GRIFFITH ECR CROSS-INSTITUTE SYMPOSIUM

Emerging Biomedical Technologies

9:00 am – 5:00 pm
Friday 5th November 2021
Nathan Campus N22 Theatre 1

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ACKNOWLEDGEMENTS

Griffith University acknowledges the Traditional Custodians of the land on which we are meeting and pays respect to the Elders, past and present, and extends that respect to all Aboriginal and Torres Strait Islander people.

The 2nd Griffith Cross-Institute Symposium organizing committee would like to extend their thanks to the Griffith students and staff for their participation and willingness to showcase their work. To the judges for their attendance, fairness and careful analysis of the research presented throughout the day – thank you.

Finally, we would like to take a moment to acknowledge and extend our thanks to Griffith Sciences, Griffith Health, Menzies Health Institute Queensland, Griffith Institute for Drug Discovery, Institute for Glycomics, Queensland Micro Nanotechnology centre, and our sponsors Bruker, Vaxxas, Rowe Scientific, invitro Technologies, Scope Scientific, John Morris Group and Eppendorf who have kindly provided financial support for this event. We would like to thank Griffith University for providing the venue.

We are extremely grateful for all of the support received, for without it we would not be able to host the event and provide lunches, tea breaks, awards and post-event celebrations.

THANK YOU

2018 event:

"Symposium"

Definition in English**:**

1. A **conference** or **meeting** to discuss a particular subject
2. A **collection of essays or papers** on a particular subject by a number of contributors
3. A **drinking party** or **convivial discussion**, especially held in Ancient Greece after a banquet

Origin:

Late 16th century (denoting a drinking party): via Latin from Greek *'sumposion'*; from *sumpotēs* 'fellow drinker', from *sun-* 'together' and *potēs* 'drinker'

Source: Oxford Dictionaries

2021 event:

"Technology"

Definition in English:

1. scientific **knowledge** used in **practical** ways in industry, for example in designing new machines
2. machines or equipment designed using technology

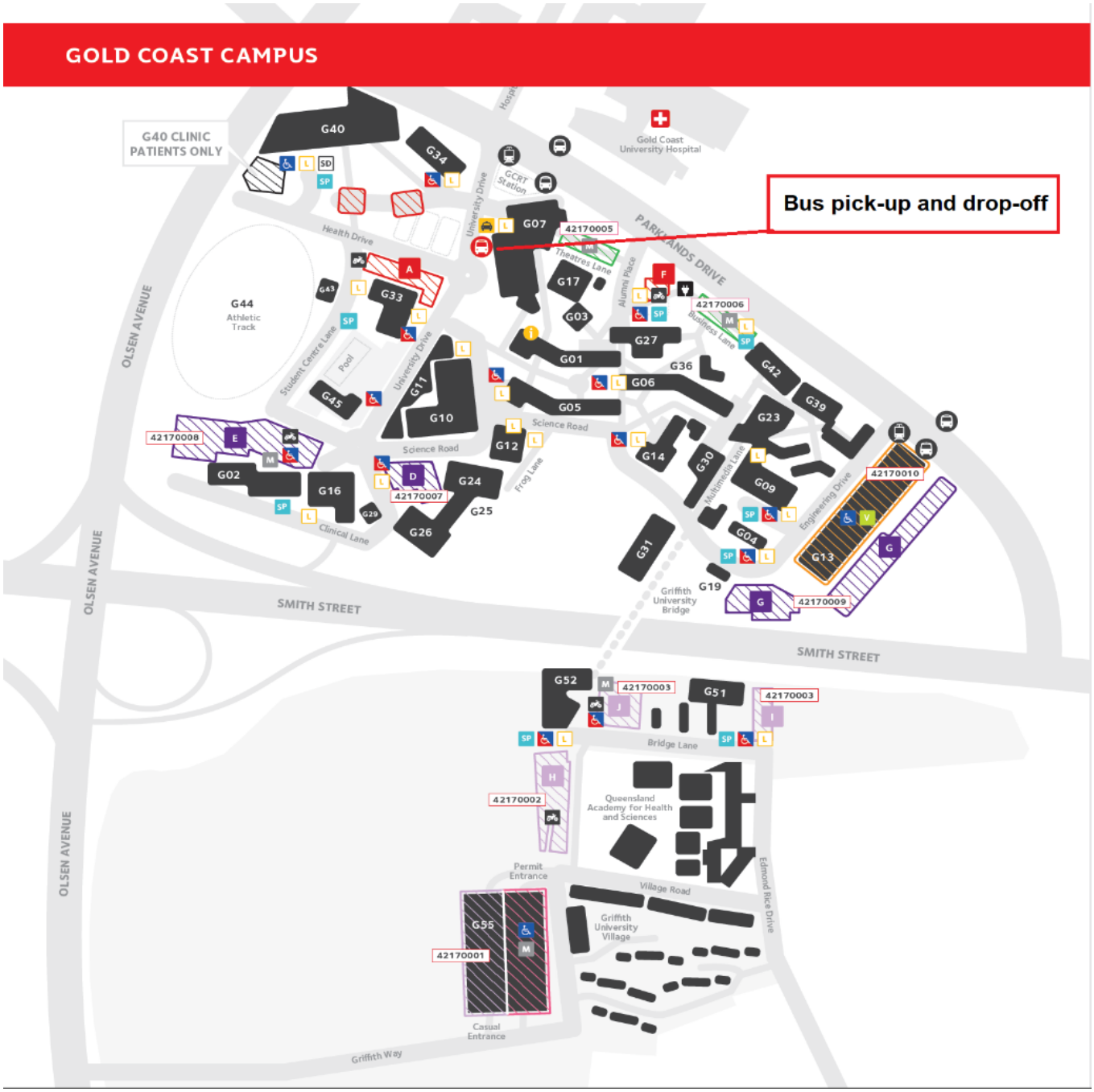
Origin:
Early 17th century from Greek *tekhnologia'* systematic treatment', from *tekhnē* '**art**, craft' + -logia (***sayings****, logy combining form denoting* field of study)

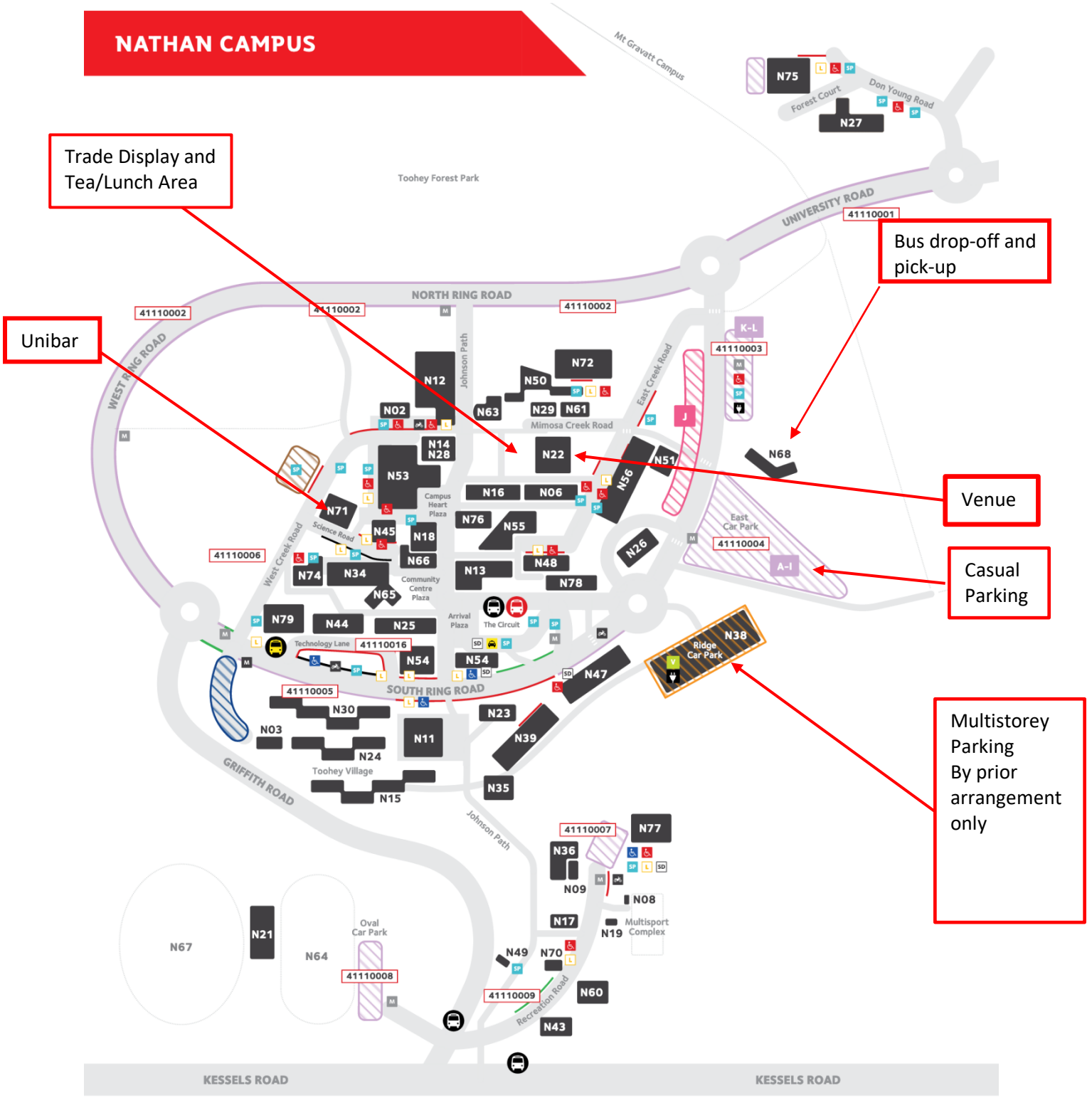
Please note, we welcome all the attendants to join us in discussing the **art** of applying our respective **knowledge** to result in **practical** solutions towards Biomedical advancements, **through the Networking and Social event which will be held at Unibar from 5:30pm onwards.**

A **bus service** for transportation between the Gold Coast and Nathan campuses is available.

Pick-up is at Griffith University Southport Campus, University Drive bus stop (opposite the Chancellery) at 08:00 am on 5th November 2021. The destination is the Griffith Uni Eco Centre, Nathan. Departure time from Nathan campus back to the Gold Coast campus will be 20:00 (8:00 pm).

