy, pain conditions and cardiac disorders, thus making Nav channels a significant pharmacological target.

Phosphoinositides are important lipids in the cell membrane and are known to modulate ion channel function. The phosphoinositide, PI(4,5)P2, was recently shown to reduce Nav1.4 activity by increasing the difficulty of channel opening, accelerating fast inactivation and slowing recovery from fast inactivation. The structural basis of Nav channel regulation by PI(4,5)P2 is yet to be elucidated.

In this work, we use molecular dynamics simulations to show that PI(4,5)P2 binds stably to the inactivated structure of Nav channels at a conserved site in the DIV S4-S5 linker, which couples the voltage sensing domain (VSD) to the channel pore. At this binding site, PI(4,5)P2 can prolong the inactivated state of the Nav channel via two proposed mechanisms - 1. PI(4,5)P2 binding stabilises the association of the DIII-IV linker to the Nav channel pore, which is required for fast inactivation; 2. PI(4,5)P2 also prevents the re-binding of the C-terminal domain at this location, which is theorised to be necessary for recovery back to the resting state. Furthermore, we show that PI(4,5)P2 binding is functional state dependent. In a resting state Nav channel model, PI(4,5)P2 can bind to VSD gating charges, anchoring them within the membrane in a way that may impede their ability to activate in response to membrane potential. Our results provide insight into how Nav channels are modulated by phosphoinositides across the different functional states, an important step for the development of novel therapeutics to treat Nav-related diseases.

Speaker Abstracts

Speaker Abstracts

Name: **Wai-Hong Tham**

Theme: Pathogens

Title: **Transmission Blocking Nanobodies against Malaria Parasites**

Authors*: Wai-Hong Tham, Melanie Dietrich, Frankie Lyons, Amy Adair, Li-Jin Chan*

Malaria is a major parasitic disease in humans with over 400,000 deaths each year, with Plasmodium falciparum being responsible for almost all deaths. P. falciparum has a complex life cycle involving the female Anopheles mosquito and a vertebrate host, the human. Within the female Anopheles mosquito, malaria parasite fertilization occurs. By stopping malaria parasite fertilization, we effectively block the transmission of parasites from mosquito to human. The 6-cysteine protein family is one of the most abundant surface antigens of P. falciparum and play important roles in parasite fertilization and transmission. We seek to understand the function and structural mechanisms of 6-cysteine proteins during the malaria parasite life cycle for development of novel anti-malarial interventions. With an immunised nanobody platform, we have characterised the first collection of nanobodies to different 6-cysteine proteins. Using a dedicated insectary, we show that our nanobodies block malaria parasite fertilization in the Anopheles mosquito midgut. Crystal structures of nanobodies with several 6-cysteine proteins show that our inhibitory nanobodies bind to distinct epitopes. Together with our nanobodies and crystal structures, we aim to further dissect the roles of the 6-cysteine proteins for a comprehensive understanding of their critical function in malaria parasite transmission.

Speaker Abstracts

Speaker Abstracts

Name: **Ana Traven**

Theme: Pathogens

Title: **Insights into the biology, immune interactions and virulence of the emerging drug-resistant pathogen Candida auris**

Authors*: Harshini Weerasinghe 1, 2 , Claudia Simm 1, 2 , Irma Tedja 1, 2 & Ana Traven 1, 2*

Candida auris is an environmental yeast that in 2009 emerged suddenly in human environments, including hospitals and nursing homes, and was concurrently detected on multiple continents. The reasons remain a mystery, but climate change-induced shifts in its environmental habitat have been proposed as one possible driver. C. auris behaves in an unusual way for a fungal pathogen by causing outbreaks in hospital wards including forcing closures of ICUs. It is intrinsically resistant to front-line antifungals, including multidrug resistance and pan-drug resistance. Mortality from systemic C. auris disease is around 30%. Together, these phenotypes have led to C. auris being classified as a critical priority fungal pathogen by the WHO, and it is the only fungus in the top antimicrobial resistance (AMR) priority group published by the CDC.

Due to its emerging nature, the biology and pathogenesis of C. auris are much less understood compared to other fungal pathogens. We have established a molecular biology program to study C. auris and are focusing on its mechanisms of immune evasion, the regulatory programs that enable it to respond to nutrient and stress challenges in host niches, and understanding how it could be inhibited by novel antifungal compounds. Results from these studies will be presented in the talk.

Speaker Abstracts

Speaker Abstracts

Name: **Navneet Singh**

Theme: Pathogens

Title: **Dicer is essential for maintenance of HSV-1 latency**

Authors*: Navneet Singh, Sherin Zachariah & David C Tscharke*

Herpes simplex virus-1 (HSV-1) is a prevalent and persistent pathogen. While the disease caused by HSV-1 is often mild and asymptomatic in the form of cold sores, the virus has the potential to cause severe forms of the diseases such as herpes encephalitis, keratitis and neonatal herpes, associated with significant life-long illness and, in some cases mortality. The ability of HSV-1 to establish a lifelong latent infection with intermittent reactivations is pivotal to its success and potential to cause disease. Consequently, understanding and developing strategies to target latency remain significant and ongoing objectives in HSV-1 research. Recent studies have shown that HSV-1 latency is not entirely inactive and may be modulated by viral and host factors. Among these, miRNAs of virus and host origin have been implicated, but it is not known if as a whole they are pro-replication, pro-latency or pro-host. To address this, we targeted all the miRNAs by removing Dicer, an essential protein for most miRNA biogenesis, in a novel in vivo model. We employed Dicer floxed mice and infected them with an HSV-1 that expresses Cre recombinase, such that Dicer will be knocked out selectively in infected (and not other) cells. Notably, Dicer deletion did not alter acute HSV-1 pathogenesis. However, between 30 and 150 days after infection there was an almost a 61% drop in HSV-1 genomes observed in Dicer floxed mice compared with Dicer-sufficient controls. Moreover, reactivation potential was significantly reduced at 90 and 150 days post- infection when Dicer was targeted. Next, we asked whether Dicer was required for the survival of latently-infected neurons. To our surprise, using a model where neurons could be marked and Dicer deleted, loss of Dicer did not result in a significant decrease in neuron numbers in latency. These striking data suggest that Dicer, and potentially the miRNA biogenesis pathway in general, are required for the maintenance of HSV-1 latency and are therefore possible therapeutic targets to affect the elimination of latent HSV-1.

Speaker Abstracts

Speaker Abstracts

Name: **Jade Forwood**

Theme: Pathogens

Title: **Unravelling Viral Strategies for Innate Immune Evasion through Nucleocytoplasmic Transport**

Authors*: Thilini S. Munasinghe, Megan R. Edwards, Sofiya Tsimbalyuk, Olivia A. Vogel, Kate M. Smith, Murray Stewart, Justin K. Foster, Loretta A. Bosence, David Aragão, Justin A. Roby, Christopher F. Basler & Jade K. Forwood*

The emergence of the MERS coronavirus (MERS-CoV) has inspired investigations into its innate immune evasion strategies. One such mechanism involves the ORF4b protein's interaction with nuclear import adapter IMPα3, inhibiting NF-κB-dependent innate immunity. The presentation will describe high-resolution structures of ORF4b bound to distinct IMPα family members, revealing unconventional binding mechanisms. Mutations within the nuclear localization signal (NLS) region transform the binding mechanism, underlining its significance for nuclear import, IMPα engagement, and innate immune pathway suppression. The study extends to closely related coronaviruses like HKU5, and describes how small ORF4b mutations can alter IMPα interactions.

Speaker Abstracts

Speaker Abstracts

Name: **Emma Watson**

Theme: Protein and Peptide Engineering and Evolution

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

Authors*: Emma E. Watson & Richard J. Payne*

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 mosquito2 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.

Speaker Abstracts

Speaker Abstracts

Name: **Jyoti Gurung**

Theme: Protein and Peptide Engineering and Evolution

Title: **Engineering Photo-Responsive Protein Domains for Regulation of Bacterial Motility.**

Authors*: Jyoti P Gurung, Gary Bryant, Pietro Ridone, Matthew AB Baker*

In this work, we engineered a photo-responsive protein domain such as proteorhodopsin and LOV (Light-Oxygen-Voltage) to regulate the activity of bacterial flagellar motor. The bacterial flagellar motor is crucial for bacterial motility, enabling movement of bacteria towards favorable environments. This study capitalizes on the unique properties of proteorhodopsin, a light-driven proton pump, and the LOV domain, a blue-light sensing module, to create a regulatory mechanism controlling bacterial motility.

Upon green light illumination, membrane protein such as proteorhodopsin pumps proton out of the bacterial cell. In response to low proton concentration, bacteria use bacteria flagellar motor to pass proton inside the cell. This ion passage generates torque, thus, resulting motor rotation. We engineered another photo-switchable protein domain, LOV, to either indirectly or directly regulate the bacterial motility. For indirect regulation, we used EL222 protein i.e., LOV domain with DNA-binding domain in C-terminal that regulated the expression of various motility genes such as MotAB, CheY, and CheZ. In direct regulation, we incorporated LOV domain directly into the stator (MotB) of bacterial flagellar motor. Upon blue-light illumination, the LOV domain undergoes structural changes that trigger the activation or inhibition of bacterial flagellar motor.

