Synthetic Biology **Australasia Conference 2025**

Building the Future of Synthetic Biology

19-21 November 2025
Te Papa, Wellington, New Zealand

Photo by Wellington City Council

Conference chair: Mark Calcott

Organisation committee: Alyesha Candy, Anna Donnan, Anwar Sunna, Jessica Chiang, Kelly Styles, Neroli Thomson, & Rosannah Cameron

Science advisory committee: Amy Cain, Claudia Vickers, Jason Whitfield, Wayne Patrick, Jenny Mortimer, Revel Drummond, Robert Speight

Executive committee: Anwar Sunna, Amol Ghodke, Florence Ly, Jason Whitfield, Maddison Mccoy, Rashika Sood

Contents

Welcome to SBA2025

Dear SBA2025 colleagues,

After two years we are back and ready to continue with a packed program of science from across New Zealand, Australia and further abroad. Perth was a great conference in 2023, and it is a privilege to be able to host the 2025 conference here in Wellington. I am sure we will meet your expectations for excellent science and opportunities to meet new researchers along with great bars and restaurants nearby to further your networking.

I am excited to see all the speakers and poster presenters. While piecing together the program, the diversity of projects within the synthetic biology space has been fascinating to see. The number and quality of abstracts submitted made it a hard job to finalize a program, but we are really pleased and look forward to learning more about the latest synthetic biology research.

This conference has been made possible by an incredible team of people, including the organizing committee, scientific advisory committee, executive committee, session chairs, judges, volunteers and sponsors. Conferences are community events, and I am greatly for all the help that everyone has, and will be, providing to help run SBA 2025. It is all greatly appreciated.

Have a great conference! I hope you have a great conference, expand your contacts and learn something new.

Kind regards,

Mark Calcott

Venue Location and Layout

The conference will be located at the Museum of New Zealand Te Papa Tongarewa. Located in the heart of Wellington, most amenities are within a 20-minute walk.

Physical address of Te Papa is:
55 Cable Street, PO Box 467
Wellington, 6011
New Zealand

Directions: How to get to Te Papa

By bus

Most Wellington buses (including those from the airport and railway station) stop along Courtenay Place and Willis Street. From these stops, it's just a few minutes' walk to Te Papa.

Metlink timetables

By car

Take the Aotea Quay exit when driving south into central Wellington along the SH1 motorway. Continue along Waterloo, Customhouse, and Jervois Quays, which lead directly into Cable Street and Te Papa's convenient car park.

Parking at Te Papa

By bike or kick scooter

The waterfront around Te Papa is bicycle and kick scooter friendly. Flamingo scooters offer provide e-scooters that are easy to hire and can be used to speed up the journey.

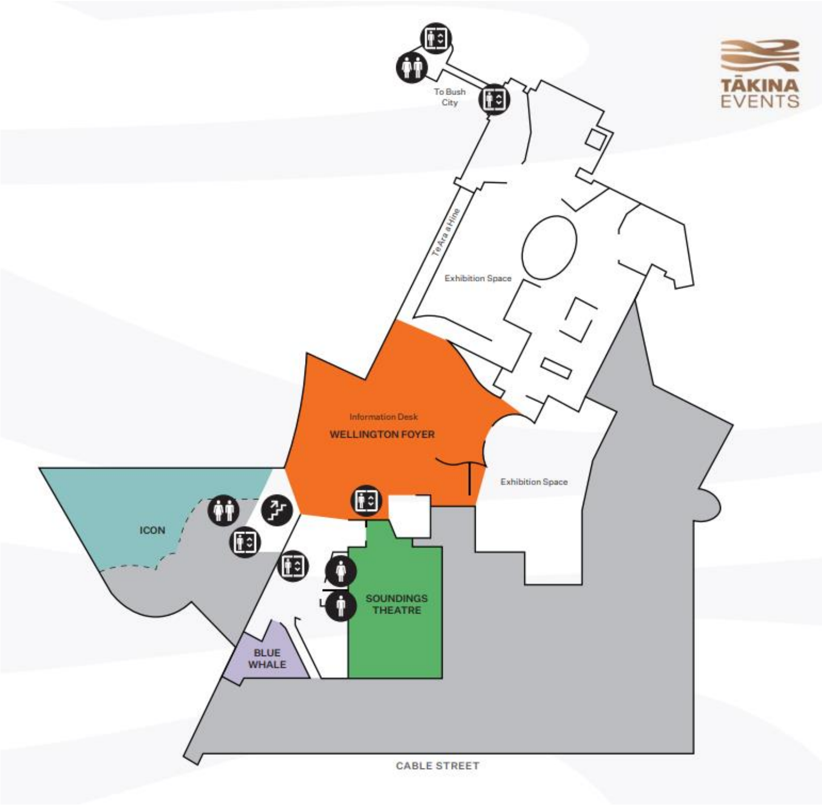
Flamingo scooters

From Wellington Airport

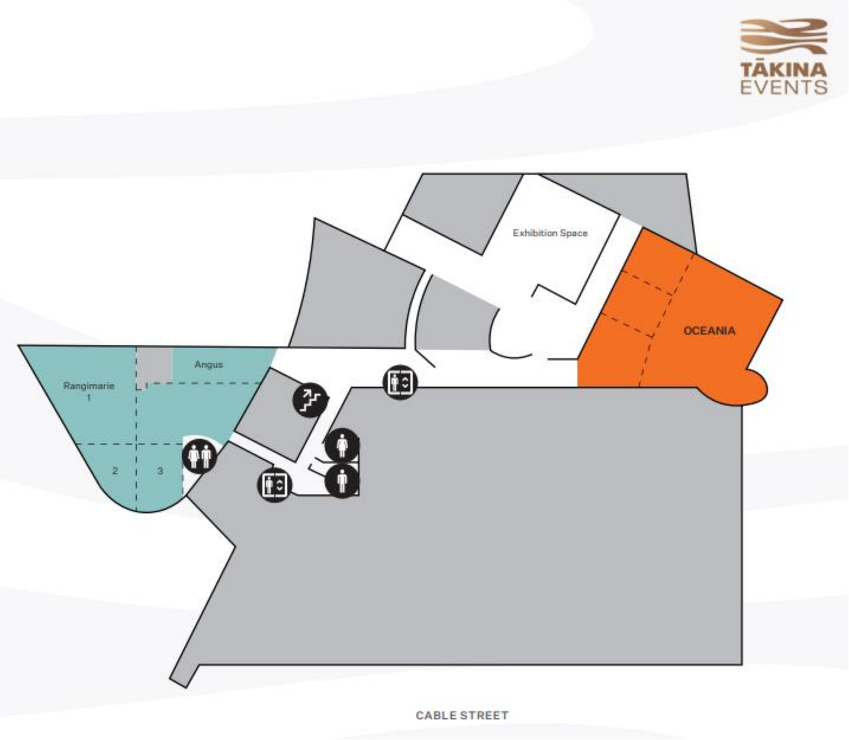
- By car
    - Driving time: Approximately 15 minutes (traffic dependent)
      1. Follow SH1, turning left along Cobham Drive.
      2. At the Evans Bay Parade lights, turn right. Continue around the harbour and along Oriental Parade.
      3. Turn right into Wakefield Street.
      4. Turn right into Taranaki Street, then right again into Cable Street. Te Papa's
- By bus
    - The Airport Express (AX) runs 7 days a week, every 10-20 minutes, except for the first few morning services and evening services. Travel time from Wellington Station to the Wellington International Airport is approximately 25-30 minutes, depending on traffic. Free Wi-Fi, USB charging and luggage racks are available on board.

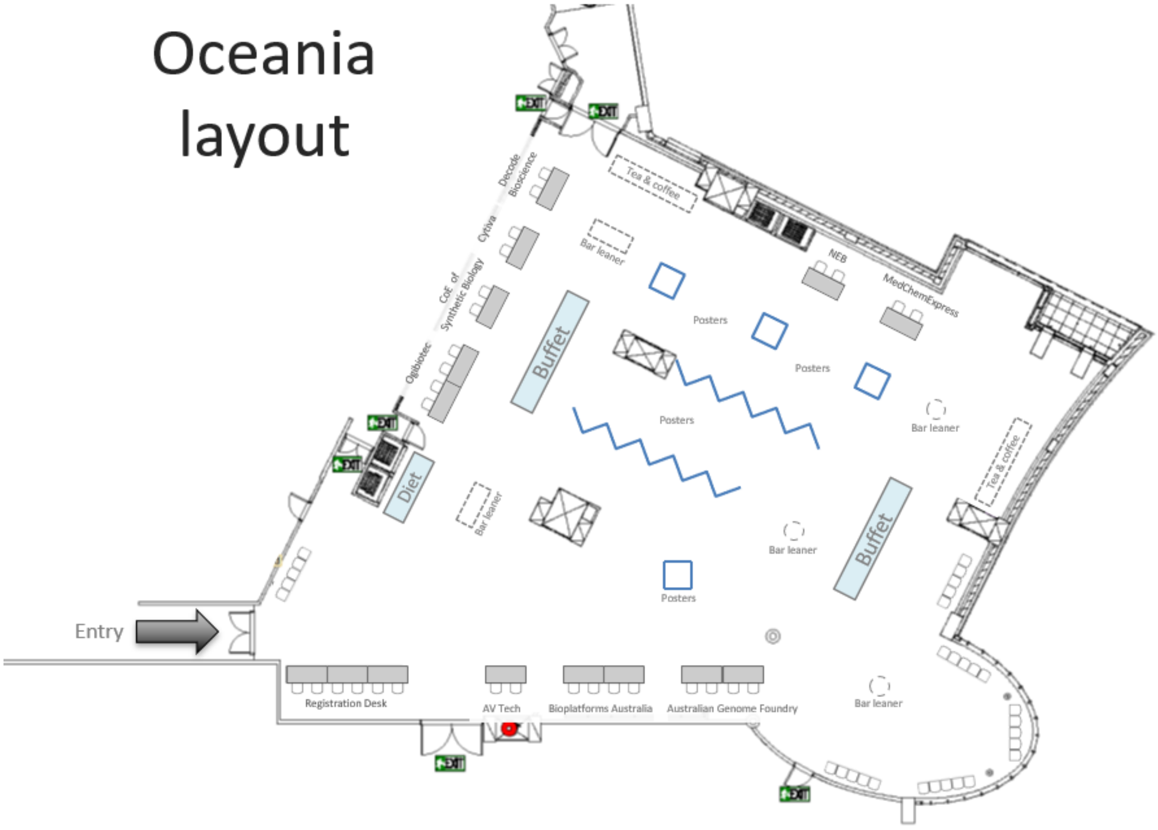
When you are at Te Papa

The main lecture theatre for all talks is Soundings Theatre. Enter from Cable Streat and take the escalator when you enter Te Papa. The room is located to the right on level 2 (coloured in green below).



The poster session and trade area are in the Oceania room on level 3. Turn right after taking the lift or stairs and walk to the end of the hall. See the room coloured in orange below.





Internet Access

Te Papa offers complimentary Wi-Fi and is shared by all function attendees. It is suitable for basic internet browsing such as checking emails and social media accounts. There is a maximum of 2GB per 24 hours allowed per user.

To access the correct Wi-Fi, connect to Tākina Events on your device. A Tākina Events internet page will pop up when you open your browser, type in the access code – events – and accept the T&Cs.

Toilets

Soundings Theatre– Toilets are outside the venues spaces near Story Place and Whale Heart display.

Oceania – Toilets are back out of the venue, turn left before the staircase. Another set of toilets available just after the bridge to Te Huinga Centre.

Accessible toilets are available on Levels 1, 3, 4 and 5. Please ask a member of the Visitor Services team for any assistance required.

Evacuation

The event organiser is responsible for advising us of any attendees that are using wheelchairs, mobility scooters and anything alike for evacuation purposes. Tākina Events is committed to providing for any accessibility needs but we will need prior notice to make sure of requirements.

Fire Evacuation

Evacuation is essential even if you cannot see or locate the fire. The continuous sound of fire alarms will be activated to notify all occupants to vacate the building immediately.

Egress is available from all floors and areas in the building via the marked emergency exits or by the main staircase. The building is also fitted with an automatic sprinkler system, manual fire alarms, fire hose reels and extinguishers on each floor.

Te Papa Visitor Services team will take full charge of the evacuation and make sure the building has been fully evacuated. Our team will follow procedures to assist disabled visitors in the case of an evacuation.

Evacuation assembly points are:

- By Te Papa Museum forecourt near Circa Theatre, or:

- At Barnett Street corner Cable Street next to Waitangi Park

Earthquake

Te Papa sits on 150 base isolators to protect the building from earthquake. The building will sway up to half a metre which is normal as it is designed to withstand an 8.5 magnitude and is a designated civil defence centre.

Te Papa would be among the safer places in Wellington in a major earthquake. All visitors must stay away from windows, find cover and remain under the shelter or in the venue. Our Visitor Services team will provide instructions should there be an evacuation.

Sponsors

A huge thank you to our sponsors — for your incredible support! Your generosity helped make SBA 2025 possible and directly contributed to lowering costs for student registrations. We're so grateful for your partnership.

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  - Macquarie University, NSW
- IDEA Bio - University of Queensland, QLD
- Plant SynBio Australia – 4 nodes: ANU, LaTrobe, Adelaide University, UWA

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- Australian Proteome Analysis Facility, NSW
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- Mass Spectrometry and Proteomics Facility, UniSA, SA
- Proteomics International and UWA, WA

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Gene Discovery and Genome Function

- Australian Genome Research Facility
- The Ramaciotti Centre for Genomics, UNSW, NSW
- Biomolecular Resource Facility, ANU, ACT
- Genomics Western Australia, WA
- South Australian Genomics Centre, SA

METABOLOMICS

Small Molecule Analysis

- Bio21 Institute, University of Melbourne, VIC
- Australian Wine Research Institute, SA
- Centre of Metabolomics. UWA, WA
- Australian Institute of Bioengineering and Nanotechnology, UQ, QLD

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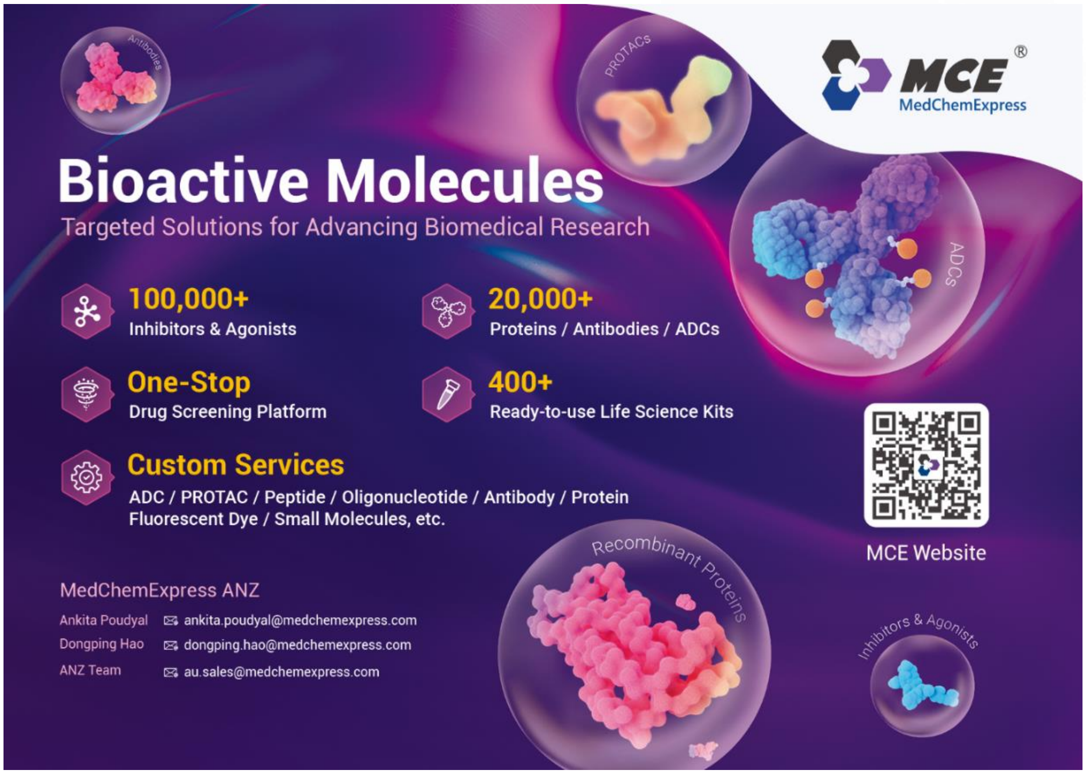

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ADCs
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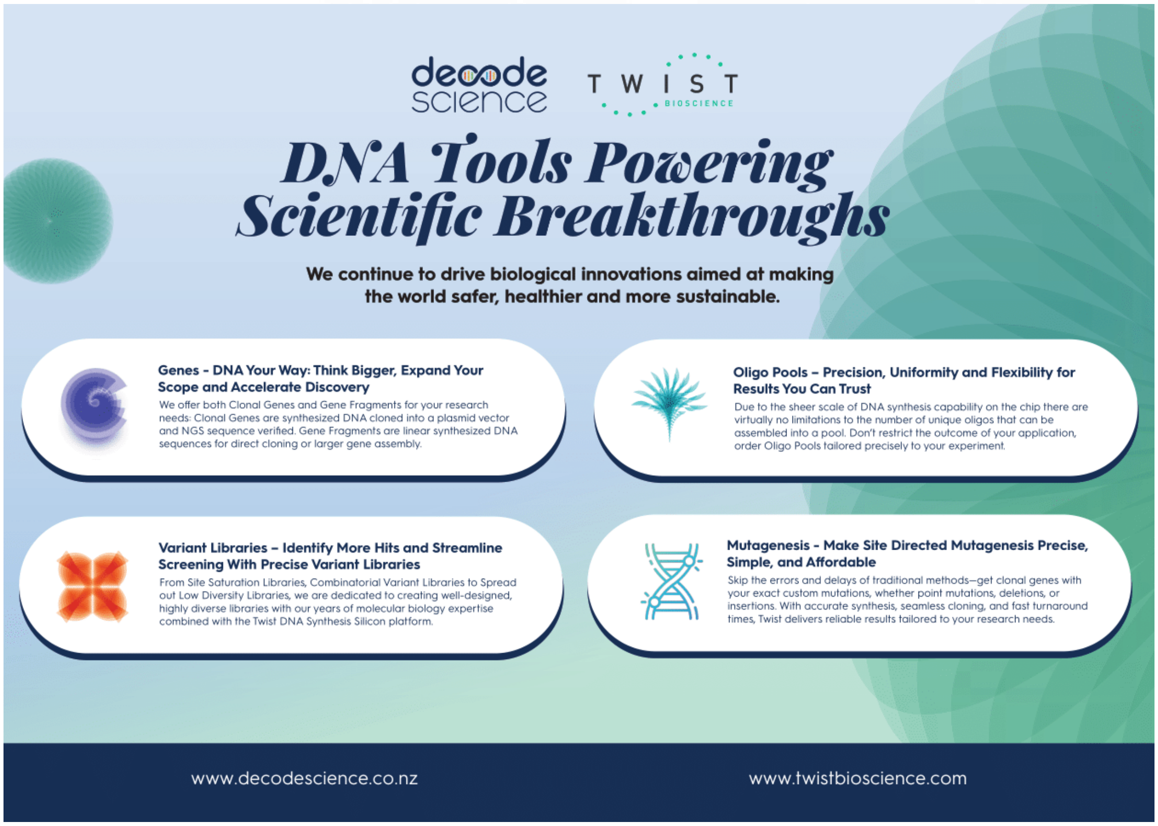