GOLD COAST CAMPUS




NATHAN CAMPUS
Trade Display and Tea/Lunch Area
Unibar
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Venue
Casual Parking
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Mt Gravatt Campus
Toohey Forest Park
UNIVERSITY ROAD
NORTH RING ROAD
WEST RING ROAD
SOUTH RING ROAD
GRIFFITH ROAD
KESSELS ROAD
Johnson Path
Mimosa Creek Road
East Creek Road
West Creek Road
Science Road
Campus Heart Plaza
Community Centre Plaza
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The Circuit
Technology Lane
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Don Young Road
East Car Park
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Recreation Road
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Program

8:30 - 9:00	***Registration and morning tea***	
9:00 - 9:15	***Opening and Introduction***	
Session 1	***Title: Biomedical devices/Biomaterials Chair: Dr Belinda de Villiers***	
9:15 - 10:15	**O1: Plenary Speaker** Dr. Alain Wuethrich University of Queensland a.wuethrich@uq.edu.au	Some liquid biopsy nanodiagnostics for monitoring cancer and the human immune system
10:15 - 10:30	**O2:** Dr. Yun Shi Institute for Glycomics y.shi@griffith.edu.au	Structure, function, and inhibition of a therapeutic target against axon degeneration
10:30 - 10:45	**O3:** Dr. Navid Kashaninejad QMNC n.kashaninejad@griffith.edu.au	Design and fabrication of an integrated microfluidic concentration gradient generator for mechanical stimulation and drug delivery
10:45 - 11:00	***Morning tea***	
Session 2:	***Title: Biomedical devices/Biomaterials Chair: Dr Sharareh (Sherry) Eskandari***	
11:00 - 11:15	**O4**: Dr. Gayathri Thillaiyampalam GRIDD g.thillaiyampalam@griffith.edu.au	Discovering new drug targets to enhance innate immune response against blood cancers using connectivity mapping analysis
11:15 - 11:30	**O5**: Dr. Shuxiong Chen GRIDD shuxiong.chen@griffith.edu.au	Innovative particle platform for development of efficacious vaccines against infectious diseases
11:30 - 11:45	**O6:** Dr. Victoria Ozbek Institute for Glycomics v.ozberk@griffith.edu.au	Towards the development of a mucosal vaccine against Streptococcus pyogenes
11:45 – 12:00	**O7: Stephen.Wilson** **Bruker's recorded scientific presentation** Stephen.Wilson@bruker.com	Bottom-up and mid-down approaches for the sequence analysis of RNA
12:00 – 12:45	***Group Photo and Lunch***	
12:45 – 13:15	***Poster session 1***	
Session 3	***Title: Biomedical Science/Biomaterial Chair: Dr Andrew Rayfield***	
13:15 – 13:30	**O8:** Dr. Benjamin Bailly Institute for Glycomics b.bailly@griffith.edu.au	Development of disaccharide receptor mimetics as inhibitors of enterovirus A71 infection
13:30 – 13:45	**O9:** Dr. Greg Tram Institute for Glycomics g.tram@griffith.edu.au	The Acinetobacter baumannii autotransporter adhesin Ata recognises host glycans as high-affinity receptors.
13:45 – 14:00	**O10:** Dr. Bilal Zulfiqar GRIDD b.zulfiqar@griffith.edu.au	Identification of potent inhibitors of Leishmania donovani, and Leishmania infantum chagasi: causative agents of Old and New worlds visceral leishmaniasis.

14:00 - 14:15	**O11**: Dr. Sanaz Behtaj MHIQ, Clem Jones Centre for Neurobiology and Stem Cell Research s.behtaj@griffith.edu.au	A perspective on the structural requirements of nerve conduits in the peripheral nervous system
14:15 – 14:30	**O12**: Dr. Anu Chacko MHIQ, Clem Jones Centre for Neurobiology and Stem Cell Research a.chacko@griffith.edu.au	Chlamydia pneumoniae infects the brain via olfactory and trigeminal nerves and triggers Alzheimer's disease pathologies
14:30 – 14:45	**O13:** Dr. Gillian Fisher GRIDD g.fisher@griffith.edu.au	In vitro resistance selection and omics approaches for the identification of anti-plasmodial drug targets
14:45 – 15:00	**O14:** Roktima Tamuli GRIDD roktima.tamuli@griffithuni.edu.au	Chemical investigation of Clerodendrum polycephalum for antimalarial compounds
15:00 – 15:30	***Afternoon tea and poster session 2***	
Session 4	***Title: Biomedical research/Bioengineering*** ***Chair: Dr Hoang Phuong Phan***	
15:30 - 15:45	**O15**: Dr. Louise Sternicki GRIDD l.sternicki@griffith.edu.au	Native Mass Spectrometry for the study of PROTAC Ternary Complexes
15:45 – 16:00	**O16**: Dr. Jun Zhang QMNC jun.zhang@griffith.edu.au	Inertial microfluidics for cell separation
16:00 – 16:15	**O17:** Dr. Melissa Sykes GRIDD m.sykes@griffith.edu.au	Imaging methods to assess the impact of Trypanosoma cruzi strain on the activity and efficacy of compounds
16:15 – 16:30	**O18**: Dr. Hadieh Eslampanah Institute for Glycomics hadieh.eslampanahseyedi@griffithuni.edu.au	Human Galectin-8 Dynamic Characteristics & Sialylated Glycan-Binding Capacity Towards Development of a Novel Cancer Drug
16:30 – 16:45	**O19: Amanda Miotta** eResearch Support Service a.miotto@griffith.edu.au	Research technologies and resources available to you at Griffith
16:45 – 17:00	***Closing, prize presentation and photos***	
17:30 – 20:00	***Social networking***	

Poster presentations

Poster session 1	**13:00-13:30**
P1- Stretchable inertial microfluidics for isolation of cancer cells with large size distributions Hedieh Fallahi QMNC, Griffith University hedieh.fallahi@griffithuni.edu.au	
P2 - Molecular models of the Stratum Cornium lipid and permeant diffusion Afshin Zamani QMNC, Griffith University afshin.zamanizakaria@griffithuni.edu.au	
P3 - Streptococcus agalactiae infects glial cells and invades the central nervous system via olfactory and trigeminal nerves Dr. Ali Delbaz MHIQ, Menzies Health Institute Queensland, Clem Jones Centre for Neurobiology and Stem Cell Research a.delbaz@griffith.edu.au	
P4 - Interleukin-17 contributes to Ross River virus-induced arthritis and myositis Helen Mostafavi School of Pharmacy and Medical Sciences helen.mostafavi@griffithuni.edu.au	
P5 - Oncogene editing technology and immune activation to treat HPV driven oropharyngeal cancers Ana Maria Salinas Menzies Health Institute Queensland anamaria.salinasmontalvo@griffithuni.edu.au	
P6- Discovering novel compounds from multidrug resistant Pseudomonas aeruginosa and MRSA to treat brain infections Tejaswini Kalkundri and Dr. Indra Choudhury MHIQ tejaswini.kalkundri@griffithuni.edu.au	
P7 - Altered Spatial and Temporal Gait Parameters in Ross River Virus-Infected Mice Eranga Abeyratne Menzies Health Institute Queensland eranga.abeyratne@griffithuni.edu.au	
P8 - Comparison of transection-type and contusion-type spinal cord injury mouse models Dr Megha Shah Menzies Health Institute Queensland, Clem Jones Centre for Neurobiology and Stem Cell Research megha.shah@griffith.edu.au	
P9 - Development of a controlled infection immunisation (CII) strategy for a malaria vaccine Dr. Reshma Jayprakash Nevagi Institute for Glycomics r.nevagi@griffith.edu.au	
P10 - High-throughput split-protein profiling by combining transposon mutagenesis and regulated protein-protein interactions with deep sequencing Md. Solayman Institute for Glycomics md.solayman@griffithuni.edu.au	
P11 - Towards pan-flaviviral protease inhibitors Dr. Crystall Swarbrick Institute for Glycomics c.swarbrick@griffith.edu.au	

Poster session 2	**15:00-15:30**
P12 - Development and pre-clinical evaluation of a whole asexual blood-stage parasite malaria vaccine formulated with cationic liposomes Winter Okoth Institute for Glycomics winter.okoth@griffithuni.edu.au	
P13 - Antimicrobial resistance and bacterial oxidoreductases Guillaume Petit GRIDD guillaume.petit@griffithuni.edu.au	
P14 - Modulation on olfactory ensheathing cell migration by liraglutide in 2D and 3D environments Dr. Yu-Ting (Tammy) Tseng Menzies Health Institute Queensland, Clem Jones Centre for Neurobiology and Stem Cell Research t.tseng@griffith.edu.au	
P15 - Identification of hematoxylin and gallic acid as potential Nsp7 inhibitors of SARS-CoV-2 Yushu Gu GRIDD yushu.gu@griffithuni.edu.au	
P16 - Activity characterization and classification of anti-plasmodial compounds. Dr. Sandra Duffy GRIDD sandra.duffy@griffith.edu.au	
P17 - Characterising heat stress in cattle using NMR-based metabolomics Alexandra Gloria GRIDD a.gloria@griffith.edu.au	
P18 - The Glitazone Class of Drugs as Carbonic Anhydrase Inhibitors – A Spin-Off Discovery from Fragment Screening Sarah Mueller, Maria Halili GRIDD sarah.muller2@griffithuni.edu.au	
P19 - Antimicrobial Bacillus probiotics: metabolites and mechanisms Charlie Tran GRIDD Charlie.tran@griffithuni.edu.au	
P20 - Using Machine Learning on the Patient derived cell phenotypes to classify Schizophrenia (oral and poster) Dr Jamila Iqbal GRIDD j.iqbal@griffith.edu.au	
P21 - Evaluation of mitochondria as a potential target in cancer using 2D and 3D breast cancer models Dr. Kah Ni Tan GRIDD kahni.tan@griffith.edu.au	
P22 - Mimicking the Tumour Microenvironment - Integrating 3D into early drug discovery assays Dr. Elke Kaemmerer GRIDD e.kaemmerer@griffith.edu.au	
P23 - High-throughput drug screens – Image Analysis Dr. Tayner Rodriguez GRIDD, T.Rodriguez@griffith.edu.au	

P24. A structure-guided drug discovery approach towards developing novel neuraminidase inhibitors against influenza A virus

Olivia Tan, Institute for Glycomics, olivia.tanhui@griffithuni.edu.au

AWARDS

Research Excellence:

Oral Presentations:

1st Prize $400

2nd Prize $300

Poster Presentations:

1st Prize $250

2nd Prize $150

Audience participation prize: $100

This award is for a member of the audience who asks the best question(s) and engages with the speakers. We want to encourage questions and engagement from all participants, as ultimately this is what makes an exciting and interesting symposium.

Don't be afraid to ask anything!

Logo design competition: $250

There will be a QR code on each seat for access to the logo designs and voting form. On the day of event, you will be asked to vote for one of 6 delivered logo designs using a QR code. The design with the highest vote will be prized to its designer at the prize session and will be used for the promotion of future Griffith ECR Cross-Institute Symposia.

Thank you for voting on the future ECR GCI Symposium Logo.
The ECR GCI Symposium Logo should represent the core ideals of the Symposium, which includes:

building cross-institute collaborations,
championing early career researchers and
promoting biomedical sciences

The logo should be easily recognisable in small scale e.g. the size of a Twitter profile picture."

Which logo design do you select to be future logo of Griffith ECR Cross-Institute Symposium?

A)

B)

C)

D)

E)

F)

- ○ A
- ○ B
- ○ C
- ○ D
- ○ E
- ○ F

Griffith ECR symposium website:

Information about Griffith ECR symposium and program booklet is available on Griffith ECR symposium website. www.gcisymp.org

FOOD ALLERGEN STATEMENT

Please note that food provided at this symposium may contain traces of milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybeans amongst other known allergens. Consumption is at your own risk and Griffith University cannot be held responsible for adverse reactions.

There will be vegetarian options, free of gluten, lactose, dairy, nuts, passion fruit, ham, mushroom, or seafood. There will also be Halal options available, please speak to the organizing team for advice.

ORAL PRESENTATIONS
Session 1

O1: Plenary Speaker Oral presentation

Some liquid biopsy nanodiagnostics for monitoring cancer and the human immune system

Dr Alain Wuethrich

NHMRC Emerging Leadership fellow and DECRA awardee at the Centre for Personalised Nanomedicine, Australian Institute for Bioengineering and Nanotechnology, the University of Queensland (UQ).