This approach of using light illumination offers remote control over bacterial motility, allowing for fine-tuning of movement patterns in bacteria. In conclusion, the ability to engineer protein domains with such tailored photo-responsive properties holds promise for designing advanced synthetic biological systems that can sense, adapt, and respond to dynamic environments.

Speaker Abstracts

Speaker Abstracts

Name: **Renae Ryan**

Theme: Membrane Transporters

Title: **The twisted link between a dual function glutamate transporter and Episodic Ataxia**

Authors*:*

Excitatory Amino Acid Transporters (EAATs) regulate excitatory neurotransmission by transporting glutamate into cells, mostly glia, to terminate neurotransmission and to avoid neurotoxicity. EAATs also conduct chloride ions (Cl-) via a channel-like process that is thermodynamically uncoupled from transport. The molecular mechanisms that allow these dual-function transporters to carry out two seemingly contradictory roles, and the physiological role of Cl- conductance of the EAATs, are not clear. I will describe the cryo-electron microscopy structure of a glutamate transporter homologue in an open-channel state, revealing an aqueous-accessible Cl- permeation pathway that is formed during the transport cycle and discuss the impact of a series of mutations in EAAT1 that have been identified in patients with the neurological disease episodic ataxia type 6 (EA6). By studying EAAT1 function and using a Drosophila melanogaster model of locomotor behaviour, our results indicate that mutations that lead to functional glutamate transport but either increased OR decreased Cl- channel activity contribute to the pathology of EA6, highlighting the importance of Cl- homeostasis in glial cells for proper central nervous system function. Our findings provide insight into the mechanism by which glutamate transporters support their dual functions and provides a framework for the rational development of therapeutics that can differentially modulate substrate transport or channel properties for the treatment of neurological disorders caused by EAAT dysfunction, such as Episodic Ataxia.

Speaker Abstracts

Speaker Abstracts

Name: **Xin Jiang**

Theme: Membrane Transporters

Title: **Mechanistic investigation of the Plasmodium falciparum lactate transporter PfFNT**

Authors*: Xin Jiang*

Plasmodium falciparum, responsible for over half of global malaria cases, is the deadliest pathogen of human malaria. Previously effective antimalarial agents, such as Chloroquine, have faced significant drug-resistant challenges in recent years. The emergence and spreading of drug-resistant Plasmodium strains highlight the pressing need for innovative antimalarial solutions. Blood-stage Plasmodium parasites heavily rely on glycolysis for their energy requirements, resulting in heightened glucose uptake and lactate production. Consequently, targeting the Plasmodium falciparum lactate transporter, PfFNT, presents an eliciting therapeutic approach for malaria treatment. Among the compounds investigated, MMV007839 has emerged as a lead candidate capable of effectively eliminating parasites at submicromolar concentrations, but the precise mechanism remains elusive. To unravel the transport and inhibition mechanism of PfFNT, we employed cryogenic-electron microscopy (cryo-EM) to determine two critical structures: one with the protein in its apo form and another in complex with MMV007839. Complementing these structural insights, our biochemical analysis of PfFNT mutants enhances our understanding of both the lactate transport process mediated by PfFNT and the inhibition mechanism employed by MMV007839. These findings not only shed light on the molecular intricacies of PfFNT but also pave the way for further advancements in antimalarial drug design.

Speaker Abstracts

Speaker Abstracts

Name: **Stefan Broer**

Theme: Membrane Transporters

Title: **Development and evaluation of amino acid transport inhibitors (the good, the bad and the ugly).**

Authors*: Stefan Broer*

The identification of high-affinity selective inhibitors for amino acid transporters requires optimization of culture conditions and cell line models to achieve optimal expression of heterologous and endogenous transporters. As a next step, isolation of the transport activity is important to achieve a robust and selective signal. This can be achieved by the addition of specific inhibitors to suppress background activity and by the selection of substrates that are preferred or selective for the transporter under investigation. We developed several methods to measure amino acid transport in cells, including fluorescent membrane potential sensitive assays (FLIPR), LC-MS assays and optimised radiolabelled uptake assays. Uptake assay conditions were optimised for the detection and expression of amino acid transporters SLC6A19, SLC38A2 and SLC1A5. This included optimisation of the transport substrate and concentration, addition of inhibitors to supress cell endogenous transport and optimising culture conditions to optimise expression.

This transporter toolbox was used for primary high-throughput screening (HTS), characterisation of initial hits and evaluation of published transport inhibitors. The FLIPR assay was the preferred method for HTS, while radiolabelled and LC-MS transport assays were used for evaluation and characterisation. The use of several independent assays is important to avoid pitfalls in inhibitor development, such as false-positives and upregulation of endogenous transport activities. Further improvements of initial hits can be achieved through medicinal chemistry and structural studies of the target protein. Using these methods, we have identified inhibitors that bind selectively and with nanomolar affinity to the target SLC6A19. Inhibition of this transporter aims to normalise plasma amino acid concentrations in inborn errors of amino acid metabolism and as a possible treatment of type 2 diabetes.

Speaker Abstracts

Speaker Abstracts

Name: **Aidan Grosas**

Theme: Membrane Transporters

Title: **Investigating the Mechanisms of Substrate Polyspecificity of the RND Transporter MexB using Cryo-EM**

Authors*: Aidan B Grosas, MariaKatarina Lambourne, Maddison Steele, David Safadi, Simon HJ Brown & James C Bouwer*

The emergence of multidrug-resistant bacterial pathogens poses a significant threat to public health, warranting a thorough understanding of the mechanisms underlying antibiotic resistance. One such pathogen, Pseudomonas aeruginosa, is a gram-negative bacterium known for causing severe infections. Multi-drug resistant strains have limited treatment options leading to increased mortality rates in individuals who are immunocompromised or suffering from chronic conditions. A critical component in the innate defence of Gram-negative bacteria against antibiotics is the Resistance-Nodulation-Division (RND) membrane transport protein. The primary RND transporter specific to P. aeruginosa is 'MexB' which is the inner membrane component of the MexAB-OprM complex and is responsible for substrate capture, binding and efflux. MexB has a high degree of polyspecificity allowing for the efflux of many different types of chemically distinct molecules including antibiotics. To understand the nature of MexB's polyspecificity, we have studied its structure, conformational plasticity, and ligand binding mechanisms via cryo-EM. We have achieved an unprecedented 2.16 Å resolution consensus structure of MexB, showing the protein adopts a canonical asymmetric trimer. Each subunit is in a different conformational state termed loose (L), tight (T) and open (O) as judged by the distance between the PC1 and PC2 sub-domains. The T-subunit harbours three DDM detergent molecules, a known substrate of MexB. Two are within the known proximal and distal binding sites, with the third in a not yet documented intermediate site. The appearance of molecules bound to these sites within the T-state is a novel finding and challenges the current conception that ligands likely transition from the proximal to distal binding sites during conformational transition from the L to T-state. 3D Variability Analysis (3DVA) shows MexB to be highly dynamic with several large-scale motions across multiple principal components observed. These conformational changes appear to show different subunits of the trimer transitioning between states which has been posited but never directly evidenced. Currently, we believe two additional states are seen in the 3DVA analysis, a resting (R) state, previously shown to be related to the O-state, and a new wide (W) state where the vestibule is further opened relative to the L-state, likely to facilitate capture of substrates with varying sizes. Finally, there are significant correlative motions of the transmembrane helices that accompanies opening of the vestibule. When the vestibule opens to achieve the W-state, much of the density related to the transmembrane helices disappears, likely indicating their transition to an unfolded state. This has bioenergetic implications for MexB as the purported proton transport mechanism resides within the transmembrane helical region. In summary, the findings herein offer a highly novel structural perspective into MexB's ligand binding modes and dynamic function. Further

comparative studies using clinically relevant antibiotics are to follow in view of elucidating the entire mechanistic cycle of polyspecific MexB efflux towards clinically relevant inhibitor design.

Speaker Abstracts

Speaker Abstracts

Name: **Ciara Wallis**

Theme: Membrane Transporters

Title: **The malaria parasite lactate/H+ symporter PfFNT: transporter or channel?**

Authors*: Ciara J F Wallis, Kasimir P Gregory, Ruitao Jin, Adele M Lehane, and Ben Corry*

The malaria parasite Plasmodium falciparum relies extensively on anaerobic glycolysis for energy production in the intraerythrocytic phase of its lifecycle. The parasites depend on their formate-nitrite transporter (PfFNT) to extrude lactate and protons, the major by-products of anaerobic glycolysis, from their cytosol to prevent lethal disruptions to cytosolic pH and cell volume. Unlike bacterial formate-nitrite transporters which are classified as channels due to a continuous pathway for substrate passage, PfFNT is thought to function as a transporter.