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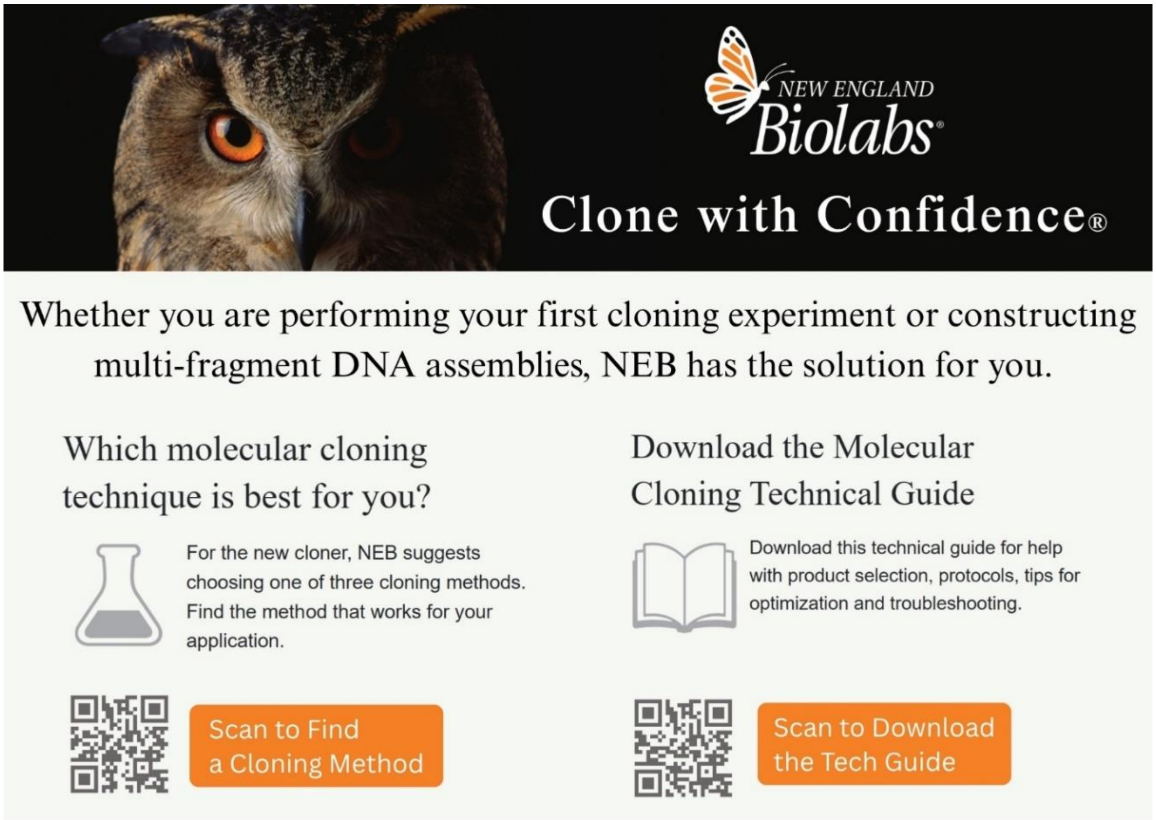

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AGF is proudly funded by Bioplatforms Australia through funding from the Australian Government National Collaborative Research Infrastructure Strategy

Biomolecular
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Program

Wednesday, 19 November 2025	
8:15	Registration (Oceania Room)
	Session I: Production of high value products - Sponsored by Bioplatforms Australia (Soundings theatre)
9:15	Welcoming Mihi Whakatau
9:30	Welcome to SBA 2025: Conference chair Mark Calcott
9:40	Plenary: Barrie Wilkinson (John Innes Centre): A platform for lantibiotic diversification
10:10	Jenny Mortimer (Adelaide University): Enabling Synthetic Biology Via NCRIS Investment
10:25	Georg Fritz (UWA): Toward Engineered Living Materials in Seawater: A New Role for Vibrio natriegens
10:40	Morning Tea (Oceania Room)
	Session II: Space, metabolism and molecular machines (Soundings theatre)
11:20	Invited: Sarah A Kessans (U Canterbury): Bringing synthetic biology to new heights: platforms for biological research in microgravity
11:50	Simona Della Valle (UWA): Synthetic starvation: combining natural and engineered responses to nutrient limitation in Vibrio natriegens
12:05	Neil O. Robertson (UNSW): Learning to walk: the first steps of an artificial protein motor
12:20	Paula de Dios Andrés (Aarhus U): Flippase-Mediated Division of Polymer-Lipid Hybrid Vesicles as Artificial Cell Scaffolds
12:35	Silvio S. Bonni (U Auckland): Engineering yeast to investigate the hidden role of 'Cheater"-suppression in the evolution of germ-soma specialization
12:50	Lunch break (Oceania Room)
	Session III: High-throughput engineering and biosensors (Soundings theatre)
13:50	Invited: Briardo Llorente (Macquire U): Shuffling the Genomic Deck: Engineering Bacterial Genome Diversity
14:20	Invited: Kirill Alexandrov (QUT): Machine Learning Meets Cross-Eyed Protein Engineering: Building Artificial Protein Switches
14:50	Isabella N. Westensee (QUT): Protein Switch-Based Screening Platform for the Optimization of Lanmodulin selectivity
15:05	Jochem N.A. Vink (Malaghan Institute): Next-generation mRNA production drives RNA research and development advances in New Zealand
15:20	Afternoon Tea (Oceania Room)
	Session IV: Engineering biosynthesis and biodiscovery (Soundings theatre)
16:00	Invited: Emily Parker (VUW): Reconstructing biosynthetic pathways for complex fungal natural products
16:30	Rosannah Cameron (VUW): Synthetic biology tools for optimising the biosynthesis of natural products in filamentous fungi
16:45	Rory Little (VUW): Natural product biodiscovery from the bacterial symbionts of Aotearoa New Zealand endemic insects
17:00	Zeyu Lu (QUT): Engineering synthetic gene circuitry for efficient terpene biosynthesis in S. cerevisiae
17:15	Juan Pablo Molina Ortiz (CSIRO): Model Driven Engineering of Stable Auxotrophic Consortia
17:30	Birgitta E. Ebert (U Queensland): Boosting Carbon Yield of Bioprocesses via Synthetic Chemolithoheterotrophy
17:45	Poster session with nibbles and drinks (Oceania Room)
20:15	End of conference day

	Thursday, 20 November 2025
	Session V: Plant synbio to advance the bioeconomy - Sponsored by the ARC Centre of Excellence in Plants for Space (Soundings theatre)
9:00	Plenary: Jennifer A. N. Brophy (Stanford): Synthetic biology tools to enable engineering of environmentally resilient plants
9:30	Gabrielle Herring (UWA): Advancing synthetic gene circuitry in plants with novel split dCas12a-based tools
9:45	Vincent Nowak (CSIRO): Strain Engineering of Actinobacteria to Uncover Natural Products for Crop Protection
10:00	Florence Ly (UWA): Building synthetic gene circuits in moss for on-demand biomolecule production in space
10:15	Maxim Harding (U Queensland): Bacteriophage P22 virus-like particles as nanoscale protein scaffolds for plant synthetic biology
10:30	Morning Tea (Oceania Room)
	Session VI: Public and social policy of synthetic biology (Soundings theatre)
11:10	Invited: Juliet Gerrard (University of Auckland): Policy, politics, and emerging technologies – navigating through the maze
11:40	Invited: Aditi Mankad (CSIRO): Understanding Public Perceptions of Synthetic Biology in Australia: Psychological Insights for Guiding Innovation
12:10	Fran Humphries (CSIRO): Regulating Bio innovation to Advance the Bioeconomy
12:25	Revel Drummond (BSI): The New Zealand system of Gene technology regulation
12:40	Lunch break (Oceania Room)
	Session VII: Metabolic engineering and synthetic biology tools (Soundings theatre)
13:40	Invited: Anthony Poole (U Auckland): Could synthetic cells operate without DNA?
14:10	Vaheesan Rajabal (Macquarie U): Uncovering the potential of integrons in synthetic biology applications
14:25	Rezwan Siddiquee (U Sydney): seekRNA – A programmable, bi-specific RNA-guided system for large-scale DNA insertion
14:40	Haosheng Shen (SynCTI): Development of a Lactobacillus-Based Autonomous Nasal Drug Delivery Systems
14:55	Brady Owen (Scentian Bio): Engineering tetrameric insect odorant receptors for biosensor development
15:10	Afternoon Tea (Oceania Room)
	Session VIII: Precision fermentation and diagnostics (Soundings theatre)
15:50	Invited: Alexander Foster (BSI): Biotech Success Paradox: Why Failing Smart Matters More Than Failing Less
16:20	Elena Eremeeva (QUT): Synthetic Nucleic Acids for Advanced Therapeutics and Precision Diagnostics
16:35	Jessica Chiang (U Auckland): The potential of New Zealand fungi as generators of biomaterials to replace expanded polystyrene
16:50	David Hooks (BSI): Precision Fermentation and Synthetic Biology for Enabling Emerging Foods in Aotearoa New Zealand
17:05	Carol J. Hartley (CSIRO): Innovation in Precision Fermentation Production of Enzymes and Proteins for Food Applications
17:20	Group photo
17:35	Networking and canape event (Oceania Room)
20:35	End of conference day

Friday, 21 November 2025

9:00	SBA Annual General Meeting
10:00	Morning Tea (Oceania Room)
	Session IX: Synthetic biology tools and climate change (Soundings theatre)
10:40	Invited: Gözde Demirer (Caltech): Synthetic biology to expand the plant genome engineering toolkit
11:10	Roy Walker (Macquarie U): Exploring the Biotechnological Potential of Refactored Killer Yeasts in the Age of Synthetic Biology
11:25	Taylor N. Szyszka (UWA): Engineering carbon-fixing protein cages for enhanced photosynthesis
11:40	Claudia Vickers (QUT): Synthetic Biology and Metabolic Engineering for the Anthropocene: Identifying Meaningful Targets to Impact on Climate Change
11:55	Henry Dixson (Western Sydney U): Climate Overshoot: Risk Ethics of Synthetic Biology for Climate Change Mitigation
12:10	Henrico Adrian (U of Technology Sydney): Accelerating the design and application of protein nanocages through cell-free protein synthesis
12:25	Lunch break (Oceania Room)
	Session X: Advances in genetic circuitry and engineering biosynthesis - Sponsored by the Centre of Biodiscovery (Soundings theatre)
13:25	Invited: Keith E. Shearwin (University of Adelaide): Rewiring bacteriophage circuits for programmable packaging and delivery
13:55	Invited: Yit-Heng Chooi (UWA): Beyond Nature: Harnessing NRPS Engineering and Enzyme Promiscuity for Novel Natural Products
14:25	Sacha Pidot: Breaking the Silence: Custom Promoter Design to Awaken Cryptic BGCs in Nocardia
14:40	Rose McLellan (VUW): Non-Canonical Type II Terpene Cyclases Deliver Rare Terpenoid Architectures
14:55	Anna Donnan (U Canterbury): iGEM in New Zealand: Unique Opportunities for Growing Synthetic Biology through Student-led Innovation
15:10	Afternoon Tea (Oceania Room)
	Session XI: Computational tools and high-throughput screening (Soundings theatre)
15:40	Helen Power (CSIRO): Integrating Conventional Methods with Artificial Intelligence for Accelerated Protein Function Discovery
15:55	Siyi Xue (U Melbourne): Yeast whole cell biosynthesis of nororipavine via N-demethylation
16:10	Tim McCubbin (U Queensland): A Metabolic-Model Driven Exploration of the Dynamic Shifts in Cyanobacterial Metabolism across a Batch Fermentation Process
16:25	Mariana Velasque (CSIRO): GEMOmics: A Fully Open Source Workflow for Omics Driven, Condition Specific Metabolic Modelling
16:40	Jessica James (U Oxford): Survival of the Brightest: Evolving Dynamic Phenotypes via Light-Based Microfluidic Selection
16:55	Poster awards - Sponsored by Bioplatforms Australia
17:10	Closing remarks
17:25	Farewell - Light refreshments and drinks (Shed 22)
18:55	End of conference

Abstracts

Abstracts are ordered by the presenting author's last name. Due to space constraints, we have generally limited the authors that are listed to only the presenting author, first five additional authors and final author.

Speaker Abstracts

Accelerating the design and application of protein nanocages through cell-free protein synthesis

Henrico Adrian,[1] Zhenling Cui,[2] India Boyton,[1] Claire Rennie,[1] Yi Wen Ng,[1] Dennis Diaz,[3] Kirill Alexandrov,[2] & Andrew Care[1]

[1]University of Technology Sydney, Sydney, Australia

[2]Queensland University of Technology, Brisbane, Australia

[3]All G Foods, Sydney, Australia

Protein nanocages are highly organised macrostructures that self-assemble from multiple protein subunits. Their modifiable inner/outer surfaces and cargo loading abilities have made them engineerable platforms for creating novel bionanotechnologies, ranging from biocatalysts, biomaterials, drug carriers, and vaccines.

Protein nanocages are conventionally produced through *in vivo* recombinant expression, which is constrained by physiological cell requirements and lengthy molecular biology procedures. Cell-free protein synthesis (CFPS) is an alternative biosynthesis method that circumvents many of these constraints. CFPS conducts protein synthesis *in vitro* using crude extracts or purified transcription-translation components, enabling rapid (≤4 hr) nanocage synthesis. Their open nature also enables facile control over reaction parameters and nanocage modification. As a result, CFPS has become an exciting tool for accelerating design-build-test cycles for protein nanocage engineering.

Encapsulins are an emerging class of protein nanocages which comprise multiple identical subunits. Their simplicity makes them highly suitable for CFPS, which remains relatively unexplored. Here, we explore novel engineering applications unlocked by CFPS of encapsulins. First, we established a cell-free synthesis workflow and produced all three encapsulin architectures in <6 hr. Importantly, cell-free encapsulins assembled identically to their *in vivo* counterparts. Following this, we proceeded to investigate three application domains:

(1) Using a modified encapsulin subunit, we site-specifically incorporated unnatural amino acids onto surface-exposed residues through simultaneous amber codon suppression and sense codon reassignment. These residues were bioorthogonally conjugated with fluorophores through click chemistry and the tagged nanocages used to track cell uptake

(2) A prospective vaccine library was created by inserting a disease-specific antigen at multiple flexible residues on a newly discovered encapsulin surface. CFPS was used to rapidly screen this library in <2 days, identifying residues most permissive to genetic inserts without disrupting assembly. Three vaccine candidates were chosen for pre-clinical testing in mice, which generated high antibody titres

(3) Cell-free produced encapsulins were loaded into liposomes, creating artificial cells with sub-cellular compartments. Our current work aims to synthesise encapsulins inside liposomes to enable selective compartmentalisation and controlled enzymatic pathway tuning for enhanced or new-to-nature bioprocesses

Our work highlights the synergistic benefits that CFPS grants in encapsulin engineering and establishes a versatile system for extending protein nanocage applicability in synthetic.

Machine Learning Meets Cross-Eyed Protein Engineering: Building Artificial Protein Switches

Zhong Guo,[1,2,3] Oleh Smutok,[4] Gyu Rie Lee,[5,6,7§] Cagla Ergun Ayva,[1,2,3] Haocheng Qianzhu,[8] Kejia Wu,[5,6,7] Colin J. Jackson,[1,8,9] Maria M Fiorito,[1,2,3] Oliver B. Smith,[1,8] Alfredo Quijano Rubio,[10] Thomas Huber,[8] Gottfried Otting,[8] Evgeny Katz,[4] David Baker,[5,6,7] & **Kirill Alexandrov**[1,2,3*]

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[2]Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, QLD, 4001, Australia

[3]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, QLD, 4001, Australia

[4]Department of Chemistry and Biochemistry, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, USA

[5]Department of Biochemistry, University of Washington, Seattle, WA, USA

[6]Institute for Protein Design, University of Washington, Seattle, WA, USA

[7]Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA

[8]Research School of Chemistry, Australian National University, Canberra, ACT, 2601, Australia

[9]ARC Centre of Excellence for Innovations in Peptide & Protein Science, Australia

[10]Monod Bio, 700 Dexter Ave N, Suite 700, Seattle, WA, 98109, USA

Allosteric regulation of proteins is often referred to as the “second secret of life” because it governs nearly every aspect of information and energy processing in biology. As a result, the construction of artificial allosteric proteins capable of converting one type of biochemical signal into another has become a central goal of synthetic biology.

We employed structure-based protein engineering to create artificial allosteric enzymes controlled by input signals of choice. Switches based on hydrolases, light-emitting enzymes, and redox enzymes required minimal optimisation and exhibited dynamic ranges of up to 9,000-fold. We further demonstrate that machine learning–based protein design can be effectively integrated into the protein switch development pipeline, enabling the generation of fully synthetic biosensors.

To evaluate the utility of the constructed switches, we employed them as the foundation for ultra-low-cost diagnostic assays compatible with high-throughput diagnostic workflows. We demonstrate the detection of proteins and small molecules in human samples using standard chemical pathology equipment. Furthermore, we utilised protein switches with electrochemical outputs to build sensory bioelectrodes capable of converting analyte binding into electric current, thereby enabling the development of low-cost point-of-care diagnostic devices.

Engineering yeast to investigate the hidden role of 'Cheater"-suppression in the evolution of germ-soma specialization

Silvio S. Bonni,[1] Austen R. D. Ganley,[1] Nobuto Takeuchi,[1] & Anthony M. Poole[1]

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

Reproductive division of labour (RDL)—where non-reproductive ‘helpers ’support reproductive individuals to maintain a larger collective—is a widespread evolutionary strategy found across life. One example is in multicellular organisms, where labour is divided between somatic cells that generate public goods to sustain the organism and germ cells that pass on genetic material to the next generation. This raises the question of why soma cells altruistically sacrifice their own reproduction to provide public goods to the collective. Contrary to the standard view that division of labour makes collectives more efficient, we think it is because RDL provides protection against ‘cheaters’—cells that benefit from the public goods without paying the costs. These cheaters threaten the stability of the collective and we suggest this can be overcome by restricting reproductive capacity to a few specialized germ cells, thus reducing the likelihood of cheaters arsing. To test this hypothesis, we are using synthetic biology to build ‘multicellular ’Saccharomyces cerevisiae collectives where individual cells can differentiate between germ and soma cells, with germ cells relying on non-reproductive somatic cells to metabolize sucrose, a public good essential for growth. Differentiation from germ to soma involves a tuneable site-specific recombination event, allowing us to regulate the germ/soma ratio. Similarly, cheaters, which reproduce but do not differentiate or produce public goods, arise by another tuneable recombination event, allowing us to independently control their frequency. By studying how the stability of our engineered multicellular collectives varies in response to germ/soma ratio and cheater production rate, we will determine whether protection from cheaters provides reproductive divisions of labour with the advantage that has driven their emergence multiple times across life.