Precision medicine is regarded as one of the most promising approaches to treat or even cure many severe diseases including cancer. Although precision medicine has delivered new and individualised treatment plans such as targeted therapy or immune checkpoint therapy, it has not yet lived up to its full promise. One reason that has limited the advancement of precision medicine is the requirement for a specific molecular profile to tailor the therapy. The creation of such a molecular profile is difficult and requires highly sensitive and specific technologies that can detect accurately multiple biomarkers in readily accessible biofluids. Nanomaterial- and nanostructured-based systems have attracted interest due to their unique physico-chemical properties that can be explored as nanodiagnostics for molecular profiling in precision medicine.
This presentation will highlight examples of nanodiagnostics for:
(1) Monitoring targeted therapy in melanoma
(2) Early detection of melanoma
(3) Tracking drug-induced epithelial–mesenchymal transition in breast cancer
(4) Monitoring the immune system with single cytokine resolution

O2: Structure, function, and inhibition of a therapeutic target against axon degeneration

Yun Shi[1], Philip S. Kerry[2], Jeffrey D. Nanson[3], Weixi Gu[3], Todd Bosanac[4], Tamim Mosaiab[1], Veronika Masic[1], Faith Rose[1], Stephanie Holt[1], Lauren Hartley-Tassell[1], Eduardo Vasquez[1], Bostjan Kobe[3], Robert O. Hughes[4], and Thomas Ve[1]

[1]Institute for Glycomics, Griffith University, Southport, QLD 4222, Australia.
[2]Evotec (UK) Ltd., 114 Innovation Drive, Milton Park, Abingdon, Oxfordshire OX14 4RZ, UK.
[3]School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience and Australian Infectious Diseases Research Centre, University of Queensland, QLD 4072, Australia.
[4]Disarm Therapeutics, a wholly-owned subsidiary of Eli Lilly & Co., Cambridge, MA, USA.

The NADase SARM1 (sterile alpha and TIR motif containing 1) is a key executioner of axon degeneration and a therapeutic target for several neurodegenerative conditions. We have characterised its catalytic functions and uncovered the molecular mechanism of a potent small-molecule inhibitor that was shown to assist recovery of injured axons. Employing an allosteric activator and an orthosteric inhibitor, we solved 3D structures of SARM1 protein in both its active and inactive states, revealing its activation mechanism. These discoveries led us to develop further small-molecule strategies against SARM1 that could result in better inhibition against axon degeneration.

O3: Design and fabrication of an integrated microfluidic concentration gradient generator for mechanical stimulation and drug delivery

Arash Yahyazadeh Shourabi[1], Mohammad Said Saidi[1], Navid Kashaninejad[2]
[1]Department of Mechanical Engineering, Sharif University of Technology, Tehran 11155, Iran:
[2]Queensland Micro- and Nanotechnology Centre, Griffith University, Nathan Campus, 170 Kessels Road, Brisbane, QLD 4111, Australia

Mechanical stimuli, including fluid shear stress, osmotic pressure gradient, and extracellular matrix stiffness, significantly affect cellular interactions with drugs in biological structures. This paper introduces an integrated concentration gradient generator (CGG) capable of providing cell monolayers with these stimuli and demonstrates its design, fabrication, and quantification procedures. The proposed multi-layer chip consists of a CGG integrated with a membrane-based cell culture chamber (MCCC) and two bubble trappers for removal of micro-bubbles. The CGG provides cultured cells in the MCCC with four different concentrations of desirable inlet drug/chemical reagents. The MCCC is able to impose adjustable shear stresses, as well as osmotic pressure gradients on cell monolayers. The stiffness of the extracellular matrix (ECM) is also accommodating by utilizing a proper membrane in the MCCC. A numerical simulation based on the finite element method (FEM) is employed to design and optimize the integrated device, and then, the chip's performance is quantified using the experimental data. Finally, the biocompatibility of the proposed device is investigated by dynamic culturing of human lung cancer cells (A549 cell line) on the chip.

ORAL PRESENTATIONS
Session 2

O4: Discovering new drug targets to enhance innate immune response against blood cancers using connectivity mapping analysis

Gayathri Thillaiyampalam [1], Karolina Bednarska [2], Sally Mujaj 4, Rohan A. Davis [1], Maher K. Gandhi 23, Alex S. Cristino [1]

1 Griffith Institute for Drug Discovery, Brisbane, Queensland
2 Mater Research, University of Queensland, Brisbane, QLD, Australia
3 Princess Alexandra Hospital, Brisbane, QLD, Australia
4 Queensland Institute of Medical Research, Brisbane, Queensland

Natural killer (NK)-cells are immune effector cells that have the ability to kill cancer cells without prior sensitization. A number of cell surface receptors and transcriptional factors are known to control NK-cell activation. However, little is known about targeting NK-cells for cancer immunotherapy. In this study, we aim to alter the function of NK-cells pharmacologically by identifying drugs that are targeting gene signatures associated with NK-cell activation. Firstly, we used human NK-cell lines as a model system to generate the gene signatures. Comparison between the transcriptome of resting NK-cells and two activation systems: direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity revealed that ~80% of the genes are upregulated during activation including the genes involved in Endoplasmic Reticulum (ER) stress, cell motility and checkpoint receptors. We then used this signature to screen for drug targets by applying connectivity mapping approach, a bioinformatics tool which analyses large perturbational datasets to accelerate drug discovery and repurposing. Our findings provide potential drug targets which can alter the gene signatures associated with NK-cell activation leading to enhanced NK-cell anti-tumoural function. This research may open up new avenues for NK-cell-based blood cancer immunotherapies.

O5: Innovative particle platform for development of efficacious vaccines against infectious diseases

Shuxiong Chen[1], Benjamin Evert[1], Adetayo Adeniyi[2], Mercè Salla-Martret[2], Linda H-L Lua[2], Victoria Ozberk[3], Manisha Pandey[3], Michael F. Good[3], Andreas Suhrbier[4], Peter Halfmann[5], Yoshihiro Kawaoka[5], Bernd H.A. Rehm[1, 6*]

[1]Centre for Cell Factories and Biopolymers, GRIDD, Griffith University, Nathan QLD 4111, Australia
[2]Protein Expression Facility, University of Queensland, Brisbane QLD 4072, Australia
[3]Institute for Glycomics, Griffith University, Gold Coast QLD 4215, Australia
[4] QIMR Berghofer Medical Research Institute, Brisbane QLD 4006, Australia
[5] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison Madison Wisconsin 53706, USA.
[6]Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia

Polyhydroxyalkanoic acids (PHAs) are naturally occurring bacterial polyesters which serve as a carbon and energy storage for bacteria. PHA synthase, PhaC, is the key enzyme required for PHA biosynthesis and PHA bioparticle self-assembly in the presence of excess carbon source, such as glucose. These PHA bioparticles are shell-core structures composed of a hydrophobic PHA core surrounded by proteins, such as PhaC. The PHA synthase PhaC remains covalently attached to the surface of PHA bioparticles after the self-assembly process and serves as an anchor protein for a variety of protein of interests. The utility of engineered PHA bioparticles has been demonstrated for applications in medicine and industry including protein purification, enzyme immobilization, diagnostics, and imaging. This talk will focus on applications of PHA bioparticles in vaccine development for induction of protective immunity against various infectious diseases, such as tuberculosis and/or SARS-CoV-2.

O6: Towards the development of a mucosal vaccine against Streptococcus pyogenes

Victoria Ozberk[1#], Mehfuz Zaman[1#], Sharareh Eskandari[1], Jamie-Lee Mills[1], Ainslie Calcutt[1], Emma L. Langshaw[1], Jessica Dooley[1], Ailin Lepletier[1], Yongbao Huo[1], Glen C. Ulett[2], Michael R. Batzloff[1], Michael F. Good[1*] and Manisha Pandey[1*]

[1]Institute for Glycomics, Griffith University, Gold Coast, Australia.
[2]Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia.
# These authors contributed equally to this work.

Background and Aims: Subunit vaccines with minimal epitopes are often poorly immunogenic and adjuvants are required to enhance and direct immune responses towards protective determinants. Most pathogens enter or colonise via the upper respiratory tract mucosa. Mucosally active subunit vaccines are an unmet clinical need due to lack of licensed immunostimulants suitable for peptide antigens. It is also critical for a vaccine to be able to stimulate both the humoral and cellular immune system to achieve long-lived protection.
Methods: Mice were immunised intranasally with liposomes incorporating: the Streptococcus pyogenes peptide antigen, J8; diphtheria toxoid as a source of T-cell help; and the immunostimulatory glycolipid, 3D(6-acyl) PHAD® (PHAD) - vaccine referred to as J8-Lipo-DT-PHAD. Antibody responses were assessed by ELISA and cytokine responses were analysed by cytokine bead array (flow cytometry). Gene knock-out mice were utilised to assess specific cell subsets required for vaccination and protection.
Results: We showed that intranasal administration of J8-Lipo-DT-PHAD, was able to induce both cellular and humoral immunity. We demonstrated long-lived humoral and cellular memory responses following vaccination. However, mice genetically deficient in either mucosal antibodies or total antibodies were protected against mucosal infection. By using IL-17-deficient mice or by depleting cellular subsets using antibody therapy, we showed that it is the cellular responses encompassing, CD4+ T-cells, IL-17 and macrophages that play the critical role in vaccine-mediated mucosal immunity.
Conclusions and Significance/Impact: Overall, the data demonstrate the utility of a novel mucosal vaccine platform to deliver multi-pronged protective responses against a highly virulent pathogen.

O7: Bottom-up and mid-down approaches for the sequence analysis of RNA

Bruker's recorded scientific presentation

Abstract is not available

ORAL PRESENTATIONS
Session 3

O8: Development of disaccharide receptor mimetics as inhibitors of enterovirus A71 infection

Benjamin Bailly, Gergely Pipa, Gael Martin, Daniel Earley, Chih-Wei Chang, Robin Thomson, Mark von Itzstein

Institute for Glycomics, Griffith University, Gold Coast, Australia

Enterovirus A71 is a major cause of hand, foot and mouth disease in children worldwide. While symptoms of disease are usually mild, the virus can sometimes cause severe neurological infections such as meningitis, encephalitis or flaccid paralysis. Despite the high socio-economic burden associated with EV71-A71-related hand, foot and mouth disease, no vaccines or drugs are yet available for prevention or treatment. It has been well established that the virus binds to glycosaminoglycans (GAG) such as heparan sulfate to initiate infection. We have recently taken advantage of this property and synthesised defined, densely sulfated disaccharide heparan sulfate analogues that efficiently blocked EV71 in vitro infection. We now have further expanded our structure-activity relationship studies to explore per-sulfated disaccharides templates made up of glycans not found in heparan sulfate, that provide improved virus binding affinity as well as synthetic feasibility. Importantly, as demonstrated by STD-NMR experiments, functionalisation of these disaccharides at the anomeric position with bulky and hydrophobic substituents further improves their binding footprint to the virus capsid, resulting in enhanced antiviral potency. Through cell-based mode of action studies and competition STD-NMR, we show that the receptor mimetics bind to the virus capsid to compete with cellular receptors and specifically block virus attachment to cells. We hereby present the first report of potent, glycan-based inhibitors of EV71-A71 infection. This study opens new ground for the future development of potent EV71-A71 binding inhibitors that can be used for the treatment of hand, foot and mouth disease.