Cryo-EM structures show each PfFNT subunit contains a transport cavity containing a critical histidine residue (His230), bordered by hydrophobic constrictions on each side. However, it is not known whether charged lactate and protons are transported independently, together as neutral lactic acid, or if the species convert during transport. To determine this, we used extensive molecular dynamics simulations covering all potential protein and substrate protonation states. We found that binding only occurs between lactate and positively charged His230. As lactate binds tightly in the cavity, we suggest that charged His230 protonates lactate to lactic acid for the substrate to be released to the extracellular medium. This is supported both by quantum mechanics calculations, which show that proton transfer from His230 is favourable, and by simulations where the newly-formed lactic acid dissociates to the extracellular side.

Subsequently, we propose a proton-transfer mechanism as the mechanism of PfFNT transport. However, we show that no significant protein conformational change is required during the transport process, unlike what occurs in most transporters. As lactate does not have a continuous pathway entirely through the protein, we suggest that the requirement for proton-transfer is what allows PfFNT to be defined as a transporter rather than a channel.

Speaker Abstracts

Speaker Abstracts

Name: **Magdalena Plebanski**

Theme: Immunology

Title: **Ageing, cancer and immunity**

Authors*: Magdelena Plebanski*

The immune system changes with age, hence increased longevity brings with it an increase in diseases associated with a dysfunctional immune system, which include chronic inflammatory diseases, as well as increased susceptibility to infections and cancer. We study the immune system across the lifespan, accounting for sex and gender, to optimise the application of vaccines to prevent infectious diseases and cancer, as well as to develop personalised vaccines and immunotherapies engaging new nanoparticle properties. We are further committed to help women with ovarian cancer, the most lethal gynaecological malignancy, using systems immunology and biomarkers to enable much needed earlier diagnosis, novel drugs and personalised immunotherapies. We work hand-in-hand with nanoengineers to translate our biomarker findings into useful point-of-care devices capable of analysing new families of complex molecules and cells in liquid biopsies. Our work has progressed to large scale human trials, currently across over 20 hospitals in Australia.

Speaker Abstracts

Speaker Abstracts

Name: **Michael Gantier**

Theme: Immunology

Title: **3-base long 2'O-Methyl oligonucleotides are potent TLR7 and TLR8 modulators**

Authors*: Arwaf Alharbi, Sunil Sapkota, W. Samantha N. Jayasekara, Ruitao Jin, Josiah Bones, Mary Speir, Daniel Wenholz, Olivier F. Laczka, Ben Corry & Michael P. Gantier*

RNA therapeutics all rely on chemical modifications, which help to stabilise these molecules against nucleases and are paramount to their drug-like properties. Critically, such modifications are also essential to blunt activation of innate immune nucleic acid sensors by such RNA therapeutics, but a detailed mechanistic understanding of how this operates remains poorly defined.

We have now made the ground-breaking discovery that degradation fragments as short as three bases (3-mers) from 2'O-methyl-modified oligonucleotides can directly bind Toll-Like Receptor (TLR) 7 and TLR8 to impair their RNA sensing activity. Systematic analyses have identified the optimal 3-mer sequences and chemistries impacting TLR7/8 signalling. Functionally, select 3-mer oligonucleotides have divergent effects on TLR8 activity, allowing for both inhibition or potentiation in a sequence-dependent manner, while TLR7 sensing is strictly suppressed by immunoregulatory 3-mers. Critically, TLR7-inhibiting 3-mer oligonucleotides showed significant protection against systemic and topical TLR7-driven inflammation in vivo.

Collectively, our findings add to the understanding of TLR7/8 sensing of RNA, indicating a complex interplay between activating and inhibiting fragments according to base and sugar modification of their nucleotides. Our studies suggest that the distinction between self and non-self RNA by TLR7/8 relies on the competitive activities of 3-base long "immune codons", acting as agonists or antagonists. Finally, our work defines a novel class of ultra-short immunomodulatory oligonucleotides with therapeutic potential in inflammation and autoimmune diseases.

Speaker Abstracts

Speaker Abstracts

Name: **Si Ming Man**

Theme: Immunology

Title: **Inflammasome sensing in inflammation and cancer**

Authors*: Si Ming Man*

Inflammation is a fundamental process that maintains our health by protecting us from infection. However, excessive or chronic inflammation can cause pathology and promotes cancer. Inflammasome sensors activate immune signalling machineries to drive inflammation and cell death processes. However, other biological functions of these sensors remain poorly characterised. Here, we show that inflammasome sensors attenuate tumour development independently of canonical inflammasome signalling. Mechanistically, we show that inflammasome sensors interact with the DNA-damage sensing ATR-ATRIP complex to promote the recruitment of the checkpoint adaptor protein Claspin, and license the activation of the kinase CHK1. Genotoxic-induced activation of this protein complex drives the tumour-suppressing DNA damage response and attenuates the accumulation of DNA damage. These findings demonstrate a non-canonical function of inflammasome sensors in promoting the DNA damage response and mediating protection against cancer.

Speaker Abstracts

Speaker Abstracts

Name: **Dimitra Chatzileontiadou**

Theme: Immunology

Title: **A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection**

Authors*: Demetra S. M. Chatzileontiadou, Lawton D. Murdolo, Danillo G. Augusto, Joseph J. Sabatino Jr, Stephanie Gras, Jill A. Hollenbach*

Throughout the COVID-19 pandemic, research has centred on understanding why some people infected with the SARS-CoV-2 virus experience severe disease. However, 10-30% of individuals with the virus do not show any symptoms. Investigating asymptomatic infection could shed light on features of the immune system that help to eliminate SARS-CoV-2. Here, postulating that variation in the human leukocyte antigen (HLA) loci may underly processes mediating asymptomatic infection, we investigated the genetics of almost 30,000 registered bone marrow donors who participated in a voluntary program to track COVID-19 infection and symptoms1. Our analysis revealed a strong association between HLA-B*15:01 and asymptomatic infection, observed in two independent cohorts. Suggesting that this genetic association is due to pre-existing T cell immunity, we show that T cells from pre-pandemic samples from individuals carrying HLA-B*15:01 were reactive to the immunodominant SARS-CoV-2 Spike-derived peptide NQKLIANQF. The majority of the reactive T cells displayed a memory phenotype, were highly polyfunctional and were cross-reactive to a peptide derived from seasonal coronaviruses (NQKLIANAF). The crystal structure of HLA-B*15:01–peptide complexes demonstrates that the peptides NQKLIANQF and NQKLIANAF share a similar ability to be stabilized and presented by HLA-B*15:01. Finally, we show that the structural similarity of the peptides underpins T cell cross-reactivity of high-affinity public T cell receptors, providing the molecular basis for HLA-B*15:01-mediated pre-existing immunity1. Our findings have major implications for public health because such knowledge could inform the design and development of vaccines and therapies for COVID-19. More broadly, identifying genetic factors that control the course of the disease could begin to explain the wide variation in how people respond to SARS-CoV-2 and other viral infections.

Reference:

1 Augusto DG, Murdolo LD, Chatzileontiadou DSM, et al. A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection, Nature, 2023, 620(7972):128-136.

Speaker Abstracts

Speaker Abstracts

Name: **Marsia Gustiananda**

Theme: Immunology

Title: **Immunoinformatic prediction of SARS-CoV-2 T-cell epitopes and validation using ELISPOT and ELISA assays on the PBMC and serum samples from Indonesian people**

Authors*: Marsia Gustiananda, Michael Jonathan, Jyothsna Girish, Vivi Julietta, Sita Andarini, Yacine Maringer, Annika Nelde, Jaturong Sewatanon, Juliane Walz*

The presence of memory T-cells recognizing peptide epitopes from SARS-CoV-2 might protect us from severe diseases due to infection caused by the new variants of coronavirus. CD8+ and CD4+ T-cells recognize peptide epitopes that are presented by HLA Class I and II, respectively. CD8+ T-cells kill the infected cells and CD4+ T-cells orchestrate the immune responses by helping other immune cells such as B-cells to produce antibodies. Immunoinformatic analysis of the entire SARS-CoV-2 proteome of Wuhan Hu-1 was conducted using netMHCpan and netMHCIIpan to predict T-cell epitopes presented by HLA Class-I and Class-II alleles, respectively. As the aim is to study T-cell responses to SARS-CoV-2 in Indonesian population, the HLA alleles which have at least 5% frequency in the population were chosen. Peptides that fulfil 2% rank threshold as binder were checked for sequence conservancy in Delta and Omicron variants using IEDB conservancy analysis tool, and conserved peptides were chosen for synthesis. Several other criteria were applied such as cysteine containing peptides were not selected for synthesis and ORF1ab peptides that span the cleavage site of the 2 consecutive non-structural proteins were also not selected. The immunoinformatic analysis generated 111 peptides presented by HLA-Class I and 34 peptides presented by HLA Class II. Initial ELISPOT assay was conducted to test the immunogenicity of 10 HLA Class II peptides. PBMC and serum samples were collected from 6 study participants: 5 with history of SARS-CoV-2 infection and COVID-19 vaccination and 1 without prior infection nor vaccination. Prior to the ELISPOT assay, the PBMC samples were stimulated with peptides for 12 days to expand the number of T-cells that recognize the peptides. IL-2 were added at day 2, 5, and 7. Six peptides were positive in IFN gamma ELISPOT assay, indicating the presence of T-cell memory among the study participants. Three peptides are reported already in IEDB, while three others are new. Serum samples were tested for the presence of IgG anti nucleoprotein antibody. Anti-NP antibody was detected in all samples from individuals who had prior infection or vaccination using inactivated whole virus such as Sinovac. Not antibody nor T-cells were detected in individuals having no prior vaccination and infection.