Synthetic biology tools to enable engineering of environmentally resilient plants

Jennifer A. N. Brophy,[1] Vivian Zhong,[1] Isabel Goldaracena Aguirre,[1] Janina Tamborski,[1] Aditya M. Kunjapur,[2] Michaela Jones[2] *et al*

[1]Stanford University, Stanford, California, United States of America

[2]University of Delaware, Newark, Delaware, United States of America

Plants lie at the heart of many potential solutions to the climate crisis. Yet, it can take years to breed plants with new, useful features. Synthetic biology could expedite the development of diverse climate-resilient crops through precise manipulation of gene expression across cell types and environments. This level of control is essential for improving complex traits, like yield and resilience, which are often determined by cell- and developmental stage-specific gene activities. We are developing synthetic genetic circuit tools to enable precise gene expression control across cell types and tissues in plants. These genetic circuits use logical operations to combine the activity of tissue specific promoters and create new patterns of gene. We are applying genetic circuits to engineer the size and shape of plant roots to better understand their contribution to environmental stress tolerance. A better understanding of the root features that are important for environmental stress tolerance should enable targeted breeding and biotechnological interventions that improve agricultural sustainability.

Synthetic biology tools for optimising the biosynthesis of natural products in filamentous fungi

Rosannah Cameron,[1] & Emily Parker[1]

[1]Ferrier Research Institute, Victoria University of Wellington, Wellington, New Zealand

Filamentous fungi are extraordinary natural chemists, synthesising structurally diverse natural products with potent bioactivities. Despite their bioactive potential, comprehensive characterisation and large-scale production of these compounds remain constrained by limited yields in native hosts. Through synthetic biology approaches and heterologous expression systems, we seek to overcome these production bottlenecks and unlock the full potential of fungal natural products.

We focus on indole diterpenes (IDTs), a family of natural products exhibiting diverse bioactivities including anti-insectan, anti-microbial, and anti-cancer properties. Our approach leverages the filamentous fungus *Penicillium paxilli* as a heterologous host, capitalising on its natural capacity for high-level IDT production as an optimal chassis for bioengineering applications.

To facilitate precise genetic manipulation and biosynthetic pathway optimisation, we have developed robust CRISPR-Cas systems for *P. paxilli*, including an innovative co-selection method for targeted genome editing that exploits melanin biosynthesis as a visual marker. This system enabled us to probe the function of PaxA, a previously overlooked enzyme that we recently identified as playing a crucial role in early IDT biosynthesis. Through targeted mutagenesis, we identified key catalytic residues essential for PaxA function.

Our CRISPR-Cas system provides a versatile platform for both strain optimisation and mechanistic investigation of natural product biosynthesis enzymes within their native cellular environment. This approach demonstrates the utility of precision genome editing for advancing our understanding and enhancement of natural product biosynthesis in filamentous fungi.

The potential of New Zealand fungi as generators of biomaterials to replace expanded polystyrene

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With an increased appreciation for using biological material as a renewable natural resource, mycelium materials have gained momentum in the last decade as environmentally friendly alternatives to plastic foams. Expanded polystyrene (EPS) is a versatile and low-cost packaging material used widely industrially. However, being nonbiodegradable and with a lack of infrastructure for recycling, the negative impact of EPS lasts much longer than its product life, especially for single-use packaging. Mycelium materials comprise fungal mycelium bonded with lignocellulosic substrates to form a spongy composite resembling EPS. The composites are biodegradable, can valorise lignocellulosic agricultural wastes, and, in bulk, can contribute to carbon sequestration.

To realise the potential of fungal mycelia in the production of new materials, this project investigates the suitability of New Zealand native fungi grown in hemp substrates to select species that produce materials with the basic physical properties (e.g., strength, density, hydrophobicity) comparable to or better than those of EPS. Furthermore, intrinsic chemical properties that provide additional commercialisation value to the bio-composite are investigated. 18 native fungal strains were selected from the New Zealand International Collection of Microorganisms from Plants (ICMP) and three native fungal strains were provided by the industry. After a series of growth tests on agar plates, three ICMP strains, along with the three industry strains, proceeded to the next stage of testing. These six fungal strains were used to create mycelium materials, which were then evaluated for their physical and chemical properties by industrial testing standards and compared with EPS.

While no single fungal strain exhibited all the desired properties as an EPS alternative, each strain demonstrated unique strengths in different areas. We hypothesise that the diverse properties exhibited by NZ native fungi make each species uniquely suited for various applications as biodegradable EPS alternatives.

Beyond Nature: Harnessing NRPS Engineering and Enzyme Promiscuity for Novel Natural Products

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Microbial natural products underpin many therapeutics, yet their structures evolved for ecological roles rather than human health. Synthetic biology offers the means to refit these molecules for improved target engagement and pharmacokinetics. Nonribosomal peptide synthetases (NRPSs) are modular megaenzymes capable of incorporating hundreds of different monomers, making them powerful platforms for structural diversification. In addition, many tailoring enzymes such as cytochrome P450s, halogenases, and glycosyltransferases exhibit remarkable substrate promiscuity, enabling further scaffold modification.

We combined NRPS domain swapping with the exploitation of naturally promiscuous biosynthetic enzymes to expand fungal alkaloid diversity. As a primary case study, anthranilate-incorporating

NRPSs from *Aspergillus* spp. were re-engineered by module exchange at defined condensation–adenylation boundaries, yielding multiple “new-to-nature” quinazolinone–ketopiperazine analogues. In parallel, we showed that the P450 in the pathway could accept the new quinazolinone–ketopiperazine analogues to modify the peptide core scaffold. In another example, we leveraged a promiscuous NRPS that ligates single amino acids to aromatic acids, halogenases, and glycosyltransferases, to generate analogues.

This dual strategy has produced a rich set of scaffolds for future structure–activity relationship studies. It showcases how engineering of NRPSs (and other “backbone” enzymes), coupled with opportunistic use of enzyme promiscuity, can systematically expand chemical space for drug discovery.

Synthetic biology to expand the plant genome engineering toolkit

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Plants are central to addressing urgent global challenges in food security, sustainability, and climate resilience. While plant genetic engineering offers a powerful route to accelerate crop improvement, it remains constrained by slow, inefficient, and technically demanding transformation and genome editing workflows.

In this talk, I will share our lab’s recent advances in overcoming key barriers to plant engineering through novel nanomaterial and synthetic biology approaches. We have developed self-assembling protein nanoparticles that enable efficient delivery of biomolecules—including proteins—into plant cells, supporting DNA-free gene editing approaches suitable for field deployment. In parallel, we have expanded the plant genome editing toolkit by establishing CRISPR-associated transposases and R2 retroelements as platforms for efficient, targeted DNA insertion. Finally, we are engineering plant–microbe interactions in the rhizosphere, designing strategies to reprogram both the microbiome and plant signaling pathways to create more resilient, sustainable crop systems.

Together, these tools advance the frontier of plant synthetic biology, offering scalable solutions for next-generation agriculture.

Synthetic starvation: combining natural and engineered responses to nutrient limitation in *Vibrio natriegens*

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The marine bacterium *Vibrio natriegens* holds great potential as a next-generation chassis for biomanufacturing. Offering a doubling time of less than ten minutes, a versatile metabolism, and the ability to be cultured in seawater-based media, *V. natriegens* is poised to deliver improved economic and environmental sustainability in industrial bioprocesses.

A systems-level understanding of this bacterium's remarkable metabolism is key to unlocking its potential. Here, we report how *V. natriegens* naturally partitions its metabolism between growth and production in response to selective nutrient limitation. We harness this trait as a case study in "synthetic starvation," demonstrating how metabolic engineering strategies can be conditionally implemented within a naturally occurring starvation response. This generalisable strategy is particularly useful when designing microbial host strains for two-stage fermentations. In such processes, engineered gene expression programs that are optimal in the production stage may be incompatible with the growth stage.

To implement synthetic starvation in *V. natriegens*, we first identified the molecular agents and mechanisms that orchestrate the starvation response. Next, we developed a suite of molecular tools that enable the selective expression or repression of both native and heterologous genes in response to nutrient limitation. Specifically, we generated a library of starvation-responsive promoters, and designed a nutrient-responsive CRISPRi system for conditional gene knockdowns. Finally, we explored how these tools can be applied to construct efficient *V. natriegens* production strains for a two-stage fermentation process.

Flippase-Mediated Division of Polymer-Lipid Hybrid Vesicles as Artificial Cell Scaffolds

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Bottom-up synthetic biology is an approach that involves the assembly of life-like units using designed building blocks, often involving the assembly of artificial cells.[1] A key application of these life-like units is the investigation of biological processes. Polymer-lipid hybrid vesicles are an alternative artificial cell scaffold to liposomes and polymersomes that combine the self-assembly ability of the lipids and the benefits of modern polymer chemistry. Recently, we illustrated the *in situ* microtubule network formation in the lumen of hybrid vesicles. In addition, we explored the use of artificial intrinsically disordered proteins that undergo liquid–liquid phase separation and recruit model proteins, in combination with hybrid vesicles, as a minimal system to gain insight into the complex cell biological process of membrane repair.[2]

Self-replication of artificial cells remains a long-standing challenge. To this end, we investigated the ability of a membrane-modulating enzyme, specifically lipid flippase, which utilizes anionic lipids as substrates, to tune the transmembrane asymmetry, thereby leading to the constriction and division of artificial cells. First, ATP depletion experiments confirmed the presence of the flippase in the hybrid vesicle membrane. Furthermore, we conducted an Annexin-binding assay on both small and giant hybrid vesicles, which provided evidence of anionic phospholipid flipping, which resulted in controlling the distribution of the membrane building blocks, i.e., transmembrane asymmetry of anionic lipids in the inner leaflet. Furthermore, the transmembrane asymmetry induced changes in the spontaneous curvature of the bilayers, resulting in constriction and division of the vesicles. Taken together, these findings illustrated that the use of a single protein was sufficient to include giant hybrid vesicle division as a first step towards a self-sustained artificial cell.

REFERENCES

1. I. N. Westensee, P. de Dios Andres, B. Städler, Adv. Mater. Technol. 2024, 10.1002/admt.202301804

2. P. de Dios Andres et al. Adv. Mater. 2025 (Accepted)

Climate Overshoot: Risk Ethics of Synthetic Biology for Climate Change Mitigation

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Synthetic biology is increasingly researched as a tool for climate intervention, particularly in response to the growing recognition that climate overshoot is now inevitable. This joint paper brings together two approaches to understanding this shift in both public and social policy: critical analysis of the ethics of risk around such SynBio interventions, and new empirical research on Australian public reasoning about SynBio for climate change mitigation.

The empirical component reports on a national public engagement study (2023 to 2026). This has included comparing views across groups divided by their agreement or disagreement with the proposition of climate overshoot and the use of SynBio for such mitigation. Participants considered a range of SynBio strategies—emissions removal via Negative Emissions Technologies, SynBio conversion of GHGs into products, infrastructural development of industrial alternatives, and social policy for multi-benefit approaches. Analysis shows contrasting narratives shaping how advanced bioengineering is perceived within Australia, as well as existential concerns raised by climate change and climate mitigation. Participants harbour concerns about ecological integrity alongside differing visions of intergenerational impact. Findings reveal how public reasoning around upstream SynBio technologies are likely to shape public discourse and policy downstream.

In parallel, the paper draws on Environmental Humanities research to examine the conditions under which SynBio is imagined as a climate change technofix. Bioengineered interventions are increasingly framed as necessities rather than options. This framing carries profound implications for social policy and the role of radical uncertainty in climate action and policy. Investigating the risk ethics of these interventions offers a counterpoint to normative visions of risk analysis and control.

Together, this work examines new ways of imagining SynBio-based climate bioengineering, by considering how it can be explored in the context of climate overshoot.

iGEM in New Zealand: Unique Opportunities for Growing Synthetic Biology through Student-led Innovation

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This talk will introduce the International Genetically Engineered Machine (iGEM) competition, a global platform where students design and implement synthetic biology solutions to real-world problems. Beyond fostering technical skills, iGEM nurtures collaboration, innovation, and entrepreneurship—contributing to both local and global bioeconomic growth. Since its inception, iGEM has catalysed over 250 startups worldwide and sparked numerous international research collaborations, providing a structured pathway for cultivating the next generation of synthetic biology researchers and innovators.

Despite these benefits, New Zealand's participation in iGEM has been historically limited. Key barriers include a shortage of principal investigators (PIs) willing and able to lead teams—an essential role for providing supervision and scientific direction —and the misalignment between New Zealand's academic calendar and iGEM's global competition schedule, which creates timing conflicts with coursework and exams.

This presentation will share insights from launching an iGEM framework initiative at the University of Canterbury in 2024. This pilot embedded iGEM into the curriculum via a credited course module, making participation more accessible and sustainable. It will highlight the advantages of investing in iGEM—for PIs, students, institutions, and the wider biotechnology community—while also providing practical information and frameworks to support the establishment of new teams. Expanding iGEM participation nationwide can help build a coordinated synthetic biology community, strengthen education pathways and grow the national synbio network.

The New Zealand system of Gene technology regulation

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In 2025-26, the way in which New Zealand regulates the use of genetic technologies will undergo enormous change. Currently two Acts directly constrain the use of genetic technologies in NZ, the Food Act (2014) and the HSNO Act (1996). After a lengthy process FSANZ will redefine the term "genetically modified food" to include only those foods that contain "novel DNA". Starting much later but rapidly catching up are the changes to the HSNO Act that will remove all reference to genetically modified organisms. Instead regulating these under their own new and bespoke Gene Technologies Bill (2024). The stated intent is to put in place a system that is enabling and risk proportionate, suggesting that the HSNO Act is neither of these. Balancing the benefits and risks of any technology requires careful consideration of science-based evidence. However, as the purpose of regulation is to protect that which society values from harm additional thought around the technology and its place in society is required. These Acts, the changes to them, and their impact on the use of genetic technologies in NZ will be discussed.

Boosting Carbon Yield of Bioprocesses via Synthetic Chemolithoheterotrophy

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In bioprocesses, NAD(P)H is the universal redox currency driving biosynthesis and ATP generation. Yet in chemoheterotrophs its regeneration is inherently tied to carbon oxidation in the central carbon metabolism—diverting 30–50% of available carbon towards CO_2 rather than product formation.

To overcome this limitation, we explore synthetic chemolithoheterotrophy—an approach where inorganic electron donors decouple NAD(P)H supply from carbon metabolism. Metabolic models predict that such redox interventions could significantly increase carbon retention, reducing the reliance on carbon-derived reducing power. Furthermore, the models suggest that pathways such as anaplerotic reactions and a threonine aldolase reaction, present in some heterotrophic organisms like *Pseudomonas putida* and *Saccharomyces cerevisiae*, can enhance CO_2 recycling and its co-assimilation, facilitating a mixotrophic growth mode within a chemolithoheterotrophic framework.

Initial experimental data with recombinantly expressed O_2-tolerant NAD+-reducing hydrogenases confirm increased biomass yields with *P. putida* cells utilising hydrogen as an inorganic electron donor. Experiments with NAD+-dependent phosphite dehydrogenases and phosphite as inorganic electron donor led to co-assimilation of CO_2 when supplied as an additional carbon source. In both cases, biomass yields from the main carbon source increased by 30 %. We further explore the application of synthetic chemolithoheterotrophy for C1 and C2 by-product assimilation for efficient lignin valorisation.

This strategy holds promise for a wide range of applications, from microbial production of highly reduced molecules such as biofuels to engineered carbon capture and bioprocesses with improved sustainability through reduced biological CO_2 emissions. By rewiring redox metabolism, we aim to push carbon yields beyond conventional limits, paving the way for novel redox engineering strategies and a new generation of highly efficient bioprocesses.

Synthetic Nucleic Acids for Advanced Therapeutics and Precision Diagnostics

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Biopolymers such as DNA and RNA form the foundational basis of genetic information across all known life forms. However, their canonical nucleotide composition inherently restricts their chemical diversity and functional versatility. To overcome these limitations, we developed novel informational polymers—termed DZA and RZA—comprising chemically modified non-canonical nucleotides like 7-deaza-adenine and 5-fluorocytosine. These artificial nucleic acids have demonstrated exceptional efficiency in encoding genetic information both in vitro and in vivo, opening new avenues for therapeutic protein expression, precision gene editing technologies such as CRISPR-Cas9, and innovative oligonucleotide-based drug design.

Building upon advances in sugar-modified nucleic acids, we engineered Xeno Nucleic Acid (XNA) aptamers exhibiting superior stability and selectivity against clinically significant targets, including VEGF and thrombin. At QUT, our team employed the powerful biorecognition capabilities of aptamers to create highly sensitive biosensors targeting challenging biomedical molecules such as methotrexate (MTX), MTX-nanobody complexes, and salivary biomarkers for heart failure, including S100A7 and galectin-3. Integrated into fluorescence and calorimetric detection platforms, these aptamer-based biosensors significantly enhance diagnostic precision, lower costs, and improve stability, driving transformative developments in portable precision diagnostics.

Biotech Success Paradox: Why Failing Smart Matters More Than Failing Less

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Conventional approaches to research and development in biotechnology emphasise minimising failure as the pathway to success. But in complex, uncertain domains like synthetic biology, success rarely comes from avoiding mistakes; it comes from discovering them early.

This presentation proposes a shift from a risk-averse mindset to one that embraces strategic failure as an accelerant for innovation. Drawing on industry case studies and practical frameworks, we explore how resource-constrained teams can adopt more adaptive, evidence-driven development processes. Emphasis is placed on early-stage flexibility, dynamic prioritisation, and the intentional design of experiments that generate insight, regardless of outcome.

By embracing the counterintuitive idea that failing more frequently, but failing smart, accelerates innovation, this talk offers a new lens on how to allocate time, money, and talent. Attendees will gain practical tools for navigating synthetic biology's inherent uncertainty with greater clarity and momentum. This isn't about avoiding failure, it's about learning fast, adjusting faster, and building better systems for discovery. In short: a no-nonsense guide to succeeding in synthetic biology and fermentation by getting comfortable with getting it wrong, early and often.

Toward Engineered Living Materials in Seawater: A New Role for *Vibrio natriegens*

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The search for sustainable strategies to repair and enhance marine ecosystems is converging with advances in synthetic biology. Here we explore the potential of *Vibrio natriegens*, a fast-growing and metabolically versatile marine bacterium, as a chassis for engineered biomineralisation in seawater environments. Our work reveals that *V. natriegens* can modulate its surrounding pH through organic acid metabolism, triggering calcium carbonate precipitation under marine-like conditions. This metabolic control may be further influenced by native enzymatic activities and offers intriguing design space for environmental tuning.

Concurrently, *V. natriegens* accumulates biodegradable biopolymers under conditions conducive to mineral formation—suggesting a dual role in carbon storage and material synthesis. These properties position *V. natriegens* as a unique candidate for developing living construction systems in the ocean, such as programmable microbial platforms for artificial reef scaffolding or carbon-sequestering marine biocomposites. We present this emerging system as a foundation for scalable, seawater-compatible engineered living materials with applications in reef restoration, carbon capture, and ocean-based biomanufacturing.

Policy, politics, and emerging technologies – navigating through the maze

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Much has been written on the need to build better governance structures around emerging technologies, and yet the reality is far from ideal. Science races ahead of the policy agenda, which struggles to play catch up to harness the benefits and mitigate the risks of new science. What role can scientists play to bridge the chasm between emerging technology and policy settings? In this talk, Juliet will outline some of the challenges we face in this domain, and offer some practical tips for how to make a difference in Aotearoa New Zealand.