O9: The Acinetobacter baumannii autotransporter adhesin Ata recognises host glycans as high-affinity receptors

Greg Tram[1], Jessica Poole[1], Felise G. Adams[2], Michael P. Jennings[1], Bart A. Eijkelkamp[2], John M. Atack[1]

[1]Institute for Glycomics, Griffith University, Gold Coast, Queensland 4215, Australia.
[2] Molecular Sciences & Technology, College of Science and Engineering, Flinders University, Sturt Road, Bedford Park, South Australia 5042, Australia

The opportunistic pathogen Acinetobacter baumannii is an organism which shows extensive resistance to traditional antibiotics and has been classified by the World Health organisation as a top priority pathogen for which new antimicrobials are urgently needed. Although many virulence factors have been studied in A. baumannii, little is known about the precise molecular interactions that occur between A. baumannii proteins and surface factors and the ligands they bind in the host. Many host-adapted pathogens utilise carbohydrates as molecular binding partners and A. baumannii has been shown to interact with heavily glycosylated host extracellular matrix (ECM) glycoproteins. A. baumannii expresses a trimeric autotransporter adhesin, Ata (Acinetobacter autotransporter adhesin), which we hypothesised would interact with host glycans, and therefore play a key role in adherence to the host. Using a range of glycobioanalytical techniques, we demonstrated high affinity binding of Ata to galactose, N-acetylglucosamine, and galactose (β1-3/4) N-acetylglucosamine. These structures are present on many human ECM glycoproteins including fibronectin. We demonstrated that Ata binds fibronectin, and that this interaction is completely dependent on fibronectin being glycosylated, indicating that the interaction between Ata and a major host ECM protein is mediated by interaction with these glycans. This is the first characterisation of the precise interactions that occur between an A. baumannii adhesin and the cognate host cell ligand. This is an important step in understanding host-pathogen interactions and the role glycans play in this process. Defining the range of host glycans that A. baumannii interacts with will enable development of novel antimicrobials to block these interactions and provide much needed new treatments for this significant human pathogen.

O10: Identification of potent inhibitors of Leishmania donovani, and Leishmania infantum chagasi: causative agents of Old and New worlds visceral leishmaniasis

Bilal Zulfiqar[1], Fabio Antonio Colombo[2], Juliana Barbosa Nunes[3], Patricia Ferreira Espuri[4], Aurea Favero Ferreira[3], Sujatha Manthri[5], Laura M. Alcantara[6], Carolina B. Moraes[6], Lucio H. Freitas-Junior[6], Manu De Rycker[5], Marcos Jose Marques[4] and Vicky M. Avery[1]

[1] Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, 4111, Australia.
[2] Department of Clinical and Toxicological Analysis, FCF, UNIFAL-MG, Brazil
[3] Department of Pathology, Medical School, USP, Brazil
[4] Department of Pathology and Parasitology, ICB, UNIFAL-MG, Brazil
[5] Drug Discovery Unit, University of Dundee, Dundee, UK.
[6] National Laboratory of Biosciences, National Center for Research in Energy and Materials (CNPEM) Campinas, Sao Paulo, Brazil.

The drug discovery pipeline for neglected kinetoplastid diseases remains sparse. In particular, the field of leishmaniasis drug discovery has had limited success in translating potential drug candidates into viable therapies. Here we describe the development of two lead compounds, which have potent in vitro anti-leishmanial activity against intracellular and extracellular forms of Leishmania parasite and are selective for various mammalian cells, based on which, they were selected for further characterization and biological profiling. In addition to the activity against L. donovani DD8 (Old World - Indian strain), these compounds also showed activity against intracellular parasites from other species and strains of the Old and New World, namely L. donovani (Old World - Sudanese strain) and L. infantum chagasi (New World). Tests in the hamster model illustrated that the activity observed in vitro for these compounds was translated in vivo, with outstanding results. Histopathological data confirms immuno-inflammatory patterns associated with infection and expression levels of both pro and anti-inflammatory cytokines were also assessed. Resistance studies are in progress to provide us an insight into potential resistance mechanism and thus an indication of the target. Our data suggests that further development of these compounds is warranted as they provide urgently needed starting points for the development of novel lead series for future anti-leishmanial therapeutics.

O11: A perspective on the structural requirements of nerve conduits in the peripheral nervous system

Sanaz Behtaj
Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Queensland, Australia;
Menzies Health Institute Queensland, Griffith University, Southport, Australia

Peripheral nerve injury is a frequent cause of lifelong impairments. The most clinically applied approaches to date have relied on surgical procedures, including autografts. However, the clinical success of the autograft is limited by a low functionality and mismatching between the damaged and donor nerves. Therefore, the disease will continue to be a serious public health problem with over one million cases worldwide annually. Artificial conduits have been shown to afford to be an alternative to autografts. These conduits are used to bridge the gap between the proximal and distal ends of a damaged nerve via orienting axonal growth in an organised fashion. However, mechanical trauma, fistula formation, extrusion, and inflammatory reaction caused by the conduit rigidity are fundamental challenges of these conduits. As such, an appropriate conduit must have a closely similar structure to the peripheral nerve (PNS) and proper biomimetic features. This presentation aims to discusses the main challenges that need to be addressed to develop and apply these nerve conduits in clinical practice. It also describes some promising solutions to these challenges, so far, have shown to promote neural regeneration in peripheral nervous system injuries.

O12: Chlamydia pneumoniae infects the brain via olfactory and trigeminal nerves and triggers Alzheimer's disease pathologies

Anu Chacko[1],Ali Delbaz[1], Heidi Walkden[1], Souptik Basu[1], Charles W Armitage[3], Tanja Eindorf[1], Logan K Trim[4], Edith Miller[1], James A St John[1,2],Kenneth W Beagley[4], Jenny A.K Ekberg[1,2]

[1]Menzies Health Institute Queensland, Griffith University, Queensland
[2]Griffith Institute for Drug Discovery, Griffith University, Queensland
[3]School of Immunology and Microbial Sciences, King's College London, United Kingdom.
[4]Centre for Immunology and Infection Control, School of Biomedical Sciences, Queensland University of Technology, Queensland

Background: Chlamydia pneumoniae is a respiratory tract pathogen but can also infect the central nervous system (CNS). Recently, the link between C. pneumoniae CNS infection and Alzheimer's disease has become increasingly evident. In mice, C. pneumoniae infection of the CNS has been shown to occur weeks to months after intranasal inoculation, but the path of infection has not been determined. However, other bacteria are known to rapidly (within 1-2 days) infect the brain via the olfactory and trigeminal nerves after intranasal inoculation. Understanding the path of C. pneumoniae infection and the cellular and molecular responses may reveal whether C. pneumoniae contributes to Alzheimer's disease.

Method: We investigated whether C. pneumoniae could invade the CNS via the olfactory and/or trigeminal nerves in mice, and if this resulted in any alterations in Aβ deposition or molecular pathways involved in Alzheimer's disease. We also determined whether injury to the nasal epithelium affected C. pneumoniae infection. Using in vitro cell cultures, we investigated whether C. pneumoniae could infect and survive in cultured primary mouse glial cells including olfactory ensheathing cells, trigeminal Schwann cells, astrocytes and microglia.

Results: By isolating live C. pneumoniae from tissues and using immunohistochemistry, we show that C. pneumoniae can infect the olfactory and trigeminal nerves, olfactory bulb and brain within 72 hours in mice. Injury to the nasal epithelium using a chemical insult resulted in increased peripheral, but decreased CNS infection. Amyloid beta accumulations were detected adjacent to C. pneumoniae in the olfactory system. 28 days after intranasal inoculation, analysis of gene expression at the transcriptional level revealed that multiple pathways associated with Alzheimer's disease were modulated. Examination of cellular responses to the bacteria using in vitro cultures revealed that C. pneumoniae was able to infect peripheral nerve and CNS glia.

Conclusion: The nerves extending between the nasal cavity and the brain constitute invasion paths by which C. pneumoniae can rapidly invade the CNS likely by surviving in glia, and leading to Aβ deposition and changes in gene expression associated with Alzheimer's disease.

O13: In vitro resistance selection and omics approaches for the identification of anti-plasmodial drug targets

Macdonald JR[1], Arnold MSJ[1], Ruth MR[2], Quinn RJ[1], Winzeler E[2], Marcus CS Lee[3], Skinner-Adams TS[1], and Andrews KT[1]* and Fisher GM[1]*

[1]Griffith Institute for Drug Discovery, Nathan, Queensland, Australia
[2]University of California, San Diego, USA
[3]Wellcome Sanger Institute, Cambridge, UK
* Co-senior authors

Malaria, caused by Plasmodium parasites, continues to be a devastating global health issue, causing 409,000 deaths and 229 million cases in 2019. Due to the burden of malaria and the lack of a broadly effective vaccine, antimalarial drugs are heavily relied upon for treatment and prevention. However, malaria parasite resistance has emerged to all currently used antimalarial drugs, driving the discovery and development of new drugs, those with novel modes of action to limit cross-resistance with existing drugs. Although antimalarial drug leads can advance through the drug discovery pipeline without any knowledge of their target, target identification studies can provide important information for the development of hit anti-plasmodial compounds. Recent reductions in the cost of whole genome sequencing have begun to yield some exciting progress in the antimalarial target identification arena. When combined with selection of drug resistant malaria parasite lines, this approach has resulted in the identification of new anti-plasmodial targets and resistance mechanisms. Using this approach, we have identified potential mechanisms of action/resistance associated with several novel anti-plasmodial compounds. This approach will be discussed along with how it was instrumental in identifying a novel resistance mechanism associated with the antiplasmodial activity of a natural product compound.

O14: Chemical investigation of Clerodendrum polycephalum for antimalarial compounds

Roktima Tamuli[1], Jacinta R. Macdonald[1], Gillian M. Fisher[1], Yunjiang Feng[1]

[1]Griffith Institute for Drug Discovery, Griffith University, Nathan, QLD

Clerodendrum from family Lamiaceae (Verbenaceae) is spread around the world and known for its ethnomedicinal properties. Clerodendrum polycephalum grown in Nigeria has been traditionally used for malaria treatment. Extract of the leaves tested for in-vivo antimalarial activity showed better chemo-suppressive, and prophylactic activity against standard drug chloroquine and pyrimethamine, respectively. Curative assay has also been good, and extract showed no toxic effects.[1] However, no compounds have been reported for anti-plasmodial activity of the extract. So, isolation, purification, and structure elucidation of active compounds is the focus of this project. Chromatographic separation, HPLC purification, LC-MS, and 1D-/2D-NMR led to the isolation of eleven pure compounds, including five known, namely, acacetin, loliolide, methyl-pheophorbide a, abietane diterpene and bis(2-ethylhexyl) phthalate,5 new diterpenes and one new aromatic compound. Isolated compounds were tested against Plasmodium falciparum with methyl pheophorbide a showing an IC50 of 4.49 micromolar. Our results supported the traditional use of the plant as anti-malarial agent.

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ORAL PRESENTATIONS
Session 4

O15: Native Mass Spectrometry for the study of PROTAC Ternary Complexes

L. M. Sternicki[1], J. Nonomiya[2], M. Liu[1], M. M. Mulvihill[2], R. J. Quinn[1]

[1]Griffith Institute for Drug Discovery, Griffith University, Australia
[2]Genentech Inc., United States of America

PROteolysis TArgeting Chimeras (PROTACs) initiate target degradation as a mechanism for therapeutic treatment. Bifunctional PROTAC molecules simultaneously bind both the target protein and an E3-Ubiquitin ligase, bringing these two proteins into close spatial proximity to promote target protein ubiquitinylation and degradation via the cell's endogenous protein degradation pathways. Native mass spectrometry (MS) was utilised to study ternary complexes promoted by the previously reported PROTAC GNE-987 between Brd4 bromodomains 1 and 2, and Von Hippel Lindau (VHL) E3-Ubiquitin Ligase. Brd4 is a validated drug target for many cancers, whilst VHL has routinely been recruited by PROTACs for the efficacious in vitro and in vivo degradation of many different target proteins. High resolution native MS, utilising a non-modified SolariX 12T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker), allowed further characterisation of GNE-987 ternary complexes. Native MS allowed 1) direct measurement of non-adducted ternary complex formation, 2) accurate mass measurements to confirm correct ternary complex stoichiometry, 3) single measurement detection of all species present at equilibrium (including apo-subunits and binary PROTAC-interactions) to understand target and ligase engagement, and the balance between binary and ternary interactions, and 4) semi-quantify PROTAC ternary complex strength and stability based on relative MS intensities. Higher ratios of ternary complex were formed at lower PROTAC concentrations when GNE-987 engaged Brd4 bromodomain 1 compared to bromodomain 2, revealing the complex with bromodomain 1 had increased affinity and/or stability. This supported previous literature where surface plasmon resonance ternary complex half-life measurements revealed Brd4 bromodomain 1 ternary complex was more stable with an approximately 100-fold longer half-life. This study highlights native MS as a direct screening method to measure ternary complexes for PROTAC development.