Speaker Abstracts

Speaker Abstracts

Name: **Amber Willems-Jones**

Theme: Education

Title: **Perspectives of an Assessment Literacy Module from students and staff- a pilot study**

Authors*: Amber J Willems-Jones, Sarah Frankland, Melissa J Saligari, Saw Hoon Lim, Jennifer Fox, Angelina Fong, Claudia Munoz, Rosa McCarty & Piers Howe.*

Tension often exists between academics and students with regard to the clarity of instructions provided to students for their assessments. This often results in a disparity of outcome expectations leading to student dissatisfaction accompanied by complaints from the student that they followed the assignment task brief and marking criteria, yet did not achieve the scores that they expected. The Assessment Literacy Module (ALM), used in many subjects - including second- and third-year level Biochemistry & Molecular Biology - at The University of Melbourne, promotes the development of student evaluative judgement skills in relation to their assessment tasks. The module allows students to evaluate sample assignments as an assessor, utilising the provided assignment marking criteria to understand how each criterion relates to the assignment outcome. The process of using the ALM itself can highlight discrepancies in student academic judgement. We surveyed staff and students across multiple biomedical science subjects to gauge their perceptions of the impact of the ALM. Key feedback from the survey indicated that 46.7% of students typically looked at assignment rubrics before the introduction of the ALM. After using the ALM, 85.7% of students reported a better understanding of assessment criteria and 87.6% of students indicated they found using the ALM helpful in preparing their subsequent assessments. Sixty-nine percent of staff perceived that “students were able to use the feedback comments on the exemplars to better understand the assignment rubric”, and 61.5% of staff indicated that the ALM was also used to train tutors regarding assignment expectations to improve marking consistency. This presentation will highlight the benefits of the ALM in the development of skills associated with evaluative judgement, with a focus on how the ALM is used in Biochemistry-related subjects.

Speaker Abstracts

Speaker Abstracts

Name: **Matthew Clemson**

Theme: Education

Title: **Plagiarism and Generative AI-Resistant Online Practical Assessments in 2nd Year Biochemistry**

Authors*: Matthew Clemson, Gareth Denyer & Alice Huang*

Practical experience and laboratory-based learning activities are essential components of undergraduate biochemistry and molecular biology. Laboratory sessions enable students to explore key scientific techniques, to become familiar with specialised equipment, to record observations and measurements, interpret scientific data and to communicate with peers and instructors using the specific language and 'culture' of the discipline.

A substantial number of virtual laboratory tools currently exist, and some of these are available as open educational resources, but all have limitations that do not permit sufficient scope for enquiry-based learning within our specific context. Most are constrained to a limited number of scenarios, permit very limited deviation from predetermined (and often passive) protocols, and do not give students sufficient opportunity to influence the result outputs or to analyse, interpret and reflect upon unexpected or weak data.

Within our large cohort (n=765), 2nd year Biochemistry and Molecular Biology course there are two learning outcomes that have been particularly challenging to authentically assess. Namely, (1) to adapt, develop and trouble-shoot recognised procedures for novel contexts and requirements, and (2) to assess the quality of, interpret and draw conclusions from data obtained in the laboratory.

For their assessment task, students are challenged to take the procedures learnt in hands-on practical classes and to adapt these to measure the blood-alcohol concentration in an experiment performed online in a data generator. The gaming engine Unity was used for the development and the data generator program made available via https://garethdenyer.github.io/BCMB3X01/. The online experiment involves estimating the blood-ethanol concentration for a set of 'driver samples' and determining whether their suspect drivers are guilty of exceeding the legal 0.05% blood-alcohol concentration.

Students can test their experimental designs in an online environment without the constraints of time, resources and safety, to gather data and repeat experiments until they are confident with the accuracy and reproducibility of the results they have obtained. After performing biochemical calculations, students are tasked to reflect on their results, to evaluate the quality of their data and to troubleshoot several challenging situations (such as highly concentrated samples, high background absorbance and non-linear relationships).

The critical link between their experimental design, execution and the results output are retained in a way that is impossible when providing students with pre-existing or randomly generated datasets for analysis. In this way, students retain a sense of ownership over the data, and any results obtained are a consequence of their own design or action within the online data generator.

Each time an experiment is commenced, students are provided with a unique set of unknown samples, and these are linked to a unique ‘Run ID’ that is displayed during the experiment and recorded within the results output. Result outputs are consistent with the decisions made by students within the program. Students can work collaboratively to carry out the procedures, keep records of their actions, export results, perform calculations, interpret results, formulate conclusions and reflect on and troubleshoot their experimental approach.

Speaker Abstracts

Speaker Abstracts

Name: **Jacqui Matthews**

Theme: Education

Title: **Learning by design - combining experiment and data generation for senior undergraduate biochemistry student research projects.**

Authors*: Jacqui Matthews and Gareth Denyer*

The Laboratory Data Generator (LDG) is a Web-based program written using Unity, that was originally developed during COVID to fill gaps left by lack of access to laboratory classrooms. The LDG

allows productive struggle by incorporating a decision/consequence moderator. That is, the data generated reflects both the experimental design and implementation, in proportion to the importance of each step. There are no predefined outcomes. Rather relationships between components and the outcomes are defined, along with how that will be reflected in the readout (e.g. how many colony forming units post transformation, the migration and appearance of bands on a gel).

The LDG also has great potential for achieving student learning outcomes now that students are back in the laboratory. We have developed a project task for third year Biochemistry students based on a combination of a well-established practical laboratory experiment, and LDG modules that are programmed based on actual and extrapolated scientific data. Students design and carry out their own experiments, bypassing the need to generate and maintain a huge pool of experimental resources or running additional laboratory sessions.

The experimental practical laboratory is based on uncovering the molecular basis of human disease caused by a series of mutations in the transcription factor GATA1. It relies on yeast-two hybrid analysis (Y2H) to determine if the mutations affect binding to a protein partner, FOG, and electrophoretic mobility shift assays (EMSAs) to establish if GATA-DNA interactions are perturbed. Students learn the theory, perform, and analyze the class experiment over three laboratory sessions.

In the subsequent assessment task, they are challenged to come up with a research project to explore any aspect of GATA-FOG or GATA-DNA binding using Y2H and EMSA modules in the LDG. They can select from a wide range of GATA mutants (or propose other mutants), carry out the experiments to obtain data, and present their findings. The ability to design and test their own ideas creates a great opportunity for our third-year students to implement a range of skills and Biochemistry concepts.

Speaker Abstracts

Speaker Abstracts

Name: **Reece Sophocleou**

Theme: Education

Title: **Cells at War: A Global WIL-Based Partnership to Develop a Biology Game for Undergraduate Teaching**

Authors*: Reece Sophocleous (1), Jean-Paul Amore (2), Rosa Da Silva (3) and Tracey Kuit (1)*

1 School of Chemistry and Molecular Bioscience, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, New South Wales, Australia

2 School of Design, Center for Art, Design & Information Technology, George Brown College, Toronto, Ontario, Canada

3 Department of Biology, McMaster University, Hamilton, Ontario, Canada

In the rapidly changing landscape of higher education, the need for more engaging and interactive teaching models is increasing. Despite institutional encouragement to adopt technology-enhanced lessons and assessments, the effectiveness of such approaches is often hindered by limited resources, poor implementation, or negative perceptions among educators. Through an international collaboration between the University of Wollongong, McMaster University, and George Brown College, this project aimed to innovate undergraduate educational resource design, foster student employability skills, and enhance discipline-specific knowledge in students. Our interdisciplinary team developed a biology-based video game, "Cells at War: Cells vs. Type 1 Diabetes," as a pedagogical tool to enhance student engagement. Utilising the PEAR (Pedagogy, Experience, Assessment, Reflection) learning model and a Work-Integrated Learning (WIL) framework, the game serves as both an educational and a professional development exercise. Students from the University of Wollongong and McMaster University will evaluate the game through in-class trials and subsequent surveys (using both closed- and open-ended questions). Feedback will also be gathered from student partners involved in the game's design and development through focus group interviews. This pioneering WIL initiative will continue expanding the “Cells at War” game series, providing the frameworks to empower students and prepare them for future professional challenges, but also aims to set a new standard for curriculum innovation in biochemistry and molecular biology education.

Speaker Abstracts

Speaker Abstracts

Name: **Barry Pogson**

Theme: Plant Biochemistry

Title: **SynBio Cellular Signaling Circuitry and Gene-editing Solutions for Crops**

Authors*: Barry Pogson*

Is there reason for optimism about yield and food security against a backdrop of challenges led by climate change in light of the suite of emerging technologies from synthetic biology to gene editing? We have revealed new signaling pathways of cellular communication that drive changes in protein translation in response to oxidative stress. This includes changes to global loading onto ribosomes and 5- UTR motifs in mRNAs that alter rates of translation. One set of mRNA motifs and their interacting binding factor GAPDH, serve as integrators of energetic cues and RNA biology in context of retrograde signaling in cells. This set of modules and motifs for regulating chloroplasts proteins, alongside canonical stress sensing provides a new tool kit for SynBio optimisation of photosynthesis and resilience. Consequently, we are designing synthetic circuits for the sensing of oxidative stress, improved drought resilience and increased photosynthesis.