Bacteriophage P22 virus-like particles as nanoscale protein scaffolds for plant synthetic biology

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Advancements in synthetic biology continue to expand our ability to produce valuable molecules at reduced environmental and economic costs. Plants are promising biosynthetic hosts due to their minimal input costs, diversity of targetable tissues for product expression (i.e. leaves, seeds, fruits, roots), and their sophisticated subcellular environment which can be targeted for engineering production of secondary metabolites, or proteins and peptides with complex post-translational modifications. *Nicotiana benthamiana* is the most extensively employed biofactory plant due to its quick growth, high biomass, capacity for high protein expression and ease of transient expression which can avoid the need for developing stable transgenic events. High-value therapeutic products such as monoclonal antibodies, vaccines, peptides, and small molecule drugs and their precursors have all been recombinantly produced in *N. benthamiana*. Though there are a diversity of protein engineering approaches for optimizing recombinant production in plants, an expanded repertoire of protein engineering tools will enhance the durability of plants as biofactory organisms. Here, we explore the expression of P22 bacteriophage virus-like particles (VLPs) in *N. benthamiana* and demonstrate the ability of these proteins to serve as self-assembling nanoscale scaffolds, amendable to tailored bioengineering applications in planta.

Transient expression of P22 structural proteins in *N. benthamiana* delivers correctly assembled VLPs, with the P22 coat protein accumulating at over 7% of total soluble protein. Moreover, we showed that two different species of cargo proteins can be co-encapsulated in *N. benthamiana* produced P22 VLPs at similar loading densities to those achieved in *Escherichia coli* – exemplifying the programmability of plant-produced P22 VLPs and opening the possibility for future metabolic engineering applications. Beyond this capacity, we demonstrated the ability to modify the external surface of P22 VLPs in planta through engineering a SpyTag domain to the exterior of the P22 coat protein and co-expressing a SpyCatcher-cargo fusion for highly selective protein ligation – demonstrating the ability for both internal and external scaffolding with P22 VLPs in planta. Finally, we explore the capacity of plant-produced P22 VLPs to act as cancer-targeting payload delivery vehicles by engineering cellular tropism through external functionalization with a receptor-targeting affibody. This is the first report of a bacteriophage VLP being applied to protein engineering in *N. benthamiana* and establishes P22 VLPs as a versatile protein scaffolding system for synthetic biology applications in plants.

Innovation in Precision Fermentation Production of Enzymes and Proteins for Food Applications

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"Precision fermentation," combining synthetic biology and fermentation advances to develop enhanced yeast and fungal production systems, is transforming the way in which food ingredients and products are produced, creating a wide array of possibilities for producing food more sustainably.[1]

We have optimised and developed a number of precision fermentation production strains for enzyme and recombinant protein food applications. This talk will discuss key elements in synthetic biology strain engineering, fermentation process development, and downstream processing for extracting proteins and enzymes.

Some of the key issues for bringing precision-fermentation-derived protein ingredients to market will also be covered, including challenges relating to regulation, sourcing alternative sustainable feedstocks, and scaling of the fermentation process. Examples of research applications advancing precision fermentation for food ingredients will be highlighted.

[1] Augustin MA, Hartley CJ, Maloney G, & Tyndall S. Innovation in precision fermentation for food ingredients. *Critical Reviews in Food Science and Nutrition*. 2023.

Advancing synthetic gene circuitry in plants with novel split dCas12a-based tools

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A central aim of plant synthetic biology is to develop sophisticated, programmable gene circuits that not only enhance stress resilience and optimise agricultural performance, but also transform plants into biofactories for the on-demand synthesis of valuable products such as pharmaceuticals. Construction of complex gene circuits requires molecular tools that are modular, orthogonal, and highly programmable, enabling precise spatio-temporal control of native or engineered gene expression. However, the availability of such tools validated for robust use in plants remains limited.

My research addresses this critical need by expanding the plant synthetic biology toolkit with the development of novel split dCas12a-based synthetic transcription factors (synTFs). This represents the first successful demonstration of a split dCas12a-based system in plants. I systematically optimised these synTFs in *Arabidopsis thaliana*, where they were found to be highly potent, achieving up to 474-fold activation of transcription. I also demonstrated the inducibility and reversibility of the split dCas12a system via its rapid and dynamic response to the key environmental signal of heat stress. I further validated the function of these split dCas12a-based synTFs across diverse synthetic and endogenous promoters, plant tissues, and species, confirming their versatility and broad utility as robust tools for regulating gene expression in plants.

A significant advance in genetic circuitry was achieved by demonstrating that the split dCas12a-based CRISPR activation system can be seamlessly integrated with existing dCas9-based CRISPR interference circuit modules to construct multi-layered hybrid circuits. Here, split dCas12a and dCas9 function alongside each other to enable simultaneous activation and repression within the one circuit, unlocking more sophisticated genetic programming. Additionally, I developed split dCas12a-based synTFs for CRISPR interference that achieved up to 93% repression of transcription, and were successfully implemented in constructing a universal Boolean logic NOR gate, providing an essential foundation for scalable circuit design.

Together, these innovations unlock new possibilities for the construction of synthetic gene circuits in plants that possess the complexity and precision required for real-world applications. They provide a robust foundation for addressing key challenges in agriculture through the development of crops with enhanced and novel traits for improved yield and stress tolerance. In parallel, these circuits open new opportunities for sustainable plant-based bioproduction, positioning plants as programmable multi-purpose biofactories capable of synthesising a range of valuable products on demand, thus contributing significantly to the growing bioeconomy.

Precision Fermentation and Synthetic Biology for Enabling Emerging Foods in Aotearoa New Zealand

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Globally, demand for foods with lower environmental impact, enhanced health benefits, and ethical sourcing is rising. BSI|AgResearch Enabling Emerging Foods programme is advancing New Zealand's food systems by developing a precision fermentation platform that leverages synthetic biology, fermentation engineering, and local feedstocks to produce novel food ingredients and functional proteins.

This talk outlines our integrated approach to a precision fermentation platform that capitalises on New Zealand's agricultural strengths, supports participation in the global bioeconomy, and enables innovative, high-value food products beyond traditional animal and plant sources.

Our programme focuses on scientific pipelines spanning synthetic biology, bioprocessing, and regulatory frameworks essential to sector growth. Central to this is a yeast-based production platform targeting the dairy-identical proteins lactoferrin and osteopontin. We utilise expression systems in *Komagataella phaffii* (*Pichia pastoris)* and related hosts equipped with synthetic promoters designed for food-safe production. Strain development incorporates mutagenesis and adaptive laboratory evolution to enhance yield, substrate tolerance, and robustness in cost-effective agricultural hydrolysates under varying process conditions.

A unique advantage is our alignment with New Zealand's diverse feedstock landscape. We explore valorisation of pasture species side streams via pre-treatment and hydrolysis to produce sugar-rich media for microbial fermentation. Partnerships with biomass processors and forestry-derived woody biomass technologies enable a modular, substrate-flexible platform operable across regions and integrated with existing supply chains. Additionally, leveraging sugar-rich dairy permeates promotes circularity and relevance in our local context.

This presentation will share progress, from technology development and scaling challenges to strain optimisation insights and commercialisation pathways. We invite collaboration within the Australasian synthetic biology community to collectively build a resilient regional ecosystem that drives food system transformation through science-led innovation.

Regulating Bio-innovation to Advance the Bioeconomy

Fran Humphries[1]
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Access and Benefit Sharing (ABS) is a legal tool for governing the collection and use of genetic resources, associated traditional knowledge, and, in some cases, digital sequence information for research and development (R&D) purposes. ABS law and policy promote the equitable sharing of monetary and non-monetary benefits from the use of genetic resources and knowledge. There is little evidence that ABS modelled under the Nagoya Protocol framework is achieving benefit sharing, sparking calls to rethink governance in ways that better support bio-innovation and ABS objectives.

This presentation argues that regulatory inefficiency is driven by linear misassumptions about R&D that underlie the frameworks and procedures. It rethinks ABS decision-making and legal tools through a circular economy lens because:

(a) circular bioeconomy systems are designed to accommodate non-linear R&D, value chains, and resource flows, offering lessons for ABS regulation to enable more effective benefit sharing; and

(b) bio-R&D plays a key role in the transition towards a circular economy, requiring transformation in benefit-sharing systems.

Drawing from 2025 survey data about Australian researcher engagement with benefit sharing and regulatory frameworks, it proposes a pathway for transforming the existing linear 'single use' regulatory model towards a generative value chain model, supported by a range of legal tools that facilitate bio-innovation acceleration. This proposed model is part of a broader CSIRO project on a Circular Bio-economy System for more Equitable Benefit Sharing, which aims to accelerate long-term benefit sharing for bio-innovation, the planet, and its people.

Survival of the Brightest: Evolving Dynamic Phenotypes via Light-Based Microfluidic Selection

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Harnessing the functional potential of engineered proteins is a goal across medicine, manufacturing, and agriculture. Directed evolution—an iterative process of mutagenesis and selection—enables the discovery of novel proteins without requiring prior knowledge of sequence-to-function relationships. Traditionally, high-throughput selection in directed evolution relies on Fluorescence-Activated Cell Sorting (FACS), which captures only a single snapshot of cellular behaviour.

In contrast, the platform presented here leverages microfluidic chips to trap and monitor over 10,000 individual cells for up to 24 hours, allowing detailed observation of dynamic phenotypes. Selection is then performed with high precision using targeted UV light (<1 µm resolution). This approach enables the evolution of time-dependent behaviours that are effectively invisible to conventional methods like FACS. By allowing cells to be observed across time and under varying conditions prior to selection, the platform is especially well-suited for the directed evolution of biosensors.

Bringing synthetic biology to new heights: platforms for biological research in microgravity

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Over the last two decades, biological research on the International Space Station (ISS) has proven the value of the microgravity environment for developing groundbreaking pharmaceutical formulations, enabling drug discovery, cultivating microbes for precision fermentation, understanding human physiology in extreme conditions, and developing life support systems for low-resource environments, with the results of these experiments translated into novel solutions on Earth. With decreasing costs and increasing launch frequencies over the last decade, microgravity is becoming a tenable domain for delivering valuable innovations not possible on Earth, with the sector rapidly expanding as access to microgravity has become more regular and cost-effective. Despite the cutting-edge opportunities that microgravity experimentation can provide, executing microgravity experimentation is currently orders of magnitude more challenging than analogous experiments on Earth, with costs and extended experimentation timelines cited as leading reasons preventing most potential researchers from utilising microgravity. To address these challenges, our team is developing fully automated biological laboratory facilities which will enable high-throughput experimentation with real-time monitoring and data transfer from orbit. Our partnership with world-leading Axiom Space will ensure regular, frequent, and cost-effective missions to both the International Space Station (ISS) in the near term and the first commercial space station from 2027. This talk will outline the success of New Zealand's first mission to the ISS in 2024 testing our prototype experimentation facility and will also provide an overview of our team's upcoming plans to create opportunities for a diverse range of researchers to become involved in microgravity research.

Natural product biodiscovery from the bacterial symbionts of Aotearoa New Zealand endemic insects

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Microbial pathogens are increasingly developing resistance to our clinically used antimicrobial drugs. Many of the most successful antimicrobial to date are derived from soil actinobacteria such as Streptomyces sp., prolific producers of bioactive natural products. However, after decades of sustained study, effort put into isolating new drugs from soil actinobacteria is experiencing diminishing returns. One approach to find new antimicrobial is to search in alternative places. An underexplored source for new bioactive natural products are the bacteria that live in symbiosis with animals—particularly insects. Many insects are hosts for symbiotic bacteria, whose natural products may aid in digestion, communication or defense from pathogens. New Zealand is rich in insect diversity, boasting 18,000 endemic species which represent ca. 90 % of all its insect species, a proportion rivalled by few other countries. Considering such abundance and diversity together, it is surprising that there is yet to be any deep investigation of New Zealand insect symbionts as sources of new medicines. In my talk, I will discuss the challenges and successes of my team thus far, including obtaining iwi permission, collecting over 150 invertebrates, isolating bioactivity-screening over 300 insect-associated bacteria, and beginning the process of whole-genome sequencing for genome mining.

Shuffling the Genomic Deck: Engineering Bacterial Genome Diversity

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The ability to generate genomic diversity expands opportunities for understanding and engineering biology. Here, we present a molecular system for generating genomic diversity in bacteria. We engineered the fast-growing bacterium *Vibrio natriegens* to enable large-scale genomic rearrangements that produce populations of cells with diverse genome architectures. This approach provides a framework for exploring both natural and synthetic genome design principles.

Engineering synthetic gene circuitry for efficient terpene biosynthesis in *S. cerevisiae*

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Terpenoids are a highly diverse class of natural products with important applications in pharmaceuticals, fragrances, and renewable chemicals. Over the past decade, we have developed programmable *Saccharomyces cerevisiae* platforms for the high-level biosynthesis of mono-, sesqui-, and diterpenoids using synthetic biology approaches. A major focus has been the design of genetic circuits that regulate cell metabolic pathways in response to environmental or user-defined inputs. We here highlight our recent innovations in gene circuity design: 1. auxin-inducible expression systems, adapted from plant auxin inducible protein degradation system, allow for fine-tuned, orthogonal control on metabolic enzymes; 2. cold-inducible switches, which activate gene expression at low temperatures, separate cell growth and secondary metabolite production; 3. a tetracycline/temperature/auxin three-input gene circuit that regulates gene expression, metabolism, and growth in the yeast. Applying these synthetic regulatory circuits may facilitate efficient precision fermentation process to produce a wide range of valuable products (e.g., terpenoids) in yeast platforms.

Building synthetic gene circuits in moss for on-demand biomolecule production in space

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Implementing gene circuits in the plant chassis *Physcomitrium patens* (Physco) for targeted gene expression has many applications in agriculture, healthcare, and space exploration. While constitutive expression of transgenes may be useful in certain cases, energy and resources are typically used inefficiently which may damage the overall health of the plant. Instead, gene circuits offer the ability to spatiotemporally control gene expression patterns so transgenes are only turned on in certain tissue or cell types and in response to external stimuli, such as light, temperature, and chemical induction. One form of gene circuit uses site-specific recombinases to permanently alter gene regulatory components which may include promoters, coding sequences, or terminators. By driving recombinases with various inducible promoters, recombinases can be precisely controlled to rearrange or excise gene regulatory components and in turn, activate or repress a transgene. My research focusses on testing and comparing different parts required to construct gene circuits in Physco, with the goal of building a gene circuit capable of synthesising useful biomolecules for space.

As longer-term space missions are being planned, availability of common therapeutic products is a major concern for maintaining astronaut health. Physco is an attractive option for sustainable production of medicines as it is easier to stably transform than most plants and can be grown in large bioreactors. Constructing effective gene circuits in Physco for space applications requires a combination of gene circuit parts that can efficiently alter gene expression as well as a responsive induction method. Gene circuit parts including promoters, 5' untranslated regions, terminators, and multiple recombinases have been tested and compared to build our gene circuit toolkit. In addition, heat inducible systems are being characterised as localised temperature changes are a safe, feasible induction method. Thus, our lab is testing numerous gene circuit components and heat inducible systems in Physco with the goal of building a platform for precisely controlled, renewable production of therapeutic biomolecules to sustain the health of astronauts on space missions.

Understanding Public Perceptions of Synthetic Biology in Australia: Psychological Insights for Guiding Innovation

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Synthetic biology (synbio) offers promising solutions to address a range of critical environmental and industrial challenges facing Australia. Yet, its success depends not only on demonstrating the technical efficacy of innovations but also on ensuring public perspectives are integrated into the development and delivery of such innovations. Drawing on data from over 17,000 Australians, including key stakeholders, our research reveals that although public knowledge of synbio remains limited, there is widespread openness and curiosity. Support is strongest for applications that address clear public health or ecological needs—such as treating complex medical conditions or remediating environmental pollution. Conversely, applications perceived as non-essential or lacking a compelling value proposition tend to elicit lower approval. Importantly, public attitudes are shaped more by affective and value-laden responses such as emotions and perceived benefits, rather than by factual understanding alone. Risk perception plays a nuanced role, underpinning the benefit-risk trade-offs individuals make when evaluating synbio solutions. Ethical concerns (usually raised by a minority), including notions of 'playing God', often reflect deeper psychological reactions to uncertainty, perceived risk, and institutional trust. These concerns are often layered, masking sometimes intangible anxieties about unintended consequences and the adequacy of risk management. To ensure synbio innovations align with societal expectations and serve the public good, interdisciplinary collaboration is essential. Mechanisms that embed psychological insights from the outset can foster trust, enhance relevance, and guide responsible development. By integrating human perspectives into the innovation process, synthetic biology can more effectively meet the needs of our rapidly evolving society.

A Metabolic-Model Driven Exploration of the Dynamic Shifts in Cyanobacterial Metabolism across a Batch Fermentation Process

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The photosynthetic, autotrophic metabolism of cyanobacteria makes them an attractive platform for the production of high value chemicals. Owing to their photosynthetic nature, cyanobacterial metabolism varies greatly across the cultivation owing to the phenomenon of self-shading, where higher density cultures reduce light penetration, leading to dark zones in the bioreactor where respiratory metabolism or fermentation can occur. Most studies to date characterise cyanobacteria in early exponential, non-light limited growth, which is not reflective of the higher density conditions required of an industrially relevant bioprocess. We argue that the lack of characterisation of metabolism under these conditions reduces the ability to rationally design metabolic engineering strategies.

In this work, we seek to create a mechanistic model of the cyanobacterial batch bioprocess. We first designed and manufactured flat-panel bioreactors, which were subsequently used to culture wild-type freshwater and marine *Synechococcus elongatus* strains. Metabolomics and proteomics data collected over the batch cultivation process demonstrates distinctly different metabolisms between the freshwater and marine strains. Using metabolic models, we are able to deconvolute the differentially expressed proteins between phases to predict distinct metabolic operation modes which occur in different regions of the bioreactor. To better explore these metabolic modes, we next seek to couple protein-constrained models with mixing models of the photobioreactors. The resulting mechanistic model of the photobioreactor will allow the prediction of metabolic fluxes in a spatially defined way, and allow for the prediction of more global optimisation strategies across the alternative metabolic modes; facilitating more optimal strain design strategies.

Non-Canonical Type II Terpene Cyclases Deliver Rare Terpenoid Architectures

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Incredible structural diversity is a hallmark of secondary metabolites. Such diversity of core structures, and subsequent decoration chemistry is responsible for a molecule's bioactive properties. Terpene cyclases are renowned for creating enormous structural diversity from simple polyprenyl carbon chains. Using synthetic biology techniques, we have discovered alternative biosynthetic componentry from a filamentous fungus that delivers an indole diterpenoid (IDT) skeleton that requires a highly complex and elegant cyclisation cascade. To our knowledge, no other cyclases are known to perform an equivalent skeletal rearrangement. A further biosynthetic modification to this indole-terpenoid core delivers a molecule with reported anti-cancer activity. Additionally, we found another genome-encoded cyclase that generates an alternate IDT scaffold, revealing linkages to the wider meroterpenoid class of natural products. This work solves the final piece of the biosynthetic puzzle to access the full suite of known IDT architectures, informs type II cyclase engineering efforts, and expands the repertoire of impressive chemistry catalyzed by noncanonical TCs.