O16: Inertial microfluidics for cell separation

Jun Zhang

Queensland Micro- and Nanotechnology Centre, Griffith University, Nathan Campus, 170 Kessels Road, Brisbane, QLD 4111, Australia

Particle separation is indispensable in many microfluidic systems and holds a broad range of biomedical applications. Inertial microfluidic devices that work solely on intrinsic hydrodynamic forces and inertial effects can offer label-free, high throughput and high-efficiency separation performance. Out work specifically studied the fundamental mechanism of inertial focusing in serpentine microchannels and explore their applications. Regarding the fundamental study, we discovered that particle focusing positions could shift from channel sidewalls to the centreline with increasing Reynolds number and this transformation is highly sensitive to particle size. we developed a competition theory model for particles focusing and experimentally investigated the effects of particle size, Reynolds number and particle inertia, channel dimension on the focusing process [1-3]. Regarding the applied research, we applied the discovered differential focusing phenomenon for many biomedical applications. For example, we developed inertial microfluidic platforms for blood cell separation[4-6], nerve cell sorting[7], and isolation of circulating tumour cells [8]. Furthermore, we explored a new concept of inertial microfluidics: multiphysics inertial microfluidics. We implemented the physical coupling of dielectrophoresis (DEP) and inertial lift forces for real-time tuneable particle manipulation[9,10]. We developed an analytical theory to explain the physics coupling of DEP and inertial focusing in curvilinear channels. Finally, I will discuss some perspectives on the future direction of inertial microfluidics.

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O17: Imaging methods to assess the impact of Trypanosoma cruzi strain on the activity and efficacy of compounds

Melissa Sykes[1] and Vicky Avery[1]

[1] Discovery Biology, GRIDD Institute, Griffith University, Nathan, Queensland, Australia

Chagas disease, caused by the kinetoplastid parasite Trypanosoma cruzi results in approximately 12, 000 deaths per year. T. cruzi is endemic to 21 countries in the Americas, however incidence of the disease worldwide also occurs due to emigration. Treatment of Chagas disease is restricted to the nitroheterocyclic drugs benznidazole and nifurtimox, which both cause adverse side effects and show variable treatment efficacy. To identify compounds with improved safety and efficacy, T. cruzi early drug discovery campaigns frequently utilise a panel of laboratory adapted, geographically diverse parasite strains to aid the in vitro prioritisation of compounds. To investigate if commonly used T. cruzi laboratory strains may influence the activity of compounds, we utilised fluorescent imaging to determine the number of T. cruzi parasites in host cells and assess parasite survival. The sensitivity of a selection of compounds and drugs with known activity against the Tulahuen strain parasite were compared to a larger panel of T. cruzi strains. To reduce the influence of experimental variables on the outcome, the same multiplicity of infection was utilised for each strain. To determine the impact of the replication of strains on compound activity, fluorescence imaging was used to assess the incorporation of the DNA biomarker, 5-ethynyl-2'-deoxyuridine. Salvage of this nucleoside analogue into T. cruzi DNA is detected using click chemistry, by covalently linking incorporated structures to a fluorescently labelled azide. This is the first time that the replication of these strains has been directly compared using this method. Despite differences in strain geographical location thus potential genetic backgrounds, and observed differences in intracellular parasite replication, no significant differences in compound activity or efficacy were observed across strains. Therefore, the necessity of utilisation of T. cruzi laboratory strain panels in vitro should be questioned further.

O18: Human Galectin-8 Dynamic Characteristics & Sialylated Glycan-Binding Capacity Towards Development of a Novel Cancer Drug

Hadieh A. Eslampanah Seyedi[1], Lauren E. Hartley-Tassell[1], Helen Blancahrd[1,2], and Thomas Haselhorst[1]

[1.] Institute for Glycomics, Griffith University, Gold Coast Campus, QLD 4222, Australia.
[2.] School of Chemistry & Molecular Bioscience (SCMB), Faculty of Science, Medicine & Health, University of Wollongong, NSW 2522, Australia

Human galectin-8, as a glycan-binding protein, plays imperative roles in tumour cell metastasis, angiogenesis, immune and inflammatory response. Given its involvement in a variety of cancers, galectin-8 has been recognised as a potential target for drug discovery and development. From a structural perspective, galectin-8 binds diverse glycans, such as galactose and terminal sialosides like Sialyllactose, through two non-identical carbohydrate recognition domains (CRDs) that are joined by a peptide linker of various lengths determining the different isoforms of galectin-8. Despite galectin-8 relevance to cell function and pathogenesis, the characterisation of full-length galectin-8 isoforms and glycan binding behaviour has remained elusive. We have investigated the structure and binding of galectin-8's two full-length isoforms with the minimal binding moiety, Methyl N-acetyl-α-neuraminide (Neu5Acα2Me). We were able to identify a unique binding mode of Neu5Acα2Me, compared to its related trisaccharide Sialyllactose, whilst maintaining essential interactions. We found that Neu5Acα2Me mimics natural sialosides present on the cell surface and therefore provides an excellent lead structure for the development of novel galectin inhibitors that could lead to potential cancer drugs in the future.

Biochemical and biophysical analysis revealed galectin-8L's high tendency to self-associate mainly via its N-terminal CRD, while galectin-8M was mostly monomeric. Surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) assays disclosed the binding of galectin-8M to Neu5Acα2Me at KD of ~ 4 μM, but no binding was observed for galectin-8L. This lack of affinity could be directly correlated to galectin-8L's self-associate via N-CRD with picomolar affinity (~ 3.3 pM) and hence blocking the Neu5Acα2Me's binding site. The results described here, for the first time, suggest the differential role for N- and C-CRD with alteration of the linker size between galectin-8 isoforms. This shed light on the dynamic characteristics of the mechanism of carbohydrate recognition and the role of the linker-peptide among tandem-repeat galectins.

O19. Research technologies and resources available to you at Griffith

Amanda Mioto

Learn about the resources available to you as Griffith Researchers on Bioinformatics, Medical and Microscope imaging, working with sensitive data and bringing together technical information for grant writing.

Symposium ECR Menzies_Gly_GRIDD.pptx

POSTER PRESENTATIONS

P1: Stretchable inertial microfluidics for isolation of cancer cells with large size distributions

Hedieh Fallahi, Jun Zhang, Nam-Trung Nguyen

Queensland Micro- and Nanotechnology Centre, Griffith University, Nathan Campus, 170 Kessels Road, Brisbane, QLD 4111, Australia

Rare-cell isolation is an effective method for early detection and monitoring of cancer. Circulating tumour cells (CTCs) are valuable biomarkers for the diagnosis of metastatic cancer. These rare cells have an extremely low concentration of less than 10 cells per millilitre of blood, making detecting and evaluating them very challenging. In the last decade, inertial microfluidics has shown promising results in isolation of CTCs. Inertial microfluidics is a passive size-based separation technique with superiority to other separation methods in terms of simplicity, low cost, high throughput, and high efficiency. However, the current rigid design of inertial microfluidic devices with fixed geometries are not optimal for CTC detection and isolation. The sizes of CTCs vary widely and cannot be sorted with one rigid device that has a limited performance in terms of size range. Here we introduce a stretchable microfluidic device for isolation of cancer cells of varied size distributions with high separation thresholds. Stretchability brings about the possibility of dynamically adjusting the dimensions of the microchannels. This unique feature provides the size-tuneable separation with a high separation resolution. The cancer cells used in our study have similar sizes as the WBCs making it difficult to isolate them. Using the stretchable devices, cancer cells were isolated from WBCs with high recovery rates and purities.

P2: Molecular models of the Stratum Cornium lipid and permeant diffusion

Afshin Zamani Zakaria[1], Alpeshkumar Malde[2], Tim Gould[1], Owen Jepps[1,3,4]

[1]School of Environment and Science, Griffith University, Queensland 4111, Australia
[2]Institute for Glycomics, Griffith University, Southport, Queensland 4215, Australia
[3]Menzies Health Institute QLD, Griffith University,
[4]Griffith Institute for Drug Discovery, Griffith University

The outer-most layer of the human skin comprising of the flattened dead cells in the form of bricks surrounded by several lipidic bilayers. For most of the permeants, especially the hydrophilic ones, it is believed that the lipid bilayers act as barriers against molecular diffusion. Molecular understanding of the lipid conformation and arrangement is vital to understand the transverse and lateral permeability of the skin lipids. Here a molecular dynamics study of the lipid bilayers over different molar ratios of its constituents (Ceramide, Cholesterol, Free fatty acid) and their conformation had been conducted. Equilibration protocols were optimized to converge the output characteristics of the lipids. Molecular dynamics results agree with the conducted experiments in terms of area per lipid (APL). Furthermore, nematic order parameter, lipid thickness and density profiles of the lipid molecules are introduced as a result.

P3: Streptococcus agalactiae infects glial cells and invades the central nervous system via olfactory and trigeminal nerves

Delbaz A[1,2], Chacko A[1,2], Choudhury I [1,2], Eindorf T[1,2], Shah M[1,2], Godfrey C[1,2], Sullivan MJ[1], St John JA[1,2,3], Ulett GC[1], Ekberg JAK[1,2,3]

[1]Menzies Health Institute Queensland and School of Pharmacy and Medical Sciences, Griffith University, Queensland
[2]Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Queensland
[3]Griffith Institute for Drug Discovery, Griffith University, Queensland

Background: Streptococcus agalactiae causes neonatal meningitis and can also infect the adult central nervous system. S. agalactiae can cross the blood-brain barrier but may also reach the central nervous system via other paths. Several species of bacteria can directly invade the central nervous system via the olfactory and trigeminal nerves, which extend between the nasal cavity and brain and injury to the nasal epithelium can increase the risk/severity of infection. Preterm birth is associated with increased risk of S. agalactiae infection and with nasogastric tube feeding. The tubes, also used in adults, can cause nasal injuries and be contaminated with bacteria, including S. agalactiae.
Objective: To determine whether S. agalactiae could invade the central nervous system after intranasal inoculation in mice and to determine cellular responses to the bacteria.
Method: We investigated whether S. agalactiae serotype III, which is epidemiologically the most relevant in neonatal and adult Group B streptococcal meningitis, can infect the brain via the olfactory and/or trigeminal nerves after intranasal inoculation in mice. Because epithelial injury is associated with increased infection of the olfactory nerve, we also determined the effects of prior experimental injury to the nasal epithelium on S. agalactiae infection via this path. As the ability to infect glia is thought to be important for bacterial invasion of both cranial nerves and the brain, we also determined how the glial cells of the olfactory/trigeminal nerves and glia limitans layer responded to S. agalactiae.
Results: S. agalactiae rapidly infected the olfactory nerve and brain. Epithelial injury led to increased bacterial load in these tissues, as well as trigeminal nerve infection. S. agalactiae infected and survived intracellularly in cultured olfactory/trigeminal nerve- and brain-derived glia, resulting in cytokine production, with some differences between glial types. We also found that the S. agalactiae capsule significantly altered cytokine and chemokine responses and affected intracellular survival in trigeminal glia.
Conclusion: This study shows that S. agalactiae can infect the central nervous system via the nose-to-brain path with increased load after epithelial injury, and that the bacteria can survive in glia.