With respect to advances in gene editing the ARC Training Centre for Future Crops seeks to accelerate development of new technologies alongside contributing to (inter)national dialogues on the appropriate level of regulation and elucidating the attitudes of societies to genetic technologies. Consequently, our vision includes training a new generation of R&D leaders to be cognizant of the

need to co-design with industry and societies technologies that can deliver socially-responsible climate resilient crops for healthier foods, feeds and alternative fuels. Strategies include research into optimising CRSPR, tuning transformation technologies ranging from developmental regulators to nanotechnology. Progress, plans, constraints and opportunities for the Centre, the sector and society will also be presented.

Speaker Abstracts

Speaker Abstracts

Name: **Min Chen**

Theme: Plant Biochemistry

Title: **Biochemistry of photopigment and photopigment-binding protein complexes -- Heterodimer of allophycocyanin gives the most red-shifted absorption at 730 nm**

Authors*: Min Chen*

Phycobilisomes, the main light-harvesting complexes in cyanobacteria, absorb light ranging from green to red, where chlorophylls have poor absorbance. Allophycocyanin (Apc) is located in the core of phycobilisomes and functions as the energy terminal emitters intermediating the energy of bulk phycobilisomes to the chlorophyll-binding protein complexes in the photosynthetic membranes. Here, we present a newly isolated phycobiliprotein complex that absorbs red and far-red light. According to proteomic analysis, this special red-shifted allophycocyanin is encoded by gene ApcD4, which has 4 cys residues, the potential chromophore binding sites. We confirmed phycocyanobilin (PCB) is the chromophores covalent bound in the red-shifted allophycocyanin at to the conserved Cys78. Site-directed mutagenesis revealed that Cys 61 and Cys78 are essential for binding chromophore, although only one chromophore bound at Cys78. Additionally, the recombined chromophylated monomer ApcB2 showed absorption of 615 nm and the chromophylated monomer ApcD4 has a red-shifted absorption of 688 nm. Interestingly, the heterodimer of ApcD4/ApcB2 demonstrated absorption of 730 nm and fluorescence emission peak at 742 nm. These red-shifted phycobiliprotein complexes were also isolated from the chlorophyll f-containing cyanobacterium, Halomicronema hongdechloris, grown under far-red light conditions. This observation indicated that heterodimer of ApcD4/B2 likely functions as energy terminal emitter for Chl f-binding protein complexes. Spectral analysis of dissociated and denatured phycobiliprotein complexes show that the same bilin chromophore PCB is exclusively used in ApcB2 and ApcD4, suggesting that the redshifted absorption features are due to altered states of this chromophore within the protein complexes. These new red-shifted phycobilisomes have implications for flexible strategies in chromatic acclimation, which would improve light-harvesting efficiency. The biochemical mechanism of red-shifted spectral feature will be discussed.

Speaker Abstracts

Speaker Abstracts

Name: **Peter Solomon**

Theme: Plant Biochemistry

Title: **Exploiting the dual functionality of the Tox3 effector to dissect plant immunity**

Authors*: Peter Solomon*

It had long been thought that necrotrophic plant pathogenic fungi use a barrage of lytic enzymes to break down host cells releasing nutrients for growth. However, in recent years it has emerged that some necrotrophic fungi facilitate disease through a strict gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen Parastagonospora nodorum, the basis of this host specific interaction is small cysteine-rich effector proteins secreted during infection (ToxA, Tox1 and Tox3). These effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, whilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood.

To characterise these necrotrophic effectors, a search for potential host protein binding partners for the Tox3 effector was conducted. From this work, the wheat TaPR1-1 protein was validated through three independent approaches to interact with Tox3. We have now generated high-resolution crystal structures of several PR-1 proteins as well Tox3 and have used these these dissect the basis and function of this protein interaction.

In this talk I will present our latest findings on dissecting the dual functionality of the Tox3 effector protein. Together with its function in causing cell death through its interaction with Snn3, we demonstrate that Tox3 has an important role in mediating PR-1 defence signalling and is required for disease development. Furthermore, I will discuss new and exciting data on how PR1 proteins function and contribute to disease resistance. Collectively, these data have not only significantly advanced our understanding of necrotrophic diseases, but also provided a rare insight into the function and mechanism of the enigmatic plant PR-1 proteins.

Speaker Abstracts

Speaker Abstracts

Name: **Megan Maher**

Theme: Plant Biochemistry

Title: **Novel insecticidal proteins from ferns resemble insecticidal proteins from Bacillus thuringiensis.**

Authors*: Megan J. Maher, Jun-Zhi Wei, Narayanan Eswar and Marilyn A. Anderson.*

Lepidopterans affect crop production worldwide. The use of transgenes encoding insecticidal proteins from Bacillus thuringiensis (Bt) in crop plants is a well-established technology that enhances protection against lepidopteran larvae. Concerns about widespread field-evolved resistance to Bt proteins have highlighted an urgent need for new insecticidal proteins with different modes or sites of action.

We discovered a new family of insecticidal proteins from ferns. The prototype protein from Pteris species (order Polypodiales) and variants from two other orders of ferns, Shizaeales and Ophioglossales, were effective against important lepidopteran pests of corn and soybean in diet-based assays. Transgenic corn and soybean plants producing these proteins were more resistant to insect damage than controls.

This presentation will describe the structural and functional characterisation of these fern insecticidal proteins, including the crystal structure of a variant of the prototype to 1.98 Å resolution. Remarkably, despite being derived from plants, the structure resembles those of Cry proteins from Bt (a bacterium), but has only two out of three of their characteristic domains. These structural differences may be linked to differences in sites or modes of action of these fern proteins versus those from Bt. Accordingly, two of the fern proteins were effective against lepidopterans that were resistant to Bt 3-domain Cry proteins.

This therefore represents a novel family of insecticidal proteins that have the potential to provide future novel tools for pest control.

Speaker Abstracts

Speaker Abstracts

Name: **Hilary Hunt**

Theme: Plant Biochemistry

Title: **A role for fermentation in aerobic tissues?**

Authors*: Hilary Hunt*

Roots play an essential role in the physiology of higher plants. Much work has been done characterising the structure and composition of roots in order to understand and optimise their role in plant growth. However, the internal biochemistry and metabolic roles of different root cell types are hard to discern experimentally. Building on a structurally accurate model of root cell growth and measured respiration, cell composition, and soil uptake rates, we have constructed a computational model of the metabolism in growing lateral maize roots to better understand the roles of the many cell types involved. In doing so we discovered a distinct metabolic pattern in cells of the cortex, endodermis, and pericycle that differs from the epidermal cells; and a model preference, but not obligation, for aerobic fermentation that matched metabolite analysis of root exudates.

Speaker Abstracts

Speaker Abstracts

Name: **Bostjan Kobe**

Theme: Structural Biology

Title: **Innate immunity signalling across phyla: open-ended assemblies and enzyme activities by TIR domains**

Authors*: Bostjan Kobe, Jeffrey D. Nanson, Mohammad K. Manik, Sulin Li, Weixi Gu, Mengqi Pan, Yan Li, Timothy W. Muusse, Parimala R Vajjhala, Katryn J. Stacey, Susannah Holmes, Connie Darmanin, Max T. B. Clabbers, Hongyi Xu, Yun Shi, Thomas Ve*

TIR (Toll/interleukin-1 receptor) domains are widely distributed in animals, plants and bacteria, and function through self-association and homotypic interactions with other TIR domains [1]. Across phyla, these domains feature in proteins with immune functions - TLRs (Toll-like receptors), IL-1Rs (interleukin-1 receptors) and their adaptor proteins in animals; NLRs (nucleotide-binding, leucine-rich repeat receptors) in plants; and antiphage defence proteins in bacteria. Although long assumed to only have protein interaction functions, the TIR domains across kingdoms also feature self-association-dependent enzymatic activities, namely cleavage of nucleotides such as NAD+ [2,3]. We used an integrated structural biology approach to characterize the signalosomes formed by different TIR domains. We reconstituted large assemblies of the TLR/adaptor TIR domains (not known to have enzymatic activities); the structures of the filamentous assemblies of the TIR domains of TLR adaptor MAL [4], TRAM and the TLR4:MAL complex (unpublished) were determined by cryo-electron microscopy (cryoEM) helical reconstruction, and the structures of crystalline arrays of MyD88 were determined by micro-electron diffraction and serial femtosecond crystallography [5]. We further stabilized the active assemblies of enzyme TIR domains from the mammalian protein SARM1 (involved in axon degeneration; octameric complexes) [6] and the bacterial protein AbTir from Acinetobacter baumanii [3] (filamentous assemblies) with NAD+ mimics and determined their structures using single-particle cryoEM and helical reconstruction, respectively. We found that all these TIR domain assemblies feature a head-to-tail arrangement of TIR molecules, with the enzyme active site located in the interface between two molecules, explaining the requirement for self-association in enzyme activity. However, such head-to-tail row of molecules in stabilized by another row associating in an antiparallel fashion in enzyme assemblies such as those from SARM1 and plant NLRs, and in a parallel fashion in both scaffold assemblies (in TLR adaptors) and bacterial enzyme assemblies. In all cases, we validated the observed interactions by structure-guided mutagenesis and functional assays (e.g. [7]). The products of enzymatic reactions have downstream signalling functions in immune pathways or their suppression. Our studies will form the foundation of applications ranging from the treatment of inflammatory disorders and bacterial infections in humans to the prevention of plant diseases.