Model-Driven Engineering of Stable Auxotrophic Consortia

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Synthetic microbial consortia are increasingly used in biotechnology, biomedicine, and environmental applications due to their potential for enhanced resilience compared to monocultures. Engineering metabolic interdependencies is a powerful strategy for enforcing cooperation and stability in synthetic microbial systems. However, the design space is immense: for any given pair of strains, there are numerous potential gene knockouts and donor–recipient configurations to consider, and exploring these possibilities experimentally is time-consuming and context-dependent.

We present a computational pipeline leveraging genome-scale metabolic models (GEMs) to rationally navigate this design space and identify robust auxotrophic–prototrophic pairings. The pipeline simulates auxotrophic candidates and tests whether growth can be rescued by exogenous metabolite supplementation. For each viable rescue, the minimal uptake flux needed to restore wild-type growth is calculated, along with shadow prices to assess strain sensitivity. Donor strains are evaluated for their ability to export the essential metabolite without significant growth penalties. These metrics allow ranking of design pairs based on resource demand, donor burden, and metabolic fragility.

To improve mechanistic understanding, the workflow integrates cofactor balance profiling (ATP, NAD(P)H) to explain variations in donor burden across designs. Co-culture simulations are then used to assess whether candidate pairs form stable mutualisms under trade-off constraints.

A proof-of-concept implementation focused on amino acid cross-feeding and single-gene knockouts demonstrates the method's scalability. The approach is modular and generalisable to other metabolite classes and more complex engineering strategies.

By combining metabolic modelling, mechanistic insight, and rational prioritisation, this pipeline offers a scalable, interpretable tool for designing stable synthetic consortia.

Strain Engineering of Actinobacteria to Uncover Natural Products for Crop Protection

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Pesticide overuse and the emergence of resistance have driven demand for new agricultural control agents. This project screens CSIRO's in-house collection of Actinobacteria, mostly of endophytic origin, for natural products with pesticidal activity.

High-throughput genetic manipulations of *Streptomyces* species were performed by inserting global regulatory genes, such as sporulation and antibiotic-related gene A (*sarA*), and precursor or co-factor–related genes, such as the pathway for pyrroloquinoline quinone (PQQ). Gene insertions were carried out with a phiC31 integrase-based system, which was also trialled in *Micromonospora*, *Microbispora*, and *Rhodococcus*. While effective in some *Microbispora* and *Rhodococcus* strains, efficiency was lower than in *Streptomyces*. This limitation led to the development of a plasmid toolkit containing alternative integrases, selectable markers, and modified conjugation protocols, tested across all four genera.

Metabolomic analysis of engineered strains revealed both overproduction of known metabolites and novel metabolite signals. Engineered strains and their chemical extracts were tested for insecticidal, antifungal, and herbicidal activity against the *Spodoptera frugiperda* Sf9 cell line, eGFP-tagged *Verticillium dahliae*, and *Arabidopsis thaliana*. These bioassays, combined with advanced metabolomics, are enabling the identification of new bioactive molecules.

Long-term, this work will contribute to assessing and potentially deploying Actinobacteria-based strains as sustainable biocontrol agents.

Engineering tetrameric insect odorant receptors for biosensor development

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At Scentian Bio we are developing novel biosensors that utilise insect olfactory receptors (ORs) to detect volatile organic compounds (VOC). This biomimicry approach offers the promise for a highly sensitive universal screen that could be used in diverse applications, including non-invasive point-of-care health diagnostic tools, pest management and food production. The core of our technology is the ability to recombinantly produce >70 insect ORs and functionally incorporate these receptors into biosensing platforms.

Insect ORs are heteromeric ligand-gated ion channels. They function as tetramers composed of one odorant-binding OR subunit and three copies of the odorant receptor co-receptor (Orco). Orco is structurally similar to ORs, having evolved via gene duplication, but it lacks an odorant-binding site. The functional 3:1 Orco:OR stoichiometry is essential for receptor activity. However, it poses a significant synthetic biology challenge: the ORs and Orco can form homotetramers when expressed alone, and probably other non-functional complexes, as their structural similarity permits interchangeable assembly.

Conventional protein purification methods cannot distinguish between complexes with different stoichiometries because of their nearly identical physical and biochemical properties. We have developed tagging strategies to identify and isolate the functional 3:1 Orco:OR complex. In parallel, we have optimised the expression system to favour the assembly of the target complex. These developments allow us to produce high yields of functional receptor complexes for biosensor development. Our approach may be a generalisable strategy for isolating multimeric membrane protein complexes with a defined subunit composition.

Reconstructing biosynthetic pathways for complex fungal natural products

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Many organisms, including plants and microbes, utilise unique armouries of bioactive compounds provide protection against predators and enable establishment of new ecological niches. Although bioactive compounds are used widely for industrial and pharmaceutical applications their diversity remains largely untapped as many compounds are produced in very low yields and the complexity of their biosynthesis is yet to be fully understood.

The indole diterpenes (IDTs) class of fungal natural products that have attracted widespread interest due to their remarkable chemical diversity and attendant bioactivities, which include anti-bacterial, anti-viral, anti-cancer and insecticidal properties. By using heterologous expression and employing our new molecular biology tools, we have reconstructed and reprogrammed biosynthetic pathways for some unusual and potentially valuable IDTs in an alternative fungal host. Our work has uncovered large natural product compound arrays, allowing access to many known and novel compounds.

References:

McLellan, R. M., Berry, B., Cameron, R. C., **Parker, E. J.** (2025), Non-canonical type II Terpene cyclases deliver rare terpenoid architectures *J. Am. Chem. Soc* **147**, 13108–13113

Bundela, R., Cameron, R. C., Singh, A. J.; McLellan, R. M., Richardson, A. T., Berry, D.; Nicholson, M. J., **Parker, E. J.** (2023) Generation of alternate indole diterpene architectures in two species of aspergilli. *J. Am. Chem. Soc.* **145**, 2754–2758

Richardson, A. T., Cameron, R. C.; Stevenson, L. J., Singh, A. J., Lukito, Y., Berry, D., Nicholson, M. J., **Parker, E. J.** (2022), Biosynthesis of nodulisporic acids: A multifunctional monooxygenase delivers a complex and highly branched array. *Angew. Chem. Int. Ed.*, **61**, e202213364.

Cameron, R. C., Berry, D., Richardson, A. T., Stevenson, L. J., Lukito, Y. Styles, K. A., Nipper, N. S. L., McLellan, R. M., **Parker, E. J.** (2025), An overlooked cyclase plays a central role in the biosynthesis of indole diterpenes, Chem. Sci., **16**, 9441–9446

Breaking the Silence: Custom Promoter Design to Awaken Cryptic BGCs in Nocardia

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While Nocardia species are recognized for producing bioactive natural compounds, their full biosynthetic potential, particularly for secondary metabolites with therapeutic promise, remains largely untapped. Despite being part of the Actinomycetes family, a well-established group of secondary metabolite producers, the identification of new compounds from Nocardia strains is limited by a lack of tools to activate silent biosynthetic gene clusters.

In this study, we aimed to identify strong promoters in Nocardia to enhance natural product discovery. We constructed a *Nocardia abcessus* genomic library in a mCherry-based promoter probe vector and screened this library using fluorescence-based assays to identify and select strong promoter sequences. We selected >240 clones exhibiting varying levels of fluorescence intensity and sequenced each of their cloned promoter sequences. The selected sequences were further characterised by testing their strength across different Nocardia strains and growth conditions. We then performed promoter engineering to optimize and validate the minimal promoter region and strength of each sequence.

Preliminary findings suggest that certain promoter sequences in *Nocardia abcessus* are stronger than other previously used and well-characterised promoters, including ermEP and kasOP. Further optimization and testing are underway to determine the efficacy of the identified promoters in activating silent biosynthetic gene clusters under a range of conditions, which may lead to the discovery of novel natural products.

Could synthetic cells operate without DNA?

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All life stores genetic information within DNA. DNA is made up of four building blocks, commonly referred to by their bases: A, G, C and T. RNA is similar to DNA, but differs in two important respects. One difference is the chemistry of the backbone sugar that gives RNA and DNA their names. In RNA, this is called ribose while DNA uses deoxyribose. The second difference is that, in DNA, the fourth base is thymine (T) while in RNA it is uracil (U).

It has been proposed that, early in the history of life on Earth, RNA was the genetic material, not DNA. This suggests two key changes were needed for transitioning from RNA to DNA. First, ribonucleotides would have had to be replaced with deoxyribonucleotides. Second, U would have had to be replaced by T. Thus, the evolution of genetic material may have occurred in two steps: RNA to 'U-DNA' to DNA.

If this transition occurred then, at some point in the deep past, cells would have made use of either U-DNA or RNA for storage of genetic information. It is known that this is possible as there are both U-DNA and RNA viruses, but whether complex cells could make use of these alternative genetic materials is an open question. To this end, we have created bacterial lines that either cannot synthesise the fourth base, T, or cannot make deoxyribonucleotides de novo. We have been evolving these lines to examine how cells cope without these processes. I will report on our progress towards answering the key question: can life operate without DNA?

Integrating Conventional Methods with Artificial Intelligence for Accelerated Protein Function Discovery

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Understanding protein function unlocks solutions for a wide range of applications, from enzyme engineering to disease target identification to the design of synthetic biology systems. Conventional annotation methods rely on identifying proteins of known function with similar sequences or folds. However, these approaches fail when small changes occur at key functional residues or when similar proteins have no known function. Recent advances in deep learning models can overcome these limitations by capturing patterns linked to specific functions. However, these models perform poorly for certain functional classes, particularly where training data is sparse or biased.

To overcome limitations of different annotation methods, we developed an integrated pipeline that combines similarity-based annotations with a suite of deep-learning models trained to predict diverse functional properties. Outputs from these tools are synthesised using a large language model, which contextualises predictions with information extracted from scientific literature. The pipeline leverages recent breakthroughs in protein language models and protein structure prediction, enabling superior function annotations to be drawn from a range of features.

We have applied the pipeline to identify novel antibiotic targets missed by standard knock-out assays. Focusing on *Klebsiella pneumoniae* KP13, a highly virulent multidrug-resistant strain, we annotated its complete proteome and used these predictions to refine a genome-scale metabolic network model. The model was first validated against experimental knock-out data, proving to be successful in identifying known essential genes. We then investigated essential metabolic reactions, where the protein driving the reaction was not known. The pipeline notably discovered single proteins or protein pairs for each unannotated reaction, and we found that both similarity and deep-learning methods were required for complete coverage. Remarkably, we discovered a pair of functionally redundant proteins with a common druggable pocket, representing promising targets for the development of a mechanistically novel antibiotic. By integrating conventional and deep learning-based methods, this approach improves upon the completeness and accuracy of annotations, making it broadly applicable not only to antibiotic target discovery, but to diverse protein function prediction tasks.

Uncovering the potential of integrons in synthetic biology applications

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Integrons are natural genetic engineering systems in bacteria that capture, incorporate, and express mobile genes within gene cassettes. While often studied for their role in spreading antimicrobial resistance, integrons also harbour diverse gene cassettes with varied functions from various environments, indicating broader contributions beyond antibiotic resistance.

This work explores ways to co-opt the natural ability of integrons to incorporate and express genes tagged with a gene cassette signature recombination site, to introduce new beneficial traits into bacteria. Our current work sets out to engineer plant-beneficial bacteria that are naturally resident within plant microbiomes for improved capacity to aid plant growth and stress tolerance. We show that plant-associated microbiomes are rich in gene cassettes, with different plant species hosting specific sets of gene cassettes with potential roles in plant-microbe interactions. We developed a gene cassette trap system to capture gene cassettes into an expression vector, facilitating functional characterisation with a focus on identifying gene cassettes with plant-beneficial functions. Additionally, we developed a synthetic gene cassette system to identify integron-carrying bacteria from environments of interest such as plant rhizosphere. Using these two systems, we aim to introduce natural and/or synthetic gene cassettes with plant-beneficial traits into environmental bacteria utilising integrons' natural genetic engineering capacity. Synthetic gene cassettes are not limited to single genes but can carry entire operons, indicating potential to introduce a variety of beneficial traits via this technique. This approach has wide-ranging applications, allowing introduction of diverse beneficial traits to integron-carrying bacteria without standard artificial genome modifications.

Learning to walk: the first steps of an artificial protein motor

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Nature uses an array of remarkable protein motors and machines to facilitate life. However, the complex and highly evolved nature of these protein motors makes it difficult to answer the fundament question of how to link chemical and mechanical action on the molecule scale. Attempts to address these issues using artificial motors made from small molecules and nucleic acids have been made. The production of artificial motors made of protein, the material selected by nature for the synthesis of molecular machines, has remained a challenge to the artificial motor and synthetic biology fields. Here we present the Tumbleweed (TW), an artificial protein motor that walks along a DNA track under external control. TW is built using a modular design approach, assembling three legs, each with a ligand-gated DNA-binding domain that enables selective interaction with specific sites along a DNA track. TW operates via a Brownian ratchet mechanism where steps are affected by diffusion and then rectified by controlling ligands. Using a combination of small angle X-ray scattering, mass photometry and surface plasmon resonance (SPR), we show that TW assembles correctly and interacts with DNA in a specific, stoichiometric and ligand-gated manner. Single-molecule Förster resonance energy transfer (smFRET) experiments demonstrate that TW steps along a DNA track in response to a defined sequence of ligand inputs. By creating TW using a modular approach, we show that motor function can emerge from an assembly of individual functional components. Our challenge now is to improve the performance of TW, as well as using components and lessons of TW in the design of increasingly more complex artificial protein motors.

Rewiring bacteriophage circuits for programmable packaging and delivery

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Bacteriophage use a combination of individually simple control mechanisms to infect, reproduce within and lyse their specific bacterial hosts. Understanding the molecular basis of these fundamental phage control mechanisms has driven the development of many familiar synthetic biology and molecular biology tools. I will describe ways in which we have rewired various phage control circuits to generate additional tools. For example, by converting prophage induction from a UV sensitive circuit to a chemically-inducible one, we have built an efficient phage-based lysis system, that can be combined with a rewired DNA packaging mechanism, to produce a range of tools for programmable gene expression and delivery.

Development of a Lactobacillus-Based Autonomous Nasal Drug Delivery Systems

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The human nasal cavity harbors a diverse community of commensal microorganisms that engage in close interactions with the nasal mucosa. Through long-term co-evolution with the host, these native microbes have adapted to occupy distinct anatomical niches within the nasal passages. Leveraging this natural tropism, we developed a commensal Lactobacillus-based autonomous nasal drug delivery platform capable of targeting both the central nervous system (CNS) via the olfactory epithelium (OE) and nasopharyngeal carcinoma (NPC) in the respiratory epithelium.

In the first application, we identified *Lactobacillus plantarum* WCFS1 (Lp), a commensal strain with a natural affinity for the OE and engineered it to function as a living therapeutic vector for cerebral drug delivery. The blood-brain barrier (BBB) remains a major obstacle for CNS-targeted therapeutics due to its restrictive permeability. We show that intranasally administered Lp can localize to the OE, release therapeutic payloads, and enable their subsequent transport and accumulation in the brain. This bacteria-mediated intranasal route provides a non-invasive alternative that effectively bypasses the BBB. Proof of concept validation was achieved by engineering Lp to produce and secrete appetite-regulating hormones, which significantly alleviated obesity-related phenotypes in a murine model following intranasal administration.

In a parallel approach, we expanded the platform for targeted cancer therapy by engineering Lp to deliver chemotherapeutics to NPC tissues. The engineered strain autonomously (i) bind selectively to NPC cells via OppA-mediated recognition of tumor-associated heparan sulfate and (ii) release the chemotherapeutic prodrug SN-38 in response to tumor-specific biosignals. This system enabled precise microbial colonization and localized drug activation within NPC xenografts, resulting in marked tumor growth inhibition and enhanced therapeutic outcomes.

Together, these studies demonstrate a Lactobacillus-based nasal drug delivery system that combines tissue-specific microbial targeting with autonomous therapeutic release. This platform lays the groundwork for next-generation microbial therapeutics capable of addressing both CNS associated diseases and epithelial malignancies via in situ, on demand and precision drug delivery.

seekRNA – A programmable, bi-specific RNA-guided system for large-scale DNA insertion

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Transposases are proteins encoded within insertion sequences (IS) and are a critical component of mobile genetic elements. These insertion sequences can transpose themselves into genomes through a variety of mechanisms. While investigating IS biology, we serendipitously discovered a novel non-coding RNA forms a complex with its associated transposase. Bioinformatic analysis revealed that the RNA guides the insertion of the IS to very specific target sites, thus behaving like a guide RNA. Unlike canonical guide RNA from CRISPR-Cas9 systems that pair with single DNA strand, this guide RNA is bi-specific and pairs with both strands of target DNA. This makes it more specific and reduces the chances of off-target effects – thus we named it seekRNA. We demonstrated that this seekRNA is programmable and perform large-scale DNA insertions (~0.8 kb to ~5 kb) to new user-defined target sites. This establishes seekRNA as a powerful and versatile platform for targeted DNA insertion for a variety of biotechnology applications.

This presentation will detail the discovery, characterization, and engineering of the seekRNA system. I will discuss its significance as the first bi-specific RNA guide to be described, a finding corroborated by recent, independent discoveries of the related bridgeRNA and TIGR-Tas systems, heralding a new class of bi-specific RNA-guided tools for genome engineering.

Engineering carbon-fixing protein cages for enhanced photosynthesis

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Ribulose 1,5- Bisphosphate Carboxylase Oxygenase (Rubisco) is one of the most important enzymes on the planet. Responsible for the carbon fixation step of photosynthesis, it underpins all of life. Despite its crucial role, Rubisco is still an incredibly inefficient and poorly selective enzyme, often confusing its CO2 substrate with O2, leading to the energy-intensive and wasteful photorespiration pathway. Autotrophic organisms have developed numerous mechanisms to overcome Rubisco's limitations including carbon-concentrating mechanisms (CCMs) such as pyrenoids and carboxysomes. However, critical C3 plants such as rice and soybean simply over-produce Rubisco to the point that it represents up to 50% of soluble leaf protein. This comes with a significant energy cost to the plant and results in a bottleneck in the growth of important food crops.

In this presentation, I will outline our work designing a novel synthetic carbon-fixing organelle based on encapsulin protein cages. I discuss our results engineering Rubisco isoforms from plants and prokaryotes for encapsulation into the encapsulin from *Q. thermotolerance* while retaining catalytic activity. I will go on to discuss the value of temporally separating the protein expression of each component, the kinetics of encapsulated Rubiscos, and outline our next steps toward engineering a complete, novel CCM compatible with C3 plants.

GEMOmics: A Fully Open-Source Workflow for Omics-Driven, Condition-Specific Metabolic Modelling

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Genome-scale metabolic models (GEMs) are powerful tools in synthetic biology, enabling the simulation and optimisation of microbial metabolism for applications ranging from bioproduction to therapeutic development. However, without extensive curation, GEMs are often inaccurate, failing to capture the context-specific metabolic states driven by genotype, environment, or process variables.

GEMOmics is a fully open-source, species-agnostic workflow designed to address this gap by transforming generic GEMs into condition-specific models through integration of transcriptomic, proteomic, and metabolomic evidence. The pipeline accepts SBML-formatted GEMs and standardised omics tables, harmonises identifiers, and assigns reaction confidence scores via gene–protein–reaction logic. Reactions lacking empirical support are down-weighted or limited in activity, while metabolite evidence is used to introduce new biologically plausible reactions. This condition-specific weighting prioritises active pathways and reduces the influence of inactive ones, allowing the model to better reflect the actual metabolic landscape without manual intervention.