P4: Interleukin-17 contributes to Ross River virus-induced arthritis and myositis

Helen Mostafavi [1,2,3], Kothila Tharmarajah [1,2,3], Jelena Vider [2,4], Nicholas P. West [4], Joseph R. Freitas [1,2,3], Barbara Cameron [5], Paul S. Foster [6], Linda P. Hueston [7], Andrew R. Lloyd 5, Suresh Mahalingam [1,2,3] and Ali Zaid [1,2,3]

[1] Emerging Viruses, Inflammation and Therapeutics Group, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia.

[2] School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia

[3] Global Virus Network (GVN) Centre of Excellence in Arboviruses

[4] Mucosal Immunology Group, Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

[5] Viral immunology Systems Program, Kirby Institute, University of New South Wales, Australia.

[6] School of Biomedical Sciences, Faculty of Health Sciences and Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia.

[7] Arbovirus Emerging Diseases Unit, Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West - ICPMR Westmead, Australia.

Arthritogenic alphaviruses are mosquito-borne viruses that are a major cause of infectious arthropathies worldwide, and recent outbreaks of chikungunya virus and Ross River virus (RRV) infections highlight the need for robust intervention strategies. Alphaviral arthritis can persist for months after the initial acute disease, and is mediated by cellular immune responses. A common strategy to limit inflammation and pathology is to dampen the overwhelming inflammatory responses by modulating proinflammatory cytokine pathways. Here, we investigate the contribution of interleukin-17 (IL-17), a cytokine involved in arthropathies such as rheumatoid arthritis, in the development RRV-induced arthritis and myositis. IL-17 was quantified in serum from RRV-infected patients, and mice were infected with RRV and joints and muscle tissues collected to analyse cellular infiltrates, tissue mRNA, cytokine expression, and joint and muscle histopathology. IL-17 expression was increased in musculoskeletal tissues and serum of RRV-infected mice and humans, respectively. IL-17–producing T cells and neutrophils contributed to the cellular infiltrate in the joint and muscle tissue during acute RRV disease in mice. Blockade of IL-17A/F using a monoclonal antibody (mAb) reduced disease severity in RRV-infected mice and led to decreased proinflammatory proteins, cellular infiltration in synovial tissues and cartilage damage, without affecting viral titers in inflamed tissues. IL-17A/F blockade triggered a shift in transcriptional profile of both leukocyte infiltrates and synovial stromal cells by downregulating proinflammatory genes. This study highlights a previously uncharacterized role for an effector cytokine in alphaviral pathology and points towards potential therapeutic benefit in targeting the IL-17 to treat patients presenting with RRV-induced arthropathy.

P5: Oncogene editing technology and immune activation to treat HPV driven oropharyngeal cancers

Ana María Salinas-Montalvo, Nigel AJ McMillan, Adi Idris

Menzies Health Institute Queensland and School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia.

Purpose: Human papillomavirus (HPV) 16 is one of the major etiologic causes of oropharyngeal squamous cell carcinoma (OPSCC) which its incidence has increased in the past decade in the United States. Our research aims to demonstrate whether oncogene targeting of HPV E7 in combination with STING agonist treatment, would result in rapid and OPSCC tumour clearance. Methods: We will assess the therapeutic efficacy of the combination of HPV16 E7-CRISPR/Cas9 inducible by doxycycline and STING agonist 2'3'-cGAMP in OPSCC cancer cell lines and xenograft models. RESULTS: In vitro experiments have shown that CRISPR of E7 shows cellular apoptosis and tumor regression, but we are sure that in combination with STING the effect will improve greatly. CONCLUSION: The combination of CRISPR E7 inducible system with STING agonist has prominent potential to be a non-invasive cancer specific treatment.

P6: Discovering novel compounds from multidrug resistant Pseudomonas aeruginosa and MRSA to treat brain infections

Choudhury, T. Kalkundri, M. Zunk, A. Chacko, M. Cheesman, J. Ekberg, G. Grant

Menzies Health Institute Queensland, Griffith University, Queensland 4222, Australia
School of Pharmacy, Griffith University, Queensland 4222, Australia

Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA) are critically prioritized organisms in the WHO 's list, reported to be the major causes for mortality, nosocomial infections and hospital acquired meningitis. The development of new, potent antimicrobial drugs and therapies are now urgently required. An estimate of 700,000 deaths annually due to anti-microbial resistant bacterial infections has been reported, and without new therapies the mortality rate could rise to 10 million by 2050.

The aim of this study was to determine the viability of MRSA treated with synthetically available metabolites, such as pyocyanin from P. aeruginosa and to test the metabolites secreted from viable MRSA on glial cells to fight bacterial brain infection.

Our study indicated potential effects of pyocyanin on MRSA with a MIC 90 at 16 mg/L. Supernatants collected from viable concentrations of MRSA were purified and analysed by mass spectrometry. We also tested the purified supernatants on glial cells (astrocytes, microglia, and neurons) for their effect on phagocytosis of bacteria causing brain infection. The significance of this study is to shed light on discovery of novel molecules from MDR organism stimulated by a component of an equally resistant organism. This may further lead to a protocol to identify natural derived from bacteria. In addition, these molecules would be used to fight bacterial infections of the brain, by stimulating glial phagocytosis. Thus, leading to the upliftment of methodologies in drug discovery and healthcare

P7: Altered Spatial and Temporal Gait Parameters in Ross River Virus-Infected Mice

Eranga Abeyratne [1,2,3], Ronak Reshamwala [3,4] Todd Shelper [3,5] Xiang Liu [1,2,3] Ali Zaid [1,2,3]
Suresh Mahalingam [1,2,3] and Adam Taylor [1,2,3]

[1] Menzies Health Institute Queensland, Griffith University, Gold Coast Campus, Southport, 4222, QLD, Australia.
[2] Global Virus Network (GVN) Centre of Excellence in Arboviruses, Griffith University, Gold Coast, QLD, Australia.
[3] School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia.
[4] Griffith Institute for Drug Discovery, Griffith University;
[5]Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University.

Infection with mosquito-borne arthritogenic alphaviruses, such as Ross River virus (RRV) and Barmah Forest Virus (BFV), can lead to long lasting rheumatic disease. Existing mouse models that recapitulate the disease signs and immunopathogenesis of acute RRV and BFV infection have consistently shown relevance to human disease. However, these mouse models, which chiefly model hindlimb dysfunction, may be prone to subjective interpretation when scoring disease. Assessment is therefore time-consuming and requires experienced users. The DigiGait™ system provides video-based measurements of movement, behaviour and gait dynamics in mice and small animals. Previous studies have shown DigiGait™ to be a reliable system to objectively quantify changes in gait in other models of pain and inflammation. Here, for the first time, we determine measurable differences in the gait of mice with infectious arthritis using the DigiGait™ system. Statistically significant differences in paw area and paw angle were detected during peak disease in RRV-infected mice. Significant differences in temporal gait parameters were also identified during the period of peak disease in RRV-infected mice. These trends were less obvious or absent in BFV-infected mice, which typically present with milder disease signs compared to RRV-infected mice. The DigiGait™ system therefore provides an objective model of variations in gait dynamics in mice acutely infected with RRV. DigiGait™ is likely to have further utility for murine models that develop severe forms of infectious arthritis resulting in hindlimb dysfunction like RRV.

P8: Comparison of transection-type and contusion-type spinal cord injury mouse models

Ronak Reshamwala [1, 2, 3], Megha Shah [2, 3], Tanja Eindorf [2, 3], Lindsay Gee [2, 3], Jia Yu Peppermint Lee [2, 3], Todd Shelper [2, 3], Graham C Smyth [2, 3], Jenny A K Ekberg [1, 2, 3], James A St John [1, 2, 3]

[1] Griffith Institute for Drug Discovery, Griffith University, Brisbane, 4111, QLD, Australia;
[2] Menzies Health Institute Queensland, Griffith University, Southport, 4222, QLD, Australia;
[3] Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Brisbane, 4111, QLD, Australia

Spinal cord injury (SCI) causes loss of sensation and paralysis below the level of injury resulting in long term disability and increased the risk of mortality. This results in physical, mental and financial burden on the individuals, their families, the society and the government. Currently, there is no cure for the SCI, hence, it is one of the most important issues under investigation both in vitro and in vivo. In vivo SCI injury models can be broadly classified as transection or contusion type. Transection type injury is complete, more precise, and easier to produce, but lacks clinical relevance. Contusion type injury is incomplete and variable, but it is clinically more significant. Rodent models, especially rats, are most widely used in the SCI research. But despite the smaller size, mice models offer distinctive advantages like ease of handling, cost and availability of genetically modified models. However, the two injury models in mice have never been compared in a single study. In this first-of-its-kind study, we followed up C57BL/6 mice with transection and contusion type injuries up to 12 weeks after the injury. Status of the motor function, body weight and overall general health conditions were recorded for the injured mice. Unlike contusion, there was no spontaneous return of functions in transection. More weight loss was observed after the complete transection injury as well as increased occurrence of formation of bladder calculi was observed. Muscle wasting was less profound in the incomplete contusion injury. The trends of recovery, weight loss and general health also correlated with the known phases of the SCI (acute, subacute and chronic).

P9: Development of a controlled infection immunisation (CII) strategy for a malaria vaccine

Reshma Nevagi[1], Tanjir Islam[2], Emily Cooper[1], Geoff Birrell[3], Mariusz Skwarczynski[2], Istvan Toth[2,4,5,] Danielle I. Stanisic[1], Michael F. Good[1]

[1]Institute for Glycomics, Griffith University, Southport, Australia,
[2]School of Chemistry and Molecular Biosciences, The University of Queensland, Australia,
[3]Australian Defence Force Malaria & Infectious Disease Institute, Australia,
[4]School of Pharmacy, The University of Queensland, Australia,
[5]Institute for Molecular Bioscience, The University of Queensland, Australia.

This abstract is not available

P10: High-throughput split-protein profiling by combining transposon mutagenesis and regulated protein-protein interactions with deep sequencing

Md. Solayman[1], Kai Zhou[1,2], Thomas Litfin[1], Huijun Zhao[3], Yaoqi Zhou[1,2]* and Jian Zhan[1]*

[1] Institute for Glycomics and School of Information and Communication Technology, Griffith University, Parklands Dr. Southport, QLD 4222, Australia
[2] Institute for Systems and Physical Biology, Shenzhen Bay Laboratory, Shenzhen 518055, China
[3] Centre for Clean Environment and Energy, Griffith University, Gold Coast Campus, Queensland 4222, Australia

Splitting a protein at a position may lead to self- or assisted-complementary fragments depending on whether two resulting fragments can reconstitute to maintain the native function spontaneously or require assistance from two interacting molecules. Assisted complementary fragments with high contrast are an important tool for probing biological interactions. However, only a small number of assisted-complementary split-variants have been identified because of manual, labor-intensive optimization of a candidate gene. Here, we introduce a technique for high-throughput split-protein profiling (HiTS) that allows fast identification of self- and assisted complementary positions by transposon mutagenesis, a rapamycin-regulated FRB-FKBP protein interaction pair, and deep sequencing. We test this technique by profiling three antibiotic-resistant genes (fosfomycin-resistant gene, fosA3, erythromycin-resistant gene, ermB, and chloramphenicol-resistant gene, catI). Self- and assisted complementary fragments discovered by the high-throughput technique were subsequently confirmed by low-throughput testing of individual split positions. Thus, the HiTS technique provides a quicker alternative for discovering the proteins with suitable self- and assisted-complementary split positions.