[1] Nimma et al & Kobe (2021) Front Immunol 12, 784484

[2] Horsefield et al & Kobe (2019) Science 365, 793

[3] Manik et al & Kobe (2022) Science, eadc8969

[4] Ve et al & Kobe (2017) Nat Struct Mol Biol 24, 743

[5] Clabbers et al & Ve (2021) Nat Commun 12, 2578

[6] Shi et al & Ve (2022) Mol Cell 82, 1643

[7] Muusse et al. & Stacey (2022) J Biol Chem, 102666

Speaker Abstracts

Speaker Abstracts

Name: **Rosemary Cater**

Theme: Structural Biology

Title: **Structural and Mechanistic Insights into Nutrient Transport at the Blood Brain Barrier**

Authors*: Rosemary J Cater, Eva Gil Iturbe, Dibyanti Mukherjee, Ting Chen, Matthias Quick, Thomas Arnold, Filippo Mancia*

The human brain comprises approximately 400 miles of vasculature. These cerebral blood vessels serve two critical functions: supplying the brain with oxygen and other important nutrients, and protecting it from toxins through properties known collectively as the blood-brain barrier. Blood vessels in the brain are formed through a process called sprouting angiogenesis, which begins early in central nervous system development and continues throughout life. Despite their importance, the molecular mechanisms underlying cerebral angiogenesis are not well understood, and there is a strong need for insights into these fundamental biological processes since cerebrovascular malformations contribute to the pathologies of various neurological diseases.

In humans, mutations in the poorly studied gene FLVCR2 (also known as MFSD7C, SLC49A2, and CCT) cause Proliferative Vasculopathy and Hydranencephaly Hydrocephalus (PVHH; OMIM 225790), a rare genetic form of hydrocephalus that is associated with gross brain vascular malformations. Recent studies have shown that FLVCR2 is specifically expressed in the plasma membranes of brain endothelial cells throughout development and into adulthood, and that inactivation of FLVCR2 in these cells severely impairs brain vascular growth. These studies highlight FLVCR2 and its substrate as central regulators of cerebral angiogenesis, although the molecular mechanisms underlying this regulation are still unknown.

The molecular function of FLVCR2 is controversial. FLVCR2 and it's close relative FLVCR1 are member of the major facilitator superfamily of transporter. Previous studies have implicated both of these proteins as putative heme transporters, however other recent studies have demonstrated that FLVCR1 is in fact a choline transporter. Whether FLVCR2 is a choline transporter or not remains unknown. Here we demonstrate that FLVCR2 is indeed a choline transporter, and present the 2.7 Å resolution structure of FLVCR2 with choline bound in the central cavity. These findings identify choline as a key regulator of angiogenesis, which poses several pressing questions regarding the cellular mechanism at play during cerebral angiogenesis.

Speaker Abstracts

Speaker Abstracts

Name: **John Bruning**

Theme: Structural Biology

Title: **Defining the Structural Mechanism of PPARG Modulators**

Authors*: Rebecca L. Frkic and John B. Bruning*

Synthetic full agonists of PPARγ have been prescribed for the treatment of diabetes due to their ability to regulate glucose homeostasis and insulin sensitization. While the use of full agonists of PPARγ has been hampered due to severe side effects, partial agonists, antagonists, and inverse agonists have shown promise due to their decreased incidence of such side effects in preclinical models. No kinetic information has been forthcoming in regard to the mechanism of full versus partial agonism of PPARγ to date and little structural and dynamic information is available which can shed light on the mechanistic difference between full and partial agonists as well as antagonists. We have used X-ray crystallography, cellular assays, Hydrogen Deuterium Exchange (HDX), and Surface Plasmon Resonance (SPR) to probe the mechanism of several PPARγ small molecule modulators to uncover PPARγ structural mechanisms and to aid in structure guided drug design. Our findings demonstrate that not only do partial agonists and inverse agonists/antagonists act through distinct transcriptional mechanisms, they also demonstrate differences in structure, dynamics, and kinetics as compared to full agonists.

Speaker Abstracts

Speaker Abstracts

Name: **Joshua Hardy**

Theme: Structural Biology

Title: **The architecture of the baculovirus nucleocapsid**

Authors:

Joshua M. Hardy1,2, Bronte A. Johnstone2*, Jungmin Ha2*, Anamarija Butkovic3, Paulina Koszalka2, Cathy Accurso2, Hariprasad Venugopal4, Alex de Marco2, Mart Krupovic3, Fasséli Coulibaly2*

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Baculoviruses are ubiquitous insect viruses that are fundamental to medical research and biotechnology, and are used as gene expression vectors in laboratories around the world. However, Baculoviridae is one of the few viral families for which the architecture of the capsid is unknown. In addition to the major nucleocapsid protein VP39 which forms the helical tube containing the genome, at least a dozen other proteins have been found associated with the capsid, possibly in the base and cap structures located at either end. Using cryo-electron microscopy, we have revealed the architecture of the prototypical AcMNPV baculovirus nucleocapsid including the tube, base, and cap components.

The ambiguous helical symmetry of the nucleocapsid tube has thwarted structure determination for years. To solve the symmetry parameters, we used a combination of subtomogram averaging, Fourier-Bessel indexing of layer lines, and real-space symmetry searches. A C14 averaged reconstruction with the correct helical symmetry reached 4.3 Å, however, the resolution was limited due to distortions from a cylindrical shape. Symmetry expansion and localised reconstruction improved the resolution to 3.1 Å, revealing a dimeric subunit with a cross-linked network of disulphide bonds that stabilise the capsid against the internal pressures of the genome.

Reconstruction of the base and cap complexes, to 4.1 Å and 6.3 Å respectively, and docking of AlphaFold2 models, revealed a 126-mer hub at both ends composed of 6 different proteins. Additional proteins involved in assembly and intracellular trafficking decorate the base hub, and the cap has an elaborate structure resembling a viral DNA portal. Almost all known eukaryotic viruses

have been classified into six viral realms according to the conservation of hallmark genes. Our structure-based phylogenetic analysis supports the proposal of baculoviruses as the representatives of a seventh viral realm with a separate origin from other viruses.

Speaker Abstracts

Speaker Abstracts

Name: **Qian Guo**

Theme: Structural Biology

Title: **Structural basis for coupling of the WASH subunit Fam21 with the SNX27-Retromer endosomal trafficking complex**

Authors*: Qian Guo, Kai-En Chen, Manuel Gimenez-Andres, Adam P Jellett, Ya Gao, Boris Simonetti, Meihan Liu, Chris M Danson, Kate J Heesom, Peter J Cullen & Brett M Collins.*

Endosomal protein sorting controls the homeostasis of essential proteins and lipids and is mediated by specific protein coats as well as the formation of endosome-associated actin filaments. The Retromer complex transports hundreds of transmembrane cargos in coordination with cargo adaptors from the sorting nexin (SNX) family including SNX27. Each of these interact with the WASH complex, an endosome-associated activator of Arp2/3 nucleation of branched actin networks that facilitate Retromer trafficking. Here, we define how the Fam21 (WASH2) subunit of WASH interacts with both Retromer and SNX27 using different mechanisms, but via the same repeat sequences in its extended C-terminal domain. Crystal structures, modelling, biochemical and cellular experiments show that SNX27 binds acidic-Asp-Leu-Phe (aDLF) repeat sequences similar to those found in other endosomal trafficking proteins SNX1 and SNX2. The same repeats, as well as a specific Pro-Leu containing motif in Fam21 bind three distinct sites on Retromer involving both the VPS35 and VPS29 subunits of the complex. These studies establish the specific molecular basis for how Retromer and SNX27 are coupled to the WASH complex via overlapping and multiplexed motif-based interactions essential for the dynamic assembly of endosomal membrane recycling domains.