Optional modules assess model viability, simulate growth in defined environments, and identify candidate genes for targeted modification in strain engineering. Applied to *Escherichia coli*BW25113 grown under 19 experimental conditions, GEMOmics generated tailored models showing 5–25% reaction rewiring and a 45% increase in metabolite coverage, with significantly improved growth-rate predictions versus the unrefined model. All code, documentation, and datasets are openly licensed.

By integrating multi-omics layers without manual curation, GEMOmics enhances the predictive accuracy of GEMs and accelerates the design–build–test–learn cycle central to modern synthetic biology.

Synthetic Biology and Metabolic Engineering for the Anthropocene: Identifying Meaningful Targets to Impact on Climate Change

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Metabolic engineering has revolutionised our ability to control biology, delivering a much higher degree of control, speed, and complexity in metabolic control. This provides us the opportunity to consider how we can apply our technology to the greatest threat humankind has ever faced: climate change. Identifying applications that can meaningfully contribute to emissions reduction and/or drawdown of atmospheric carbon requires an understanding of factors that must be included to evaluate potential interventions. Key attributes include the ability to process large carbon volume, scalability, positive lifecycle analysis, economic viability, technical and translational feasibility, and field applicability. Here we will explore intervention approaches and, using Fermi analysis, quantify their CO_2e impact potential and benchmark them against non-engineered nature-based approaches. Scalability remains a key challenge, with agricultural applications emerging as forerunners given that crops are already scaled and covering 20 % of the Earth's land surface. Results reveal that while per-hectare impacts vary by orders of magnitude (<1 to >30 t CO_2e/ha/year), deployment scale is the dominant factor determining total impact. Targeted synthetic biology strategies deployed across existing agricultural systems could deliver ~120 Gt CO_2e drawdown over a century and contribute to an additional ~140 Gt. Significant potential exists to exploit our bioengineering advances for climate sustainability, but a holistic and integrated approach will be required to achieve impact. Early-stage quantitative evaluation is critical to guide R&D toward climate-relevant solutions and deliver a prioritized portfolio of near- and long-term strategies. This work offers a framework for evaluating plant-based climate mitigation strategies and highlights a key role for synthetic biology in climate mitigation pathways.

Next-generation mRNA production drives RNA research and development advances in New Zealand

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The use of RNA technology in therapeutics and preventative vaccine development is rapidly expanding. The recent success of messenger RNA (mRNA) vaccines has been driven by advances in *in vitro* RNA production, bioinformatic design tools, and the development of ionisable lipid nanoparticles (iLNPs). Over the past year, the New Zealand RNA Platform has delivered more than 1,000 batches of mRNA to collaborators and clients. This platform offers a standardised pipeline for mRNA production and quality control, supporting the development of novel antigens and delivery methodologies.

In recent months, we have implemented several key improvements to the RNA production pipeline. Our DNA template preparation has been adapted to prevent cross-contamination and to yield templates that are more efficient in RNA synthesis. We have integrated new technologies into the *in vitro* transcription (IVT) process that reduce double-stranded RNA (dsRNA) formation and lower the required input of costly reagents. Additionally, we are investigating mRNA modifications that enhance intracellular RNA stability and protein expression. These developments allow us to produce RNA faster, cleaner, and at reduced cost. We are also working to transfer this knowledge to industry, enabling scalable production of high-quality RNA products and strengthening New Zealand's biomanufacturing capabilities.

Exploring the Biotechnological Potential of Refactored Killer Yeasts in the Age of Synthetic Biology

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Killer yeasts are defined by their ability to secrete proteinaceous toxins that are lethal to other yeast and fungi. These toxins exhibit substantial diversity in their primary sequences and possess unique mechanisms of targeted cell toxicity. Most notably, many of these toxins are encoded by dsRNA viruses and linear virus-like dsDNA particles, further compounding their diversity. These systems have evolved to gain a competitive advantage in various ecological niches, a trait that has found several biotechnological applications. However, to this day, their full potential has been largely unexplored.

In this presentation, I summarise recent work applying synthetic biology to reimagine these systems, including the development of Mo-Tox: A Modular Eukaryotic Toxin/Antitoxin System for Refactored Killer Yeasts. Mo-Tox is intended to provide a testbed to screen a standardised combinatorial library, with potential future applications in biocontrol, plasmid stabilisation without antibiotics, and synthetic microbial communities.

I additionally touch on unexpected applications of these unique killer yeast systems, which demonstrate the utility of applying an engineering mindset to real-world challenges.

Protein Switch-Based Screening Platform for the Optimization of Lanmodulin selectivity

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Rare earth elements (REEs), such as lanthanides, are widely used in electronics, medicine, energy storage, and energy generation applications, with their demand on the rise. However, the extraction and separation of REEs is environmentally unfriendly and energy consuming, which is largely due to their similar physical and chemical properties. To address this, we take inspiration from the ability of living systems to evolve selective protein-based systems for the extraction of diverse ions from the environment. The discovery of the bacterial lanthanide-binding protein lanmodulin (LanM) inspired a range of efforts in lanthanide detection and bio-extraction. This includes a lanthanide biosensor based on β-lactamase-lanmodulin (BLA-LanM) chimera developed by the Alexandrov lab (1). Its utility, however, is limited by the lack of selectivity for neighbouring lanthanides – a feature that traces back to LanM selectivity. *E. coli* cells expressing such switches exhibit coupling between lanthanide-dependent BLA activity and β-lactam antibiotic resistance, which can potentially be used to drive the directed evolution of LanM.

In this project, we aim to use the BLA-LanM switch as a platform for developing much more selective LanM variants. To achieve this, several challenges must be addressed: First, LanM possess four REE binding sites, which dramatically increases the sequence space and poses a practical challenge for developing LanM variants with altered specificity. Therefore, we constructed functional BLA-LanM variants with a single lanthanide binding site. Second, the conditions that allows for lanthanide-dependent survival of *E. coli* cells expressing single binding site BLA-LanM were identified. This establishes the foundation for the evolution of REE-selective single binding site LanM-BLA switches in *E. coli*.

1) Guo, Z. et al. (2025) Angew. Chem. Int. Ed. 64, e202411584.

A platform for lantibiotic diversification

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Cinnamycin-like, class II lantibiotics are ribosomally synthesized and post-translationally modified containing peptides (RiPPs) which specifically bind to phosphatidylethanolamine (PE) lipids causing cell membrane disruption and antimicrobial activity against gram-positive bacteria. This ability to bind PE also causes trans bilayer phospholipid movement in the cell membrane of mammalian cells and PE exposure to the outer leaflet of the membrane, and cinnamycin-like lantibiotics entered clinical trials for cystic fibrosis and have been reported to block the entry of flaviviruses such as Zika virus into human cells.

Building on our understanding of their biosynthesis and its regulation we developed a heterologous platform to rapidly produce these molecules in yields sufficient for activity screening. This includes numerous cryptic lantibiotics identified from the genomes of various Actinomycetota and Cyanobacteria. Extension of the approach enabled the ready diversification of non-conserved amino acid residues, providing ready access to hundreds of lantibiotic variants.

Yeast whole cell biosynthesis of nororipavine via N-demethylation

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The global opioid crisis has led to a high demand for treatment agents, such as buprenorphine, naloxone and naltrexone. Yet the current chemical N-demethylation process, which is a critical to synthesis, is suboptimal. Synthetic steps starting from poppy derived alkaloids thebaine or oripavine, for instance, are low yielding and use toxic chemicals. An alternative route involves the N-demethylation of oripavine to produce nororipavine using a biosynthetic system. We present a whole cell biosynthetic system that can produce nororipavine from oripavine in a bioreactor, using a novel N-demethylase isolated from a pest of the poppy plant expressed in yeast. Two modes of operation were examined: 1) fed-batch culture and batch N-demethylation and 2) continuous culture and continuous N-demethylation. Process parameters examined included varied specific growth rates, substrate oripavine concentration, oxygen transfer rate, dry cell weight and temperature. Both modes were applied to characterise and optimise process parameters to improve process productivity, with an emphasis on optimal biomass and process control. A hybrid-machine learning model was also constructed to assess the potential for reducing the number of experiments required for further optimisation of additional process parameters.

This investigation demonstrates progress towards opioid biocatalysis, in particular N-demethylation and is conducted in parallel to development at our industrial collaborator River Stone Biotechnology. It also highlights the use of different bioreactor operation modes as tools to provide insights into the whole cell biosynthetic process at laboratory scale prior to large scale industrial adoption. The study demonstrates an integrated approach that combines whole cell biosynthesis, bioreactor process design and an initial assessment of computational tools to achieve a scalable and more sustainable route to nororipavine production. The early strain clone examined demonstrates a promising time space yield and the insights obtained could help accelerate the implementation of whole cell N-demethylation in industry.

Developing Gram-Positive Hosts for Scalable Functionalised Bioparticle Production

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Polyhydroxybutyrate (PHB) is a biopolymer synthesised by many microbes as a carbon storage mechanism under high carbon but limiting nitrogen, phosphorus, magnesium, or sulphur conditions. PHB bioparticles can be recombinantly produced in a host organism via the expression of three main genes, phaABC. Acetyl-CoA undergoes a condensation reaction catalysed by PhaA (β-ketothiolase) to produce acetoacetyl-CoA that is further reduced by PhaB (acetoacetyl-CoA reductase) resulting in 3-hydroxybutyryl-CoA (3HB-CoA). PhaC (PHB synthase) polymerises the individual 3HB-CoA monomers into a polymer chain. Due to the hydrophobic nature of PHB, these naturally coalesce into a bioparticle structure with PhaC remaining covalently attached on the surface. A protein of interest can be directionally displayed on the surface of PHB bioparticles by genetically fusing its gene to the phaC gene. This process creates a functionalised bioparticle (FBP) with uses in a variety of fields including cell imaging, vaccine and drug delivery, bioseperation and purification, and enzyme purification.

Large scale FBP production platforms of these biopolymers in Gram-positive bacteria, such as *Lactococcus lactis* and *Bacillus subtilis*, remain in their infancy. PHB produced by *Escherichia coli* can equate to as much as 60% of the cell dry weight (CDW), while in L. lactis the average PHB yield is approximately 10% of the CDW. Furthermore, FBPs produced in *E. coli* are known to contain lipopolysaccharide (LPS). LPS is a known endotoxin and its presence in FBP preparations would preclude use in certain applications. Therefore, this project aims to improve the overall yield of FBPs in a Gram-positive system which will expand their application to other avenues.

In this study, we investigated the impact of separating the expression of phaAB from phaC on FBP production in *L. lactis* and B. subtilis. Separate expression of phaAB with a constitutive promoter will enable the build-up of 3-HB prior to phaC expression. This is hypothesised to improve overall FBP production. Different constitutive promoters for phaAB were also compared to determine the optimal system for FBP production in *L. lactis* and *B. subtilis*.

This work highlights preliminary findings on the impact of a sequential gene expression strategy on FBP production in both *L. lactis* and *B. subtilis*.

Optimising Kinase-Catalysed Nucleoside Phosphorylation for mRNA Vaccine Production

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C-nucleosides are a class of nucleoside analogues with enhanced metabolic stability and therapeutic potential, owing to their characteristic C-C glycosidic bond. Among them, pseudouridine (Ψ) and its synthetic derivative N1-methyl pseudouridine have become essential building blocks in mRNA vaccine production. Their presence enhances transcript stability, boosts translation efficiency, and reduces innate immune activation. However, a critical bottleneck in their use is the availability of triphosphorylated forms, such as ΨTP, required for incorporation during *in vitro* transcription (IVT). This research aims to address this limitation by developing and optimising enzymatic cascades to enable ΨTP synthesis.

We are seeking to leverage an established two-step kinase cascade previously used for uridine phosphorylation, to convert ΨMP to ΨTP. In addition, an ATP regeneration system, powered by the polyphosphate kinase chPPK from *Cytophaga hutchinsonii*, will be integrated to enable cost-effective and scalable production. This approach offers an enzymatic route to ΨTP, helping overcome the reliance on expensive, chemically synthesised nucleoside triphosphates. In parallel, we aim to expand the substrate scope of this system by testing phosphorylation of other therapeutically relevant C-nucleosides, such as oxazinomycin (OZM), a bacterial RNA polymerase inhibitor.

Overall, this research combines enzyme engineering, synthetic pathway design, and functional screening to advance the scalable, biologically relevant production of nucleoside therapeutics.

Metabolic Engineering of *Vibrio natriegens* for Scalable Biosynthesis of Poly(3-hydroxypropionate) from Glycerol

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The plastic waste crisis continues to escalate due to modern society's heavy reliance on plastic for everyday functionality. These concerns clarify the indispensable need for greener alternatives that can circumvent the ecological damage linked to conventional plastics. Poly(3-hydroxypropionate) (P(3-HP)), an intracellular energy and carbon storage compound synthesized and degraded by various microorganisms, has attracted considerable attention as a promising sustainable alternative to conventional plastics; however, achieving industrially viable bioproduction metrics remains a significant challenge. This project aims to engineer the fast-growing marine bacterium *Vibrio natriegens* as a microbial chassis for scalable, high-yield biosynthesis of P(3HP) and P(3HP-co-3HB) from renewable feedstocks, namely glycerol. Leveraging *V. natriegens*' exceptional growth kinetics, broad substrate range, and emerging synthetic biology toolkits, we will implement a multi-pronged engineering strategy: (i) construct a two-plasmid expression system with optimized aldehyde dehydrogenase activity to detoxify key intermediate; (ii) eliminate competing pathways using NT-CRISPR genome editing; and (iii) fine-tune carbon flux through the 3HP and 3HB pathway branches to enable tunable copolymer compositions. To implement this engineering strategy, we will explore a range of metabolic designs, including ATP-sparing enzyme selection, use of energy-efficient expression cassettes, and incorporation of stress-alleviating phasins to enhance polymer accumulation. The performance of engineered strains will be evaluated in fed-batch fermentations under industrially relevant conditions, and resulting polymers will be characterized for physicochemical and mechanical properties. These efforts will advance sustainable biodegradable plastic production and support the transition to a circular bioeconomy.

Recalcitrance reimagined: A cotyledonary node-based regeneration platform for epigeal medicinal plants

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Medicinal plants are high-value crops that produce a diverse array of secondary metabolites, compounds vital not only for plant defence but also for their therapeutic significance. While interest in harnessing plant synthetic biology to engineer these pathways is growing, progress remains constrained by a key barrier: the recalcitrance of many species to tissue culture regeneration and genetic transformation. *Cannabis sativa*, along with several other economically important medicinal species that undergo epigeal germination, offers a unique opportunity for developing robust regeneration systems. Their seed-derived explants, particularly the cotyledonary node, are easily accessible and represent a crucial developmental zone with significant morphogenic potential. In this study, we developed and validated a genotype-independent, direct de novo shoot regeneration protocol using cotyledonary node explants, achieving 70-90% de novo regeneration efficiency across six hemp cultivars and three medicinal cannabis lines. Histological analysis confirmed true *de novo* shoot formation from peripheral cortical cells, independent of pre-existing meristems or callus. The protocol yielded an average of 7 shoots per responding explant, outperforming existing cotyledon-based methods by 2-fold and hypocotyl-based approaches by 5-fold. Critically, this system extends beyond cannabis to other taxonomically unrelated epigeal medicinal species, demonstrating broad applicability. Our approach emphasises the significance of morpho-developmental traits, such as germination type, in guiding explant selection and regeneration strategy. The cotyledonary node's regenerative capacity makes it an ideal foundation for genetic transformation systems currently under development to enable transformative synthetic biology applications in medicinal plants.

Boosting Carbon Yields of Bioprocesses via Synthetic Chemolithoheterotrophy in *Pseudomonas putida*

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In bioprocesses, NAD(P)H is the universal redox currency driving biosynthesis and ATP generation. Yet in chemoheterotrophs, its regeneration is inherently tied to carbon oxidation in the central carbon metabolism—diverting 30–50% of available carbon towards CO_2 rather than product formation.

To overcome this limitation, we explore synthetic chemolithoheterotrophy—an approach where inorganic electron donors decouple NAD(P)H supply from carbon metabolism. Metabolic models predict that such redox interventions could significantly increase carbon retention, reducing the reliance on carbon-derived reducing power. Furthermore, the models suggest that pathways such as anaplerotic reactions and a threonine aldolase reaction, present in some heterotrophic organisms, such as *Pseudomonas putida* and *Saccharomyces cerevisiae*, can enhance CO_2 recycling and its co-assimilation, facilitating mixotrophy.

Initial experimental data with recombinantly expressed O_2-tolerant NAD+-reducing hydrogenases confirm increased biomass yields (+20 %) with *P. putida* cells utilising hydrogen as an inorganic electron donor. Model-based engineering of an NAD(P)+-dependent decarboxylase deletion mutant decoupled carbon and energy metabolism, introducing an NAD(P)H auxotrophy–essentially disabling loss of carbon in the form of CO_2. Expression of an NAD+-dependent phosphite dehydrogenase enabled fully synthetic chemolithoheterotrophy. Applying adaptive laboratory evolution further pushed yield increases by 40%, approaching the theoretical maximum biomass yields with *P. putida* on acetate as carbon source.

This strategy holds promise for a wide range of applications, from microbial production of highly reduced molecules such as biofuels to engineered carbon capture and bioprocesses with improved sustainability through reduced biological CO_2 emissions. By rewiring redox metabolism, we aim to push carbon yields beyond conventional limits, paving the way for novel redox engineering strategies and a new generation of highly efficient bioprocesses.

rcWGA: agnostic whole genome amplification for short or long read sequencing

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There is a significant gap where metagenomic sequencing is and where we want it to be. Metagenomic sequencing directly from environmental or clinical samples, particularly those with low-abundance or degraded DNA, is currently cost-prohibitive for high-throughput applications. Here we present rcWGA, an agnostic and versatile molecular method designed to overcome several limitations of state-of-the-art techniques.

rcWGA uses a hairpin DNA adapter which is ligated to the double-stranded DNA within a sample, converting it into a circular molecule. This circularized DNA is then amplified via rolling circle amplification (RCA), a mechanism which can be harnessed to enable long-read sequencing of low-abundance and fragmented DNA. Application of this methodology will significantly improve direct-from-sample molecular typing workflows and provide more comprehensive capture of all the DNA (organisms) present (metagenomics) in a sample. Unlike existing whole genome amplification (WGA) techniques like PCR-based approaches or multiple displacement amplification (MDA), rcWGA significantly reduces primer-related artifacts and eliminates problematic secondary DNA structures (e.g., cruciform DNA); these structures can adversely affect nanopore based sequencing technologies. An optional feature of rcWGA is its preferential enrichment of smaller DNA fragments /

genomes, which is highly beneficial when the targets of interest are pathogens in host (larger genome)-derived samples. rcWGA is also compatible with transposon-based protocols like ONT's Rapid Sequencing kit, enabling cost effective amplicon sequencing. Ultimately, rcWGA is an advancement in molecular diagnostics, offering an efficient and modular solution for sequencing of difficult DNA samples that lowers the barrier for advanced sequencing in applications ranging from clinical molecular typing to comprehensive metagenomic analysis.