P11: Towards pan-flaviviral protease inhibitors

Crystall M.D. Swarbrick[1,2#], Gerasimos Rassias[3*#], Vasiliki Zogali[3#], Kitti Wing Ki Chan[1], Chin Piaw Gwee[1], Mark von Itzstein[2], Minos-Timotheos Matsoukas[3] & Subhash G. Vasudevan[1,2*]

[1]Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore
[2]Institute for Glycomics, Griffith University, Australia
[3]Department of Chemistry, University of Patras, Greece
Equal first author contribution
*Corresponding authors

The Flaviviridae family includes high profile viruses dengue and Zika for which there are currently no antiviral treatments available. The flaviviral RNA genome is directly translated into a single polyprotein which is cleaved by viral and host proteases. Our research here focuses on a rational structure-chemistry approach to develop non-peptidic small molecules that can specifically inhibit these proteases. Starting with an inhibitor identified in a West Nile Virus in silico drug discovery campaign we undertook a scaffold hopping exercise to discover new lead compounds. Following a structure-activity-relationship study of the new series, compound 17 was found to inhibit DENV2 and ZIKV protease at IC50 values of 1.16 and 0.52 μM respectively which were amongst the lowest reported values in the literature. In a second iteration of this work, we decided to test the inhibitory activity of "prodrugs" of the active compounds to examine their toxicity and potencies in cellulo. This led to the discovery of a "pro-drug" derivative of compound 17 that was efficacious and potent in cellulo achieving low micromolar EC50 against DENV2, thus promising a well-tolerated series of compounds targeting the flaviviral protease. The in cellulo mechanism-of-action of the lead compound was investigated using a time-of-addition assay which suggested that the compound interfered with the early stages of replication and specifically inhibited intramolecular cleavages in NS3 that have recently been shown to have a trans-dominant inhibitory effect on DENV replication.

P12: Development and pre-clinical evaluation of a whole asexual blood-stage parasite malaria vaccine formulated with cationic liposomes

Winter A. Okoth, Michael F. Good, and Danielle I. Stanisic

Institute for Glycomics, Griffith University

Malaria is a devastating disease of great public health concern, especially in Sub-Saharan Africa where it is the cause of high morbidity and mortality in children under 5 years. The limited protective efficacy of the pre-erythrocytic sub-unit malaria vaccine, RTS,S/AS01 (Mosquirix™), and blood-stage sub-unit vaccine candidates that have been evaluated in field studies has intensified interest in pursuing alternative vaccine development strategies such as the whole parasite vaccine approach. Our research group is exploring the application of liposomes for the development of a whole parasite blood-stage malaria vaccine. This entails formulating killed, whole asexual blood-stage malaria parasites with a cationic liposomal adjuvant system. We hypothesised that a whole asexual blood-stage Plasmodium parasite vaccine formulated with cationic liposomes would induce a broad protective immunity when evaluated in rodent models of malaria. Our data demonstrates that a cationic liposomal vaccine formulated with 10^7 Plasmodium yoelii parasitised red blood cells (pRBCs) is highly immunogenic and provides strong protection against homologous parasite challenge in both inbred and genetically outbred mouse strains. Immunisation resulted in the induction of parasite-specific splenocyte proliferative responses, IgG response, and a mixed Th1 (IFN-γ, TNF-α, IL-2), Th2 (IL-10, IL-6, IL-4) and Th17 (IL-17) cytokine response. To identify immune mechanisms of protection, we next examined the role of B cells and T cells in vaccine-mediated protection. B cell deficient μMT mice succumbed to infection while mice depleted of CD4+ T cells (but not CD8+ T cells) were unable to control blood-stage infection following challenge. These data demonstrate that both B cells and CD4+ T cells contribute to vaccine-mediated protection. Future directions include: further interrogation of the immune mechanisms of vaccine-mediated protection and examining the immunogenicity of a P. falciparum CAF01 adjuvanted vaccine in mice.

P13: Antimicrobial resistance and bacterial oxidoreductases

Guillaume A. Petit[1], Russell Jarrott[1], Maria A. Halili [1], Jennifer L. Martin[2]

[1]Griffith Institute for Drug Discovery, Don Young Road, Griffith University, Nathan, QLD 4111: Vice-
[2]Chancellor's Unit, University of Wollongong, Building 36, Wollongong, NSW 2522, Australia

In these times of social distancing and lockdown, the current research has focused mainly on viruses. However, bacterial infections are still lurking, growing more resistant to antibiotics and limiting options for treatment. There is a need for innovation to tackle pathogenic bacteria and our strategy has been to study and target proteins involved in the formation and maturation of virulence factors: the bacterial oxidoreductases. These proteins are at the centre of the oxidation and isomerisation of protein disulfide bonds in the periplasm of bacteria. Disulfide bonds are like molecular staples, they provide structural stability and are essential for the activity of a multitude of membrane and extracellular proteins in bacteria, many of which are virulence factors. The most well-known proteins of this family are the Disulphide Bond forming proteins (DSB), some of which have been shown to be involved in the pathogenicity of several different bacterial species. The oxidoreductases share a conserved structural motif, a CXXC active site embedded in a thioredoxin fold, however they vary in other parts of their structure and sometimes even function. We aim to identify inhibitors of the bacterial oxidoreductases that have been characterised already, specifically inhibitors of the Disulfide bond forming protein A from Burkholderia pseudomallei (BpsDsbA), and to characterise new bacterial oxidoreductases that are emerging, such as the Suppressor of Copper Sensitivity proteins (SCS). To achieve this goal we use biomolecular techniques such as mass photometry, isomerases and oxidases activity assays, lipidic cubic phase crystallisation and PanDDA, a bioinformatics tool to detect weakly binding ligands in X-ray electron density. We have used a combination of these methods to identify small molecule fragments binding to BpsDsbA and solved the structure of the trimeric Caulobacter crescentus ScsC protein. These results will further our understanding of the role of bacterial oxidoreductases in virulence and host infections.

P14: Modulation on olfactory ensheathing cell migration by liraglutide in 2D and 3D environments

Yu-Ting Tseng[1,2,3], Mo Chen[1,2,3], James St John[1,2,3*], Jenny Ekberg[1,2,3*]

[1] Menzies Health Institute Queensland, Griffith University, Southport, QLD 4222, Australia
[2] Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Brisbane, QLD 4111, Australia
[3] Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia
* Equally contributing Senior Authors

Cell transplantation using olfactory ensheathing cells (OECs) is a promising strategy for repairing nerve injury. Improving the ideal OECs behaviours such as cell survival and migration can possess potential benefits. Liraglutide, a glucagon-like peptide-1 receptor (GLP-1R) agonists, has been known to improve cell survival and provide neuroprotection. In the present study, we further investigate the effects of liraglutide on cell migration in primary OECs (pOECs) in both 2-dimentional (2D) and 3D conditions. In 2D pOEC culture, we found that liraglutide at nanomolar concentrations could activate extracellular signal-regulated kinase (ERK), one of the important pathways in cell migration. The improvement of cell migration was also found in 3D pOECs. The migration length of pOECs was increased by liraglutide, accompanied with an increase in migration duration. However, the migration speed did not change significantly in our results. Liraglutide also induced a morphological change of pOECs towards a bipolar shape consistent with improved migration. In 3D pOEC spheroids, liraglutide not only improved the ability of pOECs to migrate out of spheroid structure, but also induced beneficial morphology for pOECs consistent with a migration morphology. In conclusion, liraglutide can stimulate OECs migration in both 2D and 3D, which may improve OEC transplantation outcomes.

P15: Identification of hematoxylin and gallic acid as potential Nsp7 inhibitors of SARS-CoV-2

Y. Gu[1], M. Liu[1], G.W Buchko[2], R.J Quinn[1]

[1] Griffith Institute for Drug Discovery, Griffith University, Brisbane, Queensland, Australia
[2]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, Washington, USA

The Coronavirus COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global pandemic with more than 200 million confirmed cases and over 4 million deaths worldwide. Vaccines are required to be modified in order to respond to variants emerged globally, and new antiviral therapeutics can take years to be approved, marking the urgent need for repurposing clinically approved drugs. Amongst the established investigation methods for studying interactions between biologically active small molecules and their protein targets, native mass spectrometry (MS) has emerged as a robust tool with high sensitivity and specificity. Native MS has the advantage of allowing direct observation of protein-ligand binding under non-denaturing conditions. Consequently, the biological functionality of the analyte molecules can be well reflected. Here, we screened an FDA-approved library containing 2400 compounds against the SARS-COV-2 viral non-structural protein 7 (Nsp7) using native MS based assay. We report the identification of 2 compounds, hematoxylin and gallic acid, in which, protein-ligand complexes were observed in both compounds with Nsp7, with hematoxylin obtaining binding affinity of 100%. The finding of the inhibitory activity of these compounds against Nsp7 demonstrates their potential to accelerate the development for COVID-19 treatment and deepen our understanding of its viral pathogenesis.

P16: Activity characterization and classification of anti-plasmodial compounds

Sandra Duffy, Vicky, M Avery

Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland. 4111

Introduction: With the possible spread of resistance to artemisinin combination therapies from the Greater Mekong subregion to Africa, it is vital to proactively identify anti-plasmodial compounds with alternative mechanisms of action: not only to artemisinin but all other previously and currently utilized anti-plasmodial drugs. Novel compound screening approaches with high throughput capabilities will enable front loading of compounds with various activity profiles prior to chemical class selection. Thus, placing potential alternative mechanism of action before chemical selection for hit to lead prioritization.
Method: The Pathogen box, an open-source compound library consisting of 400 compounds, contains 125 compounds with anti-plasmodial activity accredited to them. Accompanying the compounds set is the screening data for both asexual blood stage, the mature stage sexual forms (stage V gametocytes) and the liver forms of Plasmodium falciparum by MMV collaborating screening platforms. Utilizing small compound handling capabilities and novel image analysis screening assays, we have extended this data set to determine the onset of compound action, first or second-generation activity, and gametocyte activity profiling from the earliest forms (Ring stage gametocytes) to those of late-stage gametocytes (stage IV).
Results: Analysis of the extended data obtained has classified the 125 compounds into 9 subsets based on their onset of action, asexual blood stage and gametocyte activity profiles.
Conclusion: We believe that this data may provide a basis for selection of compounds with alternative mechanisms of action for front loading anti-malarial drug discovery efforts.

P17: Characterising heat stress in cattle using NMR-based metabolomics

Alexandra Gloria[1,5], Gene Wijffels[2], Megan Sullivan[3], John Gaughan[3,] Horst Joachim Schirra[1,4,5]

[1] Griffith Institute for Drug Discovery, Griffith University
[2] CSIRO Agriculture and Food, Queensland Bioscience Precinct
[3] School of Agriculture and Food Science, University of Queensland
[4] School of Environment and Science, Griffith University
[5]Centre for Advanced Imaging, University of Queensland

Heat stress occurs when an organism's total heat load exceeds its capacity for heat dissipation. In mammals, extreme heat stress causes catastrophic failures, including heat stroke, severe dehydration, and acute renal failure. Efficient body thermoregulatory systems are therefore necessary to combat the effects of increased heat load. When acclimatised to warmer environments, thermoregulation in Bos taurus increases but voluntary food intake is reduced. Optimum livestock health and nutrition are critical to maximise agricultural profitability and efficiency; thus, it is important to understand the metabolic changes occurring as a result of heat stress in order to develop methods of offsetting its impacts. While physiological characterisation has been undertaken before, a detailed metabolic analysis has yet to be conducted. Here we characterised the metabolic effects of a moderate (Tmax 35°C) and severe (Tmax 41°C) heat challenge over several days on grain-fed Bos taurus steers. We also compared a feed-restricted thermoneutral group with the moderate heat challenge cohort. The metabolite changes identified with NMR-based metabolomics of plasma samples and comparative multivariate statistical analyses provide insight into metabolic pathways affected by restricted feed and differing magnitudes of heat stress.