Speaker Abstracts

Speaker Abstracts

Name: **Mike Haydon**

Theme: Signalling and Cell Biology

Title: **ROS around the clock: metabolic signals in the plant circadian clock**

Authors*: Xiang Li, Ángela Román, Robert Albiston, Mike Haydon*

Rhythmic metabolism is an integrated feature of the plant circadian system. Photosynthetic metabolism is regulated by the circadian clock and metabolic signals also provide feedback to the circadian oscillator. Sugars produced form photosynthesis adjust period and amplitude of circadian gene expression and contribute to the process of entrainment. Indeed, sugars can reinitiate circadian rhythms in dark-adapted Arabidopsis seedlings. Defining sugar signalling components in plants is challenging because it is difficult to separate the influence of sugar and light in photoautrophs, and because mutants in major sugar signalling components are lethal. To identify factors required for the activation of circadian gene expression by sugars, we used an RNA-seq timecourse and a chemical screen in dark-adapted seedlings. From the combination of these experiments, we discovered that superoxide is elevated by sucrose and contributes promote expression of evening-active circadian clock genes and plant growth. Chemicals identified from the screen suggest sugar-activated superoxide is generated by NADPH oxidases and triggers Ca2+ signalling at the plasma membrane. Our data suggest that rhythmic accumulation of superoxide acts as a metabolic signal at dusk, which adjust circadian rhythms and plant growth.

Speaker Abstracts

Speaker Abstracts

Name: **Hannah Brown**

Theme: Signalling and Cell Biology

Title: **Two archaeal tubulin-like proteins coordinate cell shape changes and assembly of motility machinery**

Authors*: Hannah J. Brown, Iain G. Duggin*

The tubulin superfamily (TSF) is comprised of tubulins, FtsZs, and CetZs, which share a common fold and the ability to polymerise and depolymerise in a GTP-dependent manner. These proteins are abundant in almost all cell types and play a wide range of structural roles. In Eukaryotes, tubulin monomers assemble to form microtubules, which dynamically organise cell structure and shape. FtsZs, which are abundant in both Bacteria and Archaea, assemble into a Z-ring at mid-cell for cell division. CetZs are unique to the Archaeal domain and have been implicated in cell shape and motility. Despite being highly diverse and abundant across Archaea, CetZs are understudied compared to tubulin and FtsZ, and their basic biological functions are still being uncovered.

The model archaeon Halofereax volcanii, is a shape-shifting and motile halophilic archaeon which has and six CetZs. CetZ1 and CetZ2 are distinct paralogues and are the most highly conserved subgroups of CetZs across archaea. During the early exponential phase of growth, H. volcanii transitions from plate-shaped (discoid) cells to rod-shaped cells in a process controlled by CetZ1. These rod-shaped cells are also motile and have assembled motility structures including chemotaxis arrays and archaella (equivalent to the bacterial flagellum), which are positioned at cell poles by oscillating Min system proteins. Here we show that Min proteins differentially control the positioning of CetZ1 at either cell poles or at mid-cell. Polar CetZ1 may act as a scaffold for the assembly of the motility machinery, suggesting that it can carry out multiple roles in H. volcanii.

We also found that CetZ2 has a distinct function from that of CetZ1. CetZ2 is strongly upregulated in stationary phase and helps maintain the plate-shape of these cells, by interfering with CetZ1 function. This is likely to be via a direct interaction and could also be aided by other stationary phase-specific factors. This work is the first demonstration of a novel system for the coordination of cell shape and motility in H. volcanii and we anticipate that this system may be utilised by other pleomorphic archaea. The duplication and functional specialisation that we have discovered in archaeal CetZs is reminiscent of the coordinated activities of multiple tubulins in eukaryotes, which are thought to have evolved from the single FtsZ in bacteria and the last universal common ancestor.

Speaker Abstracts

Speaker Abstracts

Name: **Joshua Hamey**

Theme: Signalling and Cell Biology

Title: **Systematic analysis of post-translational modifications in yeast and human ribosomes reveals extensive heterogeneity**

Authors*: Joshua J. Hamey, Tara Bartolec, Marc R. Wilkins*

Protein translation is intricately controlled within the eukaryotic cell. While previously thought to be controlled exclusively through translation factors, it has recently emerged that the ribosome itself is important for translational regulation. Ribosome composition is highly varied within cells, a phenomenon called ribosome heterogeneity, and this is known to regulate selective mRNA translation. However, the contribution of protein post-translational modifications (PTMs) to ribosome heterogeneity remains poorly understood. Here we have combined ribosome profiling through Ribo-Mega SEC (size exclusion chromatography) with mass spectrometry to systematically profile ribosomal PTMs in Saccharomyces cerevisiae and human K562 cells. Ribo-Mega SEC allowed separation of distinct pools of ribosomes, from translationally active polysomes to unincorporated subunits, for downstream mass spectrometric analysis. Through use of multiple different proteases for protein digestion, we identified every yeast and human ribosomal protein (RP) except RPL41, with peptides covering every single residue for the vast majority of RPs. This includes paralogous pairs of RPs that differ by only a few residues. We identified and quantified 12 methylation sites on yeast RPs and six methylation sites on human RPs, confirming that these are all present on actively translating ribosomes. In both yeast and human, half of the methylation sites were found to be substoichiometric on polysomes, indicating that these methylation sites contribute to ribosome heterogeneity. Through phosphopeptide enrichment, we identified over 100 phosphorylation sites on actively translating yeast ribosomes. Remarkably, most of these were found on the exterior of the ribosome, suggesting they may be actively regulated on intact ribosomes. We also identified several other PTMs on ribosomal proteins, including acetylation and ubiquitination. Lastly, quantification of paralogous RPs revealed that some pairs of paralogs are differentially incorporated into different populations of ribosomes. Together, our results reveal that PTMs contribute significantly to ribosome heterogeneity and provide a foundation for detailed studies on the roles of PTMs in translational regulation.

Speaker Abstracts

Speaker Abstracts

Name: **Adam Perriman**

Theme: Biotechnology/Synthetic Biology

Title: **: Engineered Living Materials: From artificial membrane binding proteins to 3D bioprinted tissue constructs**

Authors*: Adam Perriman*

Reengineering cells to operate proactively in unnatural biological environments invariably involves the assembly of multiple components, which can only be integrated when compatible interfaces are built into the design. In practice, this can be achieved through the synthesis of hybrid materials comprising highly cooperative biological and synthetic parts that can be used to attenuate cell-host tissue interactions, drive protein self-assembly, and provide rudimentary extracellular matrices for 3D bioprinting. The systems methodology that underpins this design approach provides a gateway to the development of non-traditional approaches to tissue engineering and regenerative medicine. Accordingly, I describe an emerging research programme that spans the fields of synthetic biology, biomaterials, and regenerative medicine. Here, artificial cell plasma membrane binding protein constructs are synthesised using a twostep process: protein supercharging to give a supercationic species; followed by the electrostatic assembly of a polymer surfactant corona. Significantly, the resulting constructs spontaneously assemble at the plasma membrane of human mesenchymal stem cells, providing resistance to hypoxia during tissue engineering.1 Moreover, the methodology can be readily adapted to display a modified thrombin on stems cell, giving rise to self-contained plasma membrane nucleated hydrogels,2 or utilised to produce bacterial adhesin fusion constructs that direct stem cells to the myocardium.3 1. Armstrong, J. P. K.; Shakur, R.; Horne, J. P.; Dickinson, S. C.; Armstrong, C. T.; Lau, K.; Kadiwala, J.; Lowe, R.; Seddon, A.; Mann, S.; Anderson, J. L. R.; Perriman, A. W.; Hollander, A. P., Artificial membrane-binding proteins stimulate oxygenation of stem cells during engineering of large cartilage tissue. Nature Communications 2015, 6. 2. Deller, R. C.; Richardson, T.; Richardson, R.; Bevan, L.; Zampetakis, I.; Scarpa, F.; Perriman, A. W., Artificial cell membrane binding thrombin constructs drive in situ fibrin hydrogel formation. Nature Communications 2019, 10. 3. Xiao, W. J.; Green, T. I. P.; Liang, X. W.; Delint, R. C.; Perry, G.; Roberts, M. S.; Le Vay, K.; Back, C. R.; Ascione, R.; Wang, H. L.; Race, P. R.; Perriman, A. W., Designer artificial membrane binding proteins to direct stem cells to the myocardium. Chemical Science 2019, 10 (32), 7610-7618.

Speaker Abstracts

Speaker Abstracts

Name: **Shogo Nakano**

Theme: Biotechnology/Synthetic Biology

Title: **Design of Ancestral Sortase E that can apply to synthesize protein materials**

Authors*: Shogo Nakano*

Protein conjugation techniques hold promise for the synthesis of next-generation biomaterials, including modified antibodies and immobilized enzymes. In this study, we report the successful design of a protein conjugate enzyme, ancestral sortase E (AcSE5). This Ca 2+ -independent cysteine transpeptidase catalyzes the conjugation of acyl donors containing a C-terminal LAXTG peptide (termed “sortag”) with acyl acceptors that have an N-terminal GGG peptide. Biochemical analysis revealed that AcSE5 possesses advantageous properties for conjugation, demonstrating a k cat /K m value 1.7 times higher than those of conventional sortases, with a conjugation efficiency reaching 70%. Notably, AcSE5 exhibits broad substrate selectivity towards acyl donors, capable of conjugating various alkylamines. Due to its high activity and efficiency, AcSE5 is applicable for modified antibody synthesis. In this study, we generated two antibodies: a Venus-labeled antibody and a dual antibody conjugated via PEG diamine. Furthermore, AcSE5 is applicable for enzyme immobilization. By reacting the sortagged hyperthermostable L-amino acid oxidase (HTAncLAAO2 1,2), AcSE5, and polyaminized beads, we successfully synthesized HTAncLAAO2-immobilized beads, which can be utilized as catalysts for the deracemization of racemic Phe derivatives to their D-forms. Collectively, our results affirm AcSE5’s suitability for protein biomaterial synthesis.