Identifying and Characterizing Halophilic Carbonic Anhydrases for High-Salinity CO_2 Conversion in Industrial Biocatalysis

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Carbonic anhydrases (CAs) are powerful biocatalysts that accelerate the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO3-) by over six orders of magnitude. Their catalytic efficiency makes them attractive tools for carbon capture and utilization (CCU) technologies. However, most naturally occurring CAs lose activity under the high salinity conditions commonly found in industrial settings, such as CO2 mineralization. To make enzymatic CO_2 conversion viable at scale, we need CAs that remain active and stable in extreme environments with high salt levels. Our research targets this challenge by focusing on halophilic bacteria, organisms adapted to life in hypersaline ecosystems. We hypothesize that these microbes possess CAs naturally optimized for function under high salt concentrations. Using machine-learning tools and comparative genomics, we identified a diverse set of CA homologs from halophilic bacteria. These candidates were cloned, expressed in E. coli, and screened for stability and catalytic performance under variable salinity (0–5 M NaCl).

The hydrolysis of p-nitrophenyl acetate was used as a screening method for carbonic anhydrase activity by detecting esterase function as a proxy for CO_2 hydration. CO_2 mineralization experiments were also used as a secondary line of evidence of functional carbonic anhydrase activity under high-salinity conditions. The activity assays showed that while some predicted halophilic enzymes failed to show activity at moderate salinities, others retained robust function at NaCl concentrations up to 4–5 M. Interestingly, several successful CAs originated from species not commonly associated with known/characterized halophiles, suggesting that salt dependence in enzymes is more nuanced than current computational predictions suggest. Our workflow emphasizes iterative rounds of in-silico screening, expression optimization, and biochemical characterization to refine candidate selection. Salt-dependent kinetics were conducted to identify the most promising enzymes for downstream engineering applications. This work increases the efficiency and success rate of identifying functional halophilic enzymes using targeted bioinformatics screening. This project also highlights the importance of wet-lab experimentation in validating and improving extremophilic enzyme performance. By systematically testing and optimizing halophilic CAs, we are building a toolkit of robust biocatalysts suitable for integration into industrial CO_2 capture systems.

Indigoidine Synthetases: Driving the Synthesis of Novel C-Nucleosides and Pigments

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Indigoidine synthetases are unique single-module non-ribosomal peptide synthetases. These enzymes play a key role in synthesising 3,3'-bipyridyl blue pigments and the C-nucleoside minimycin (also known oxazinomycin). Indigoidine synthetases catalyse the cyclization of L-glutamine, which if not further modified spontaneously dimerises in the presence of oxygen to form the insoluble blue pigment indigoidine. Co-expression of additional tailoring enzymes such as a pseudouridine-5'phosphate glycosidase results in the synthesis of the water-soluble blue pigment indochrome and further modification by a HAD-family phosphatase and a questin oxidase results in the synthesis of minimycin, a potent uridine mimetic with antimicrobial and anticancer properties. C-nucleoside analogues are particularly appealing for medical applications because the carbon-carbon bond between the nucleobase and sugar confers metabolic stability, improving pharmacokinetics.

Building on our understanding of these natural biosynthetic pathways, we have engineered a strain of *E. coli* to produce minimycin and indochrome and are now seeking to synthesise novel minimycin analogues by expressing a variety of different tailoring enzymes we have mined from diverse bacterial genomes. Through our screening efforts to identify minimycin modifying enzymes we have also identified a range of enzymes capable of modifying indigoidine and indochrome, resulting in colourants with modified properties and extended hues. As we increase our understanding of the biochemical mechanisms responsible for the synthesis of these nucleosides and pigments, we have an opportunity to produce a range of new colourants and pharmacologically relevant compounds in a sustainable manner, offering advantages over traditional chemical synthesis approaches.

Mapping Allosteric Communication between Lanmodulin and β-lactamase in Rare Earth Element Biosensor

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Protein-based biosensors offer a promising strategy for metal ion detection in aqueous environments, with potential for real-time monitoring of toxic metals in wastewater and mine-impacted sites. A recent example is the lanmodulin–β-lactamase (LanM–BLA) chimera, which couples a rare earth-binding domain with an enzymatic reporter to yield a synthetic protein switch responsive to lanthanides. These constructs display high dynamic range and ion selectivity, but the molecular mechanism underpinning their allosteric control—how metal binding to LanM modulates activity at the β-lactamase active site requires further elucidation.[1]

Building on the design reported by Guo,[1] we have probed the conformational basis of this allosteric communication using molecular dynamics (MD) simulations. Specifically, we focus on how metal coordination at the LanM EF-hands (specifically EF1/EF2, with EF3 mutated for low affinity) triggers structural rearrangements that propagate to the β-lactamase domain and influence active site accessibility. These simulations seek to characterize the 'ON' and 'OFF' states of the protein switch and how they correlate with activity. With a molecular signature of these 'ON' and 'OFF' states, this enables further insight into the mechanistic reason why Fe(III), Al(III), Ga(III), and Zn(II) inhibit both β-lactamase and the chimera, while Fe(II) and Sn(II) selectively inhibit only the chimera.

The molecular simulation probe into this system is conducted using 700 ns triplicate MD trajectories (CHARMM36m + TIP3P) and inter-residue distance metrics, tracking communication between LanM loops and β-lactamase hinge/W-loop regions, known to mediate catalytically relevant motions[2]. To further probe metal specificity, we have implemented the Boltz-1 model to provide prediction of potential binding sites of inhibitory metals against Mg(II) as a non-inhibiting control for which we subsequently calculate ΔGbinding for each metal in its inhibitory state.[3]

Together, these simulations provide insight into the architecture of synthetic allostery—how metal sensing in one domain can modulate catalysis in another. This work helps address open questions left by initial biosensor studies[1] and complements mechanistic insights from β-lactamase allostery[2] and rare earth metal binding in LanM.[3] Ultimately, these findings could inform the rational design of next-generation metal biosensors with programmable selectivity and inhibition profiles.

REFERENCES

1. Guo Z. et al. Lanthanide-Controlled Protein Switches: Development and In Vitro and In Vivo Applications. Angew. Chem. Int. Ed. 2025, 64, e202411584.

2. Galdadas I. et al. Allosteric communication in class A β-lactamases occurs via cooperative coupling of loop dynamics. eLife 2021, 10, e66567.

3. Sajeevan K. A. et al. Computationally derived structural insights into Rare Earth selectivity in lanmodulin and its variants. Comput. Struct. Biotechnol. J. 2025, 27, 639–648.

High-throughput optimisation of protein secretion in yeast via an engineered biosensor

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When producing recombinant proteins for industrial use it is ideal if the target protein can be secreted from the expression system. Secretion of the target protein avoids the need to lyse the host expression cells, which is uneconomical at scale.

Optimising protein sequences for secretion can result in more efficient and profitable manufacturing of the target protein. We have generated a library of signal peptides for use in optimising recombinant protein secretion in *Saccharomyces cerevisiae*.

Using combinatorial Golden Gate assembly, these signal peptides, along with a series of promoters and terminators, can be assembled with a protein of interest to form thousands of different protein expression cassettes in a single reaction.

Using a minimal yeast strain with refactored GPCR signalling pathways, designed by Shaw et al. (2019), we created a living yeast-based biosensor for detecting secretion of any given protein of interest.

By combining the signal peptide library and combinatorial golden gate assembly method with the yeast-based biosensor, we can screen vast numbers of expression cassette combinations for successful expression and subsequent secretion of a given protein of interest.

This system allows for mass screening of protein expression cassette combinations for secreted recombinant proteins in yeast.

Protein-carrying artificial cells based on hybrid vesicles

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Artificial cells are minimal units that mimic living cells at various levels. Our work focuses on bottom-up assembly of life-like units using tools from chemistry and nanoscience to create compartmentalized systems that replicate structural and functional aspects of mammalian cells.[1] In particular, we investigate hybrid vesicles composed of amphiphilic block copolymers and lipids as scaffolds for artificial cells.[2]

Here, we report for the first time the integration of a eukaryotic cell-free protein expression system derived from *Leishmania tarentolae* into hybrid vesicles,[3] a system that enables localized expression of complex proteins within the vesicles. We systematically compared the expression efficiency of active enzymes as a function of hybrid membrane composition, with particular emphasis on the role of the block copolymer. Inspired by our initial findings,[4] we designed a polymer featuring a hydrophobic block with cholesterol pendant groups and a sugar-like hydrophilic block, drawing on native plasma membrane components for improved biomimicry.

Taken together, this work represents a step forward in integrating cell-free protein synthesis with engineered hybrid membranes, advancing these types of artificial cells toward the fundamental capabilities of life to express proteins.

REFERENCES

1. Westensee IN, de Dios Andres P, Städler B. From Single-Compartment Artificial Cells to Tissue-Like Materials. Adv Mater Technol 2024;9:2301804.

2. Brodszkij E, Städler B. Advances in block copolymer-phospholipid hybrid vesicles: from physical–chemical properties to applications. Chem Sci 2024;15:10724–44.

3. Johnston WA, Palma JA, Alexandrov K. Production and Optimization of LTE, a Leishmania tarentolae Derived Cell-Free Protein Expression System for Recombinant Protein Production. J Vis Exp 2024:e65592.

4. de Dios Andres P, Purup AB, Beaune G, Timonen JVI, Lyons JA, Städler B. Flippase-Mediated Hybrid Vesicle Division. Advanced Materials 2025:2504519.

Genome Mining and Bioprospecting of Fungal Biosynthetic Gene Clusters for Antimicrobial Compounds

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Microorganisms are prolific producers of natural products with diverse bioactivities, many of which have significant pharmaceutical potential. These metabolites are typically encoded by groups of co-localized genes known as biosynthetic gene clusters (BGCs), which enable the coordinated regulation and biosynthesis of secondary metabolites. Genome mining has become a powerful approach for identifying and characterizing BGCs from genomic data providing valuable insights into the biosynthetic potential of microorganisms.

In this study, we investigated a Basidiomycete fungus belonging to the class Agaricomycetes to explore its biosynthetic potential and antimicrobial activity. Genomic DNA was extracted, sequenced, assembled and analysed using genome mining tools to identify BGCs potentially involved in antimicrobial compound biosynthesis. The assembled genome revealed 24 putative BGCs, including 10 terpene clusters, 10 nonribosomal peptide synthetases (NRPS), one type I polyketide synthase (T1PKS), and three hybrid NRPS–PKS clusters. The presence of key core enzymes and hypothetical protein-coding genes suggests active secondary metabolite biosynthesis pathways within the genome.

Crude extracts from fungal culture exhibited antimicrobial activity against antibiotic-resistant *Staphylococcus aureus* in susceptibility assay. Fractionation of the crude extract using high-performance liquid chromatography (HPLC) followed by mass spectrometry (MS) analysis revealed a fraction with a molecular weight of approximately 261 Da. Preliminary nuclear magnetic resonance (NMR) spectroscopy indicated distinct structural features, although the compound was obtained in small quantities.

Together, these results indicate that the Agaricomycete isolate has substantial biosynthetic capacity, supported by genome mining and antimicrobial bioassays. Preliminary fractionation and MS/NMR signals suggest a low-abundance candidate molecule, but comprehensive metabolite profiling is still in progress. Our next steps are to purify the active compound, determine its structure and link it to its cognate BGC.

Bioprospecting of industrially significant specialised metabolites from medicinal plant endophyte *Microbacterium* sp.

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Andrographis paniculata, a medicinal plant widely used in Indonesian traditional medicine to treat various ailments. Notably, microbes isolated from medicinal plants have been researched and have emerged as a prolific source of specialised metabolites. We hypothesised that endophytes associated with *A. paniculata* possess bioactive specialised metabolites relevant to the plant's medicinal properties.

This study investigates a *Microbacterium* sp. strain, isolated from *A. paniculata*, to explore its biosynthetic potential. We identified key biosynthetic gene clusters (BGCs) using next-generation sequencing and advanced genome mining tools. Out of four BGCs identified, three were predicted to encode a type III polyketide synthase (PKS), a carotenoid, and a β-lactone biosynthetic pathway, supporting our claim for its contribution to the plant's medicinal property. These BGCs were cloned and successfully expressed heterologously, enabling further engineering to enhance product yield and activity.

This study demonstrates the effective integration of ethnobotanical knowledge utilising various tools like genome mining, synthetic biology, analytical instruments, and screening technologies to discover novel natural products. This study supports the rationale to modify these BGCs to produce unique compounds with potentially enhanced properties.

Reconstituting the Biosynthesis of Bioactive Hamigeran Terpenoids in *Hamigera Tarangaensis*

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Hamigerans are structurally unique secondary metabolites with promising antibacterial and antitumoral properties, yet their biosynthetic origins remain unknown. This study aims to elucidate the metabolic pathway responsible for hamigeran biosynthesis in the marine sponge *Hamigera tarangaensis* and its potential symbiotic microorganisms. By employing genomic, transcriptomic, and metabolomic approaches, we seek to identify the biosynthetic gene clusters (BGCs) responsible for hamigeran production and characterize the enzymatic mechanisms involved.

Beyond pathway discovery, this research aims to establish a heterologous expression system to enable high-yield production of hamigerans in model organisms such as *E. coli* or *S. cerevisiae*. This approach could provide a sustainable and scalable alternative to natural extraction, facilitating further pharmacological development of hamigerans as therapeutic agents. The findings from this study will contribute to our understanding of marine natural product biosynthesis and offer novel biotechnological strategies for producing structurally complex bioactive compounds. By presenting this work, I hope to engage with experts in metabolic engineering, synthetic biology, and natural product chemistry to refine my methodologies and explore potential collaborations.

Ensuring equity in the integration of artificial intelligence in synthetic biology

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Artificial intelligence offers extraordinary benefits for those working in synthetic biology, from predicting biological functions and automating the design process to streamlining drug development and monitoring biomarkers. While AI represents a significant step forward for synthetic biology and for the field of healthcare, it is critical that these technologies are used equitably in order to avoid discriminating against certain groups or perpetuating health disparities. In our recent systematic literature review exploring bias mitigation strategies for AI developers, it was discovered that no strategies were aimed specifically at those working in synthetic biology. Bias mitigation strategies also tend to be highly theoretical and lack actionable guidance on how bias mitigation can be operationalised in a real-world setting. In response to these shortcomings, we have conducted a number of interviews with Australian scientists, clinicians, and AI developers working in synthetic biology-related fields. In our presentation, we will share findings from these interviews and discuss ways that the field is currently tackling bias and what more can be done to ensure that AI tools are used equitably into the future.

A Modular Sensing and Actuating System for Detection of Farnesyl Pyrophosphate

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Nature often utilises sophisticated sensing and actuating systems to regulate essential cellular processes. However, despite significant advances in protein engineering, creating synthetic analogues that emulate these natural systems remains challenging. Chemically induced dimerization (CID) systems represent a promising approach for sensitive, real-time detection of specific small molecules. Transduction of the binding event via a reporter system allows measurement of cellular responses. In this study, we engineered artificial protein receptors to develop protein biosensors for CID systems specifically tailored for quantifying key metabolite flux within the isoprenoid biosynthesis pathway. Using a directed evolution strategy, we identified and optimized protein pairs that dimerized in the presence of farnesyl pyrophosphate. These engineered protein binders were genetically fused to custom-designed actuating domains, resulting in versatile biosensors capable of monitoring both *in vitro* and *in vivo* metabolite pools. Our modular toolkit provides a potential platform for expanding the detection and analysis of diverse small-molecule metabolites in synthetic biology applications.

Exploring *Trichoderma harzianum* for Precision Fermentation: Genomics to Bioactivity

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Precision fermentation harnesses the metabolic capabilities of microorganisms to produce high-value biomolecules. *Trichoderma harzianum* is a filamentous fungus widely known for biocontrol properties and enzyme production and secretion, making it a strong candidate for industrial biotechnology applications. This study aims to characterize a CSIRO strain of *T. harzianum*, with a focus on its potential as a host for precision fermentation.

The investigation included whole-genome sequencing and annotation, development of a transformation protocol to express a recombinant fluorescent protein, and profiling of growth and the secretome of *T. harzianaum* using various agro-industrial carbon sources. The culture filtrate exhibited strong antimicrobial activity against pathogens, highlighting its bioactive potential. The antifungal and antibacterial activities of the secreted metabolites were also evaluated, and antibiotic susceptibility assays were conducted. *T. harzianum* displayed vigorous growth on substrates including glucose, citrate, and glycerol, with enhanced esterase activity in glucose- and cellulose-rich media.

T. harzianum shows strong promise as a fungal chassis for precision fermentation, with its ability to degrade complex substrates, efficiently express recombinant proteins, and secrete potent antimicrobial compounds positioning it as a versatile platform for sustainable biomanufacturing.

Practitioner Perspectives on the Role and Feasibility of Synthetic Biology in Improving Australian Rural Health Outcomes

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Australian rural health outcomes are poorer than those of metropolitan populations and worsen with remoteness, largely due to factors such as long travel times to medical care, lack of health infrastructure, and shortages of healthcare providers. Advances in synthetic biology offer medical biotechnologies that are less dependent on centralised facilities, potentially improving these outcomes. However, rural health practitioners' attitudes toward such technologies remain largely unknown.

This scoping study canvassed the views of 12 rural health professionals on two potential tools: diagnostic biosensors and onsite bioproduction of medicines. Participants identified numerous benefits and practical considerations. Biosensors were viewed as particularly valuable for ongoing care and enabling faster pathology results in remote areas—reducing the need for long-distance travel to receive treatment. Bioproduction was seen as a potential solution to medicine shortages and wastage, though logistical concerns were expressed about production scale, location, and distribution.

Overall, the practitioners expressed in-principle support for deploying advanced medical biotechnology tools in rural settings. The study also identified priorities for future R&D, including selecting key medical substances for local production and assessing the feasibility of point-of-care manufacturing.

Directed evolution to select for protein cages with improved molecular flux

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Enzyme mediated reactions are ubiquitous, with amylase, pectinase, cellulase, lipase and glucose oxidase being some of the few enzymes that are indispensable in many industries like textile, food and medical. While biocatalysis holds enormous value in “greener” synthesis, many enzymes are not suitable for temperature and pH conditions of largescale production. Engineering enzymes is a massive field in synthetic biology, but significant gains in stability can be achieved by encapsulating an enzyme within protein nanocage – creating a nanoreactor.

Though encapsulation has been found to be useful in improving the stability of the enzyme, enzyme kinetics is not necessarily improved and may possibly be hindered. There are numerous explanations for this, with porosity being a major area of interest. Since enzyme substrate and product flow through cage pores, their size and charge are important to nanoreactor function. Our lab is interested in creating an ideal nanoreactor with at least 3 features, porosity that does not hinder enzyme kinetics, robust cage assembly and ease of enzyme loading.

Encapsulins are protein nanocages arising from the spontaneous assembly of numerous copies of a monomer in cells. Furthermore, encapsulins can encapsulate proteins fused to a cargo loading peptide. They serve as a versatile yet simple system that have the potential to act as a vessel in endeavours like imaging, drug delivery and nanoreactors. The largest to date is the *Quasibacillus thermotolerans* encapsulin with a diameter of 42 nm. Despite its large size, it has very small pores, with the largest being 7.2 Å. Thus, our lab is interested in designing pore mutants that can improve molecular flux, engineering a protein cage that can impart stability on the enzyme with improved kinetics.