P18: The Glitazone Class of Drugs as Carbonic Anhydrase Inhibitors – A Spin-Off Discovery from Fragment Screening

Sarah L. Mueller [1,2,4], Panagiotis K. Chrysanthopoulos [1], Maria A. Halili [1,2], Caryn Hepburn [3], Tom Nebl [4], Claudiu T. Supuran [5], Alessio Nocentini [5], Thomas S. Peat [4] and Sally-Ann Poulsen [1,2]

[1] Griffith Institute for Drug Discovery, Griffith University, Nathan, Brisbane, Queensland 4111, Australia
[2] ARC Centre for Fragment-Based Design, Griffith University, Nathan, Brisbane, Queensland 4111, Australia
[3] Waters Australia Pty Ltd., Rydalmere, New South Wales 2116, Australia
[4] CSIRO, Biomedical Manufacturing Program, 343 Royal Parade, Parkville, Melbourne, Victoria 3052, Australia
[5] Dipartimento Neurofarba, Sezione di Scienze Farmaceutiche Nutraceutiche, Università Degli Studi di Firenze, Sesto Fiorentino (Florence), 50019, Italy

Carbonic anhydrases (CAs), a family of zinc metalloenzymes, catalyse a reaction fundamental for life: the reversible hydration of carbon dioxide (CO2) and water (H2O) to give bicarbonate (HCO3−) and protons (H+). These enzymes affect numerous physiological and pathological processes, with different isoforms found in a wide variety of organs and tissues in the body. However, the problem of diffuse localisation and lack of selectivity of isoenzymes has led to a growing interest in the development of new CA inhibitors. It is found that approved drugs against CA consist almost entirely of primary sulfonamides (R-SO2NH2) as the zinc-binding chemotype. We have recently discovered that the thiazolidinedione heterocycle is a new zinc-binding group and an alternative CA inhibitor chemotype. Interestingly, this heterocycle, in which the ring nitrogen does not carry a substituent, is also a substructure of the glitazone class of drugs used in the treatment of type 2 diabetes. Inspired by the new findings, we used native mass spectrometry, protein X-ray crystallography and hydrogen-deuterium exchange (HDX) mass spectrometry to screen and characterise three glitazone drugs, troglitazone, rosiglitazone and pioglitazone, for their binding to CA. Furthermore, CA enzyme inhibition data demonstrate the distinctive inhibition of the glitazone drug class for different CA isoenzymes. As the thiazolidinediones are not known to be either a zinc-binding group or CA inhibitors, these results indicate that CA may be an off-target effect of these compounds when used clinically. In conclusion, thiazolidinediones may represent a new opportunity for the development of novel CA inhibitors as prospective drugs.

P19: Antimicrobial Bacillus probiotics: metabolites and mechanisms

C. Tran[1], I. Cock[1], W. Chen[2], Y. Feng[1]

[1] Griffith Institute for Drug Discovery (GRIDD), Griffith University, Nathan, QLD, 4111, Australia
[2]W. Chen - Bioproton Pty Ltd, Acacia Ridge, QLD, 4110, Australia

Antimicrobial resistance is a global health emergency that we face today. The main culprit for its accelerated spread is through the misuse and overuse of antibiotics in livestock. A safe alternative can be found in probiotics, which provide positive health benefits to its host, but do not introduce antimicrobial resistance like antibiotics do. However, probiotics must survive the acidic gastric environment to reach the small intestine and colonize the host. This has garnered interest in probiotics that are intrinsically resistant to acid such as the spore-forming Bacillus. Bacillus probiotics forms a layer to protect itself against external stresses and several antimicrobial metabolites have been identified. However, most of these metabolites remain unidentified and further research is needed to understand their mechanisms and methods to optimize their performance for commercial use. Working alongside Bioproton, a company located in Brisbane that specialises in animal feed, we have identified 6 Bacillus strains that inhibit gram-positive bacteria, (*S. Aureus*), with two of these strains also targeting gram-negative bacteria *(P. aeruginosa*). By the end of this project, I will isolate and identify the antimicrobial metabolites responsible for their antimicrobial activity, the unique characteristics behind these potent probiotics to better select candidates, and most importantly, understand how we can modify any external factors to optimize the performance of these probiotics.

P20: Using Machine Learning on the Patient derived cell phenotypes to classify Schizophrenia

Jamila Iqbal[1], Gaythri Thillaiyampalam[1], Alan Mackay Sim[1], Alex Cristino[1]

[1]Griffith Institute for Drug Discovery (GRIDD),
Don Young Road,Griffith University, Nathan Campus, Australia

The application of machine learning to image-based microscopy data is the growing line of research in biological sciences. Data from these studies can identify subtle alterations in cell traits and provide new insights into disease mechanisms or discovery of biomarkers. Here, we used a support vector machine (SVM) algorithm for the classification of Schizophrenia patients and healthy individuals based on the cell phenotypic data. We screened human olfactory neurosphere-derived cells from 9 Schizophrenia patients and 9 healthy individuals to extract single cell features from nucleus, cell, mitochondria and endoplasmic reticulum (ER). All images were acquired on the high content imaging system and image analysis was performed using the Harmony software. Single cell features such as intensity, texture and morphology of cellular organelles were subjected to SVM (support vector machine). For the purpose of analysis, the data was split into a training set (70%) which was used to train model and a test set (30%) which was used to validate the training results. The success of the model was determined by metrics such as accuracy, specificity and selectivity. Our analysis resulted in the classification accuracy of over 70% in correctly identify samples into disease group or control group. Integrating cell traits data to other 'omics' data can open new avenues for research and provide more sophisticated approaches to understand disease mechanisms with the possibility to translate this to clinical diagnostics or developing therapies.

P21: Evaluation of mitochondria as a potential target in cancer using 2D and 3D breast cancer models

Kah Ni Tan [1], Vicky M Avery [1]

[1] Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Don Young Road, Nathan, QLD 4111, Australia

Introduction/Background: Breast cancer is the most diagnosed (almost 1 in 4 women) and the leading cause of death in women. Remarkable progress in cancer research has led to the identification of numerous hallmarks of cancer and mitochondria have been identified to be instrumental in many aspects of cancer initiation and progression. Mitochondria are the powerhouses of the cells, which provide energy to support various cellular functions. In addition to energy metabolism, mitochondria have other important roles in modulating cell survival and proliferation, including the regulation of antioxidant defences and cell death cascade. Given the multifaceted roles of mitochondria, targeting the mitochondria appears to be a promising therapeutic option in breast cancer.

Methods: A novel mitochondrial-targeted compound was used to determine the roles of mitochondria in the survival of breast cancer cells using 2D and 3D culture model that better recapitulates the tumour microenvironment. Alterations in mitochondrial energy metabolism, oxidative stress as well as mitochondrial-mediated cell death following compound addition were also assessed using cutting-edge imaging platforms and real-time cell metabolic analyzer.

Results: The mitochondrial-targeted compound significantly reduced breast cancer cell viability and spheroid volume in 2D and 3D viability assays respectively. We also observed alterations in mitochondrial membrane potential and induction of oxidative stress in the presence of the compound. We are currently investigating changes in cell energy metabolism and mitochondrial-mediated cell death.

Conclusions: A mitochondrial-targeted approach appears to have detrimental effects on breast cancer cells, highlighting the significance of mitochondria in breast cancer cell survival and progression.

P22: Mimicking the Tumour Microenvironment - Integrating 3D into early drug discovery assays

Elke Kaemmerer[1], Vicky M Avery [1]

[1] Discovery Biology, Griffith Institute for Drug Discovery, Griffith University

Considerable effort in the last 40 years of cancer drug discovery has gone into miniaturising and automation of assays to detect compounds effective against cancer cells. These assays involve growing cells in an artificial environment, ie on the surface of plastic or glass, resulting in cost-and time effective high throughput screening of compounds to identify new drug candidates. However, these systems only represent cell interactions on a flat surface and don't mimic the high complexity of the actual tumour in the context of its surrounding three-dimensional (3D) microenvironment. Interactions of cancer cells with the microenvironment are not captured using these simplified models. This project assessed the suitability of bioprinted 3D cell cultures for early drug discovery assays. Therefore, bioprinted spheroid cultures were prepared in a 96-well format using PANC-1 cells embedded in synthetic matrices of different stiffness (0.5/0.9 kPa + RGD peptides) prepared with the Rastrum Bioprinter (Inventia). Spheroid growth was measured using a resazurin-based cell viability assay and an image-based cell viability assay using calcein AM/propidum iodine/Hoechst staining and the Opera Phenix high content imaging system (Perkin Elmer) over 9-days spheroid growth. Both printed matrices resulted in similar spheroid growth and showed high well to well reproducibility. Although suitable for increased throughput and automation, this system still has some limitations, e.g. 96-well format and predefined plate layouts. Incorporating automated imaging and 3D analysis of these models was then further explored, using bioprinted MCF-7 spheroids imaged with the Opera Phenix imaging system. The preciScan module was used to identify spheroids at lower magnification and to image only spheroids with more details, which significantly reduced the amount of data generated per well. Automated 3D-volumetric analysis was used to assess well-to-well reproducibility of bio-printed samples (Harmony software 4.8, Perkin Elmer), showing low well to well variation.

P23: High-throughput drug screens – Image Analysis

Tayner Rodriguez[1,2], Vicky M. Avery[1,2]

[1] Discovery Biology - GRIDD Institute, Griffith University, Australia
[2] Canthera Discovery, Australia

Phenotypic screening plays a significant role in drug discovery, primarily due to the increased complexity and functional information provided. Within Discovery Biology, Griffith University, we utilize cellular phenomics approaches to address key and critical questions relating to a diverse range of disease indications. These include the study of intracellular parasite life cycle stages, diverse targets for cancers, including programmed ribosomal frameshifting, immuno-modulatory genes, and more. This presentation will discuss programmed ribosomal frameshifting as a novel target for anticancer therapeutics. An example outlining the analysis of dual fluorescent reporters for compound selection using high content imaging identifying compounds that regulate ribosomal frameshifting utilizing intensity patterns algorithms will be described. In addition, the development of image processing algorithms on high content imaging to scrutinize a particular phenotype, such as aggregations, translocations, morphological changes, protein-protein interaction, colocalization will be discussed. Such an approach was adopted to study nuclear aggregations upon compound exposure which can be used to perform a phenotypic HTS for Multi-kinase (MK) inhibitors. MK inhibitions are a very attractive approach as overcome the limitations of mono-kinase inhibitors and can reduce the possibility of drug resistance.

P24. A structure-guided drug discovery approach towards developing novel neuraminidase inhibitors against influenza A virus

Olivia Tan[1], Linghui Li[1,2], Mauro Pascolutti[1], Yun Shi[1], Thomas Ve[1], Patrice Guillon[1], Larissa Dirr[1], Mark von Itzstein[1]

[1] Institute for Glycomics, Gold Coast, Griffith University, QLD, 4222 (Australia)
[2] University of Chinese Academy of Sciences, Beijing, 101408 (China)

Influenza viruses impose a substantial economic burden and impact healthcare systems on a global scale. Influenza viruses are respiratory pathogens known to infect epithelial cells from the upper and lower respiratory tracts and may cause mild to severe respiratory illness. Influenza A viruses (IAV) are responsible for causing seasonal outbreaks and epidemics as well as sporadic pandemics of respiratory disease, most commonly referred to as the flu. The H3N2 subtype, in particular, was responsible for the 1968 Hong Kong flu pandemic. The current class of anti-influenza drugs target the neuraminidase (NA) glycoprotein, which is responsible for releasing new viral progeny by cleaving off terminal sialic acid residues from cellular host cell receptors. The three-dimensional structure of IAV NA has been well established in literature and was used to guide the design of potential inhibitor scaffolds identified by 19F-NMR spectroscopy. This study focuses on crystallising the IAV NA glycoprotein in complex with novel fragment-based compounds to investigate protein-ligand interactions. In summary, we seek to use structure-guided drug discovery as a directional tool to develop novel neuraminidase inhibitors with new scaffolds and improved efficacy.

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