Speaker Abstracts

Speaker Abstracts

Name: **Ranjith Meemanage Cebreco**

Theme: Biotechnology/Synthetic Biology

Title: **Bacterial System for the Creation of Functional Chimeric Sensor Histidine Kinases**

Authors*: Ranjith Meemanage Cebreco*

Fiona Whelan

Keith Shearwin

Recent advances in synthetic biology have included the creation of novel biosensors. One of the initial steps required, is the characterisation of biological molecules with a ligand "sensing" mechanism. A rich source of such sensory molecules are bacteria, which express three large families of modular sensory proteins, known as Allosteric Transcription Factors (ATFs), Sensor Histidine Kinases (SHK) and Methyl-accepting Chemotaxis Proteins (MCPs). Of these, ATFs are generally well characterised with some examples commonly utilised as inducible genetic switches in creation of synthetic gene networks. In contrast, SHKs and MCPs can be more difficult to characterise, as SHK operate as a two-component system requiring a secondary protein knowns as a Response Regulator (RR), while MCPs have no cognate genetic output. One strategy which has been used to identify activating ligands of MCPs, though in a very limited manner, is the use of 'chimeric SHKs'. This required the creation of a novel fusion protein between an MCP-SHK pair (SHK-SHK fusions are also possible). In this research, we aim to develop screening capability to detect functional sensor fusion proteins, enabling large scale identification of activating ligands of uncharacterised MCPs and SHK. Using an engineered E. coli chassis, we have generated a proof-of-principle whole cell biosensor incorporating the E. coli osmolarity sensor SHK EnvZ fused to the aspartate sensing MCP Tar (S. typhimurium). The main impediment to generation of functional chimeric sensors is that the fusion point between the component proteins is not generalisable amongst all MCPs and SHKs, necessitating generations of chimeric libraries for selection of function. For library screening, we have incorporated a negative module to eliminate 'cheaters'; and a positive fluorescent reporter selection round, culminating in a quantitative colorimetric assay to determine ligand sensitivity. Using this system we have created a novel Aspartate sensing chimeric SHK by the fusion of the aspartate sensor domain of MCP Tar and the histidine kinase potion of SHK EnvZ. The novel protein was able to increase reporter activity 7-fold in the presence of L-Aspartate compared to controls. We now aim to generate novel chimeric sensors to build a library of functional biosensors as exemplars to model and predict functional fusion points for new chimeras.

Speaker Abstracts

Speaker Abstracts

Name: **Alexandra Williams**

Theme: Biotechnology/Synthetic Biology

Title: **High-throughput optimisation of protein secretion in yeast**

Authors*: Alexandra Williams, Joseph Brock*

We have been developing and testing a universal G-protein receptor (GPCR) coupled based biosensor for yeast protein secretion. We have devised a system by which any gene of interest can be screened for secretion efficiency in S. cerevisiae using high a highly diverse signal peptide library and an auto-cleavable peptide tag, in combination with a library of promoters and terminators. Combinatorial assembly is enabled by one pot golden gate cloning. This library can then be transformed into an engineered yeast strain, developed by the Ellis Lab (Imperial College, London), in which the S. cerevisiae GPCR signal transduction pathway has been heavily refactored to activate a reporter gene. This decouples receptor activation from the normal physiological responses to this signalling pathway activation and drives activation of a fluorescent reporter gene. Since the auto-cleavable tag is a GPCR peptide agonist, protein secretion is tightly coupled to reporter gene expression. Colony fluorescence is therefore a reliable proxy of secretion efficiency following transformation. We have validated our library with quantitative immunoblotting and a novel k-mer based algorithm of individual nanopore reads, to ensure roughly equimolar representation of all ~6000 combinations. We also present preliminary work in which we replace yEFGP with an anti-myotic resistance gene, effectively coupling protein secretion to cell viability. This survival-based selection enables use of a genome wide multiplexed CRISPRai screen for strain engineering. We will also discuss our research into portability of this system to higher yielding protein secretion strains such as Kluyveromyces lactis.

Speaker Abstracts

Speaker Abstracts

Name: **Vi Wickramasinghe**

Theme: Cancer

Title: **mRNA export is a new therapeutic vulnerability in cancer**

Authors*: Vihandha Wickramasinghe*

Messenger RNA (or mRNA) is part of the central dogma of biology. RNA is enjoying a renaissance in both biology and medicine due to the efficacy of mRNA based therapeutics in treating COVID and holds tremendous promise for treating many diseases, including cancer. Export of mRNA from the nucleus to the cytoplasm is a critical step in gene expression. We have identified that mRNA export factors are systematically amplified in cancer. Our findings suggest that non-essential components of the mRNA export machinery are potential therapeutic targets. Furthermore, targeting mRNA export may increase the efficacy of transcription inhibitors. We will describe our work in developing nuclear mRNA targeting therapies to treat cancer. These have the potential to be an exciting new frontier in cancer therapeutics.

Speaker Abstracts

Speaker Abstracts

Name: **Omer Gilan**

Theme: Cancer

Title: **DOT1L-mediated memory prevents polycomb silencing essential for menin inhibitor efficacy**

Authors*: Daniel Neville, Daniel Ferguson, Emily Heikamp, Graham Magor, Kathy Knezevic, Laure Talarmain, Enid Lam, Charles Bell, Andrew Perkins, Scott Armstrong, Omer Gilan*

The DOT1L enzyme is hijacked by MLL-Fusion oncoproteins to aberrantly deposit H3K79methylation at key target genes to drive leukemogenesis. While DOT1L activity is essential in these aggressive leukaemias, the mechanistic basis for this remains unresolved. Using functional genomics, we uncovered that DOT1L functionally antagonises the PRC1.1 complex to maintain transcription of critical MLL-Fusion target genes. Loss of the PRC1.1 subunits, PCGF1 or BCOR, confers resistance to DOT1L and/or Menin inhibition by preventing down-regulation of critical genes, differentiation, and apoptosis of murine and human MLL-leukaemia cells in vitro and in vivo. We find that Menin inhibition results in the PRC1.1-dependent selective induction of H2AK119ub and H3K27me3 at key MLL-Fusion target genes whilst DOT1L inhibition causes a widespread increase in H2AK119ub. Consistent with these findings, we demonstrate that the induction of H2AK119ub following treatment is specifically associated with loss of H3K79me2 rather than eviction of the MLL-Fusion protein or reduction in transcription. In addition, temporal analysis of chromatin and transcriptional changes revealed that the deposition of H2AK119ub is delayed and correlated with the gradual loss of H3K79me2, suggesting a direct functional antagonism between PRC1.1 and DOT1L. Upon this switch in chromatin modifications, polycomb target genes undergo stable repression leading to the irreversible commitment of leukaemia cells towards differentiation and apoptosis, even after early drug withdrawal. Taken together, our model establishes a fundamental and conserved function for DOT1L and the slow turnover of H3K79methylation in protecting genes from rapid PRC1-mediated repression. This epigenetic silencing is essential for the efficacy of differentiation therapies and highlights why DOT1L is hijacked in MLL leukaemia with potential implications for future therapeutic strategies using Menin inhibitors.

Speaker Abstracts

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Name: **Naisana Seyedasli**

Theme: Cancer

Title: **Single cell analysis assigns key roles to cellular plasticity and distinct DNA repair signatures in platinum-resistant ovarian cancer cells**

Authors*: George Joun, Yue Cao, James Cornwell, Anna deFazio, Jean Yang, Mohit Kumar Jolly, Naisana Seyedasli*

Platinum-resistance is a major confounding factor in the clinical management of ovarian cancer resulting in close to 70% rate of relapse of treatment-resistant disease. In this study, we have used patient-derived cells from the Australian Ovarian Cancer Study to highlight the cellular and molecular dynamics of high grade serous ovarian cancer cells during the course of platinum treatment. Using an array of techniques, including live single cell tracking and single cell sequencing, combined with quantitative proteomics, we have demonstrated inherent cellular and molecular patterns of response to platinum, stratified along the resistance spectrum. Tracking of fluorescent-tagged ovarian cancer cells with the cell cycle reporter, FUCCI, clearly demonstrated distinct patterns of cell cycle plasticity among clonal lineages with varied levels of platinum response. Further, single cell sequencing during the course of treatment, has highlighted key signatures within defined clusters with heightened response to platinum, with the remainder clusters demonstrating a clear resistance to treatment. Quantitative proteomics of chromatin-bound protein fractions in single cell-derived clones with varied response to platinum, further highlighted discrete signatures of arrest with key elements of DNA repair machinery and nuclear skeletal remodelling. Altogether, our findings provide a comprehensive single-cell resolution overview of cellular and molecular dynamics in high grade ovarian cancer cells during the course of platinum response. An image that will further elucidate the mechanisms behind development of resistance, providing biomarkers for molecular prognostics and targets for novel treatments.

Speaker Abstracts