Our lab has previously explored rationally designed pore mutants to investigate this claim. However, it was a tedious process that only allowed for a handful of mutants to be characterised. Based on those efforts, our lab was interested in designing a directed evolution selection that would allow for a much higher throughput selection of mutants. In this presentation, I will discuss the design of the directed evolution selection. I will also go through the hits from the selection and show the kinetic data of Human Carbonic Anhydrase II (HCAII) encapsulated in the selected mutants.

Engineering mosaic cell-penetrating encapsulins for drug delivery.

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Encapsulins are monomeric proteins that are capable of self-assembling into protein cages. Owing to the simplicity of sequestering cargo within these cages, and the protection from degradation that encapsulation provides, they possess enormous potential for the stabilization and delivery of therapeutics into cells. However, whilst there are isolated cases of encapsulins being shown to enter cells, they are neither optimized nor widely applicable. This project aims to address this limitation by displaying a range of motifs on the surface of the encapsulin cage to improve cellular uptake.

A library of encapsulin monomers modified to display a range of cell penetrating peptides, endosomal escape peptides, and receptor binding motifs will be synthesised. These monomers will be combined in various combinations and stoichiometries to produce a library of mosaic cages. A series of luminescence and viability assays will be performed which will identify the combinations that most efficiently enter cells. These combinations will be characterized to ensure the stability and integrity of the shells. We hope this work will allow encapsulins to be used as a modular drug delivery vehicle capable of delivering cargo into the cytosol of target cells with high specificity.

CellBauhaus: A modular computational platform for accelerated microbial strain engineering in industrial biomanufacturing

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Industrial biomanufacturing demands precise microbial strain engineering to maximise target metabolite yields, enhance metabolic efficiency, and minimise by-product formation, while ensuring process sustainability at scale. At CellBauhaus, we have developed a modular computational platform designed to accelerate the Design-Build-Test-Learn (DBTL) cycle for precision fermentation applications.

Our framework combines protein dynamics, gene regulation, and metabolic modelling through a novel algorithmic approach. Our genome-scale metabolic models are enriched through systematic integration of the UniProt protein database and the MetaNetX reaction database, generating mechanistically rich models that capture the complex interplay of cellular processes.

The platform employs hybrid kinetic and constraint-based modelling to simulate strain behaviour under dynamic process conditions representative of industrial fermentation environments. Strain optimisation utilises our proprietary evolutionary algorithm that systematically explores gene knockouts, expression modulation, and heterologous pathway integration across the complete space of characterised enzyme-catalyzed reactions. This *in silico* design and screening approach substantially reduces experimental burden by identifying viable and non-intuitive engineering strategies prior to laboratory implementation.

We demonstrate platform utility in designing novel strains across multiple industrial fermentation use cases, highlighting its ability to predict performance and uncover non-intuitive engineering targets. The platform's modular architecture and comprehensive data integration enable rapid adaptation to diverse production targets and host organisms, positioning it as a valuable tool for the expanding bioeconomy.

The OGI3 Modular Benchtop Bioreactor: Real-Time Analytics and Scalable Culturing for Synthetic Biology Applications

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The Design-Build-Test-Learn (DBTL) cycle is central to synthetic biology, yet many early-stage workflows are bottlenecked by low-throughput culturing tools and delayed analytics. To address these limitations, Ogibiotec has developed the OGI3, a compact, modular bioreactor system designed to automate and accelerate microbial experimentation. The OGI3 offers real-time monitoring, precise environmental control, and scalable operation, enabling researchers to streamline strain engineering, biosensor validation, and process development.

Each OGI3 system supports four parallel culture vessels (14–20 mL) and features integrated optical sensors for real-time optical density (OD600) and fluorescence measurements (e.g., GFP, RFP), with optional modules for pH, dissolved oxygen, and gas control. All culturing parameters—temperature, aeration, and stirring—are finely tunable and remotely managed via a Wi-Fi-connected software interface. This enables high-resolution, *in situ* data acquisition without manual sampling, significantly increasing experimental throughput and reproducibility.

The OGI3 has been applied across a range of synthetic biology applications including:

- Fluorescent reporter assays for promoter tuning and biosensor development
- Strain optimisation under variable conditions
- Temperature profiling of gene expression in E. coli
- Early-stage fermentation workflows replacing traditional shake flasks

Notably, the system promotes sustainable lab practices through its reusable glass and silicone vessels, reducing plastic waste and e-waste typically associated with single-use bioreactor systems. With Technology Readiness Level 9 (TRL 9) achieved and global availability since December 2024, the OGI3 is currently in use across academic, startup, and industrial synthetic biology labs.

This poster will showcase key performance data, experimental workflows, and use cases of the OGI3, demonstrating its value as a versatile tool for high-frequency, data-driven microbial experimentation.

Unlocking the Secondary Metabolite and Volatile Diversity of Trichoderma for Synthetic Biology and Bioactive Compound Discovery

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Trichoderma fungi produce a vast diversity of bioactive secondary metabolites and volatile organic compounds (VOCs), which have important roles in biocontrol, microbe/plant interactions, and ecological adaptation, including abiotic resilience. In this study, we used *Trichoderma atroviride* to explore the biosynthetic potential of the genus. Genome mining and transcriptomic analysis revealed a wide array of biosynthetic gene clusters (BGCs), including non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), and hybrid enzymes predicted to produce antifungal, antibacterial, and plant-interactive metabolites.

We have established a curated Trichoderma New Zealand strain collection comprising more than 1,000 isolates across over 70 species. While not yet fully characterized, this collection represents a rich reservoir of metabolic and genetic diversity. Notably, analysis of selected *T. atroviride* strains revealed distinct profiles of fungal VOCs, highlighting intraspecific variability and ecological versatility.

To enable the functional study and synthetic reconstruction of secondary metabolite pathways, we have developed TrichoGate, a modular cloning system designed specifically for Trichoderma species. This synthetic biology toolkit allows efficient assembly and expression of genes and gene clusters, facilitating the discovery and bioengineering of novel natural products.

These combined resources position Trichoderma as a valuable platform for synthetic biology and the sustainable production of bioactive compounds.

Optimising the discovery and evolution of PET-degrading enzymes

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Recycling only accounts for ~10% of annual global plastic waste disposal, with the remainder polluting the environment and harming human health. Currently, the plastic recycling industry relies on mechanical and chemical methods which are energy intensive, cannot tolerate organic contaminants, and reduce the quality of feedstock with each iterative cycle. In contrast, enzyme-catalysed polyethylene terephthalate (PET) degradation can tolerate organic contaminants and produce “virgin” feedstocks that are infinitely recyclable. To fully enable this technology, the field recognises that novel enzymes with improved industrial properties are required. To date, the discovery of novel enzymes has been limited in scope, with almost all enzymes sharing high sequence and structural homology with two of the earliest known PET-degrading enzymes, isPETase (isolated from the bacterium Ideonella sakaiensis) and the closely related leaf-branch compost cutinase (from a compost metagenome).

To efficiently access unknown PET-degrading enzymes, we have developed a novel high throughput selection method that enables us to rapidly interrogate large environmental DNA (eDNA) libraries. To do this we have combined a library construction method which promotes high-expression of metagenomic inserts, with an E. coli screening strain engineered to be sensitive to a plastic-like compound (PLC). Only E. coli cells expressing polyester-degrading enzymes can survive on PLC-supplemented agar plates. Using these techniques, we have screened tens of millions of eDNA inserts and identified thousands of novel alpha/beta hydrolases, many of which can degrade PET and other plastics. We are now testing whether this selection can be adapted for directed evolution to drive the improvement of leading plastic-degrading enzyme candidates emerging from our metagenomic screening.

Bioprospection and development of a cell factory for mycosporine-like amino acids (MAAs) production

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Ultraviolet (UV) rays from the sun are responsible for several human health issues, including skin cancers and accelerated skin ageing. Topical sunscreens are designed to filter UV rays that reach the skin. However, UV-filtering agents in most existing sunscreens are chemical in nature and have long-term negative effects on human skin as well as detrimental effects on marine biodiversity. Mycosporine-like amino acids (MAAs), native UV filters produced by cyanobacteria, lichens, algae, and fungi, are promising safe, environmentally friendly UV filters that can be applied as active ingredients in sunscreens, cosmetics, pharmaceutical, and material products. In addition to UV-filtering functionality, MAAs exhibit more physicochemical and biological properties that position them as natural sunscreen agents with functional benefits. However, MAAs suffer from extremely poor supply chains, and we still haven't appreciated the full diversity and capabilities of MAAs from nature. Isolation and purification of MAAs is still a challenge.

In this work, we bioprospected MAAs from several cyanobacteria and are making efforts in developing a robust cell factory for their heterologous production and isolation. In addition, we are using integrated omics studies to understand the regulation of MAA synthesis in engineered heterologous hosts in an effort to increase production titres to commercial levels. We report current efforts in engineering, isolation, and production of MAAs using synthetic biology approaches.

Characterising Dynamic RNA–Protein Interactions on Intact, Native RNA with Nanopore Direct RNA Sequencing

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RNA–protein interactions are foundational to gene regulation, yet our ability to study them remains constrained by current technologies. CLIP-based methods, while impactful, often fragment RNA and capture only short binding footprints, severing the interaction interface from its broader epitranscriptomic landscapes.

To retain full transcript information, we are developing Sen-siBORG-seq, an innovative approach that enables direct mapping of RNA–protein interaction sites on intact, native RNA molecules. The core innovation of Sen-siBORG-seq is its use of “cyborg” proteins—genetically engineered RBPs equipped with a photocrosslinkable amino acid—to enable precise RNA and siBORG protein crosslinking at their interaction interface in living cells. Unlike ribonucleotide-based crosslinking, our amino-acid-based approach ensures that covalent linkages form exclusively between RNA and the targeted RBP. After controlled proteolysis, a small peptide stub remains attached to the RNA, physically marking the site of interaction, hence the name siBORG.

Sen-siBORG-seq leverages Oxford Nanopore Technologies (ONT) molecular sensing capability for real-time, single-molecule analysis of bound RNAs. As each RNA molecule translocates through the nanopore, ONT’s platform detects subtle changes in ionic current induced by canonical bases, natural base modifications, and crosslinked adducts. This enables the simultaneous identification of RNA sequence, epitranscriptomic modifications, and the precise locations of protein interaction sites, all on full-length, native transcripts.

By integrating rational site-targeted RBP design (siBORGs) with ONT direct RNA nanopore sequencing, Sen-siBORG-seq aims to provide a comprehensive, context-rich view of ribonucleoprotein complexes—revealing not just where, but how, proteins engage with RNA in living cells.

Solvent resistance of ancestral CYP2Ds for applications in biocatalysis

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The CYP2D subfamily is an important group within the cytochrome P450 enzyme family in vertebrate animals, with forms catalyzing a broad range of oxidative reactions and metabolizing various drugs and endogenous compounds. This versatility makes CYP2D enzymes promising candidates for drug discovery and development. High substrate concentrations can enhance enzyme catalytic rates–a crucial factor in industrial-scale reactions, thus employing elevated solvent levels is advantageous for dissolving lipophilic target substrates. However, most extant CYP2D forms rapidly lose activity in organic solvents, even at very low concentration. This study aimed to characterize the solvent resistance of highly thermostable ancestral CYP2D enzymes and elucidate the basis to their solvent tolerance. Ancestral CYP2D variants demonstrated enhanced stability in organic solvents compared to their extant counterparts with the solvent effect order being DMSO < MeOH < EtOH < acetonitrile < acetone. Solvent resistance (RTC50) correlated broadly with the evolutionary age of the enzymes suggesting a relationship between resistance to solvents and heat (T50). While extant CYP2D forms lost activity at around 3% v/v methanol, the most robust ancestral CYP2D metabolized testosterone at methanol concentrations of up to 20% v/v. Overall, this study sheds light on resistance mechanisms of ancestral CYP2Ds underscoring the potential usefulness of these enzymes for industrial biocatalysis.

Engineering of *Aspergillus oryzae* for the production of nutraceutical omega-6 oils

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Aspergillus oryzae is a fungal model organism with high total lipid content, an extensive history of use in industrial production and an existing suite of techniques and tools for genetic modification and strain optimisation, and generally recognised as safe (GRAS) status. *A. oryzae* is used in the commercial production of products including but not limited to soy sauce, miso paste, sake, shochu, and mirin; as well as citric acid and other valuable industrial biomolecules. GRAS status extends to those products where *A. oryzae* biomass is incorporated and forms part of the final product. Selection of a production strain amenable to regulations may simplify downstream processing.

Omega-6 poly-unsaturated fatty acids represent valuable target biomolecules for production and potential feedstock for downstream production of prostaglandins.

To achieve the engineering of an auxotrophic ΔpyrG RIB40 Aspergillus oryzae, a suite of Golden Gate plasmids was designed using the Dueber Lab's "Yeast Toolkit" MoClo overhang syntax. Constitutive and inducible promoters were tested, and plasmid backbones were designed for episomal expression, genomic integration, and marker recycling for repeated integration. Protocols for the generation of A. oryzae protoplasts were optimised, allowing for the freezing and storage of pre-prepared aliquots.

The conversion of natively present linoleic acid (LA, 18:2 Δ9,12) to omega-6 poly-unsaturated fatty acids was attempted via two pathways using heterologous FA-modifying desaturases and elongases. The addition of a desaturase to increase the pool of available LA by conversion of natively present oleic acid (OA, 18:1 Δ9) was also attempted.

Modified strains were grown in shaking flasks and lipids were extracted from the collected biomass. Fatty-acid methyl esterification was used to render the extractions amenable to GC-MS.

Further engineering of strains to increase FA titre would include overexpression of transketolases or downregulation of the beta-oxidation pathway.

Exploring Oxidative Stability in Ancestral Cytochrome P450s: Toward Robust Biocatalysts for Pharmaceutical Applications

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Cytochrome P450 enzymes (P450s) are versatile proteins with potential as biocatalysts in pharmaceutical synthesis due to their ability to perform regio- and stereo-selective oxidations. However, their industrial application is often hindered by the reliance on a redox partner and an expensive cofactor, such as NADPH. The use of the peroxide shunt pathway is a cheap alternative that bypasses the need for redox partners, NADPH, and oxygen. However, this leads to oxidative damage due to the use of reactive peroxides. In this study, we investigated the resistance of both ancestral and extant P450s to inactivation by the organic peroxide, cumene hydroperoxide (CuOOH), and measuring P450 stability via the characteristic Fe(II).CO vs Fe(II) difference spectrum. Ancestral P450 variants exhibited markedly higher resistance to peroxide-induced inactivation compared to their extant counterparts, suggesting inherent structural features that confer oxidative resilience. Molecular dynamics simulations and in silico analyses of hypothetical protein structures were performed using both ancestral and extant enzymes to elucidate the basis of this enhanced robustness. Preliminary results suggest that specific residues located near the haem pocket and access tunnels influence peroxide accessibility and structural integrity. These findings contribute to a better understanding of the structure-function relationships in P450s and highlight the potential of ancestral sequence reconstruction as a strategy for generating robust biocatalysts. By identifying the key structural determinants of oxidative stability, this study aims to facilitate future protein engineering efforts to develop efficient peroxide-driven P450 systems for the manufacture of fine chemicals.

Synthetic Biology Applications: Future-repairing or Future-making

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This project examines how SynBio startups and ecosystem stakeholders shape sustainable futures through narratives and anticipation of unpredictable times. In entrepreneurial ecosystems (EEs), enabling technologies such as synthetic biology are often viewed as solutions to complex problems, with their applications aimed at ‘future-repairing’ extractive systems, such as fossil fuels and intensive agriculture.

Future repair involves developing applications to address issues such as climate change, energy shortages, food scarcity, biodiversity loss, and waste. The concept of future making, beyond tackling wicked problems, aims to support innovative business models and alternative systemic frameworks that also create and deliver value in fair and inclusive ways, promoting sustainability and prosperity.

EEs are complex systems that emerge and adapt through entrepreneurial intentionality, activity coherence, and resources. EEs evolve and mature through competing narratives within the start-up community in a region. With a synthetic biology ecosystem still emerging, I will argue that future-making, as a collectively learned action, can lead to deliberately and purposefully guiding roadmaps toward creating new ventures and preferred transformative futures. My project investigates if and how actors collaboratively shape future scenarios through narratives and strategic foresight, while responding proactively to external stressors. This involves developing adaptable frameworks to manage unpredictable events, such as geopolitical shifts, as well as linear changes, including regulatory frameworks. The aim is to support startups in swiftly pivoting and adapting their strategies to secure profitability, enhance sustainability, and work towards their preferred futures.

The synthetic biology market is rapidly expanding, especially in North America and the Asia Pacific, driven by recent advances in large-scale biomanufacturing and other breakthroughs. A global mapping of 562 synthetic biology companies reveals that most are in the food and health sectors, primarily B2B and manufacturing. Growing venture capital funding makes up nearly 50% of the total USD 47 billion.

Australasia represents less than 4% of global startups. Additionally, only 82 out of 287 companies prioritize sustainability in their value propositions.

Questions from this data concern the emergent SynBio EE and its capacity to support new ventures, as well as make sustainable futures a reality. I argue that we require approaches that purposefully and continually ‘make’ futures. At the conference, I plan to engage with participants in conceptual discussions, explore collaboration in futures thinking at the ecosystem level, and identify individuals willing to participate in data collection and ecosystem foresight workshops.

The human tumour suppressor p15(INK4b) forms amyloid fibrils upon oxidation

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Cancer growth is driven by a set of specific hallmarks, one of which is the evasion of growth suppressors, resulting in unregulated cell growth. The INK4 family of cyclin-dependent kinase inhibitors is essential for preventing uncontrolled cell growth. These proteins act at the G1/S phase checkpoint and induce cell cycle arrest. The INK4 family of proteins include p15(INK4b), p16(INK4a), p18(INK4c) and p19(INK4d).

Amyloid fibrils are protein aggregates often associated with neuropathological conditions and are characterised by their fibrillary morphology and cross ß-sheet secondary structure. We recently reported that p16(INK4a) forms amyloid fibrils through a thiol-dependent oxidation mechanism due to a conserved cysteine residue [1]. Here, we study the potential amyloid formation of the homologous protein p15(INK4b), which shares 86% sequence identity with p16(INK4a).

p15(INK4b) also harbours a conserved cysteine residue, and we are using the cysteine-specific synthetic oxidant diamide to induce aggregation of the protein. We are characterising these aggregates by mass-spectrometry, gel electrophoresis, amyloid-specific fluorescent dyes, and atomic force microscopy. We find that p15(INK4b) forms a dimeric intermediate species similar to the thiol-dependent amyloid formation mechanism of p16(INK4a). Kinetic Thioflavin-T binding assays reveal that upon oxidation, p15(INK4b) converts into amyloids about four times faster than p16(INK4a). We are currently investigating the mechanistic differences leading to the rapid formation of amyloids. Understanding p15(INK4b) amyloid fibril formation helps elucidate a potential mechanism for cancer progression, where important tumour suppressors can lose their function through conversion into amyloid structures.

1. Heath, S.G., et al., Amyloid formation and depolymerization of tumor suppressor p16(INK4a) are regulated by a thiol-dependent redox mechanism. Nat Commun, 2024. 15(1): p. 5535.

Engineering microbial strains to produce platform chemicals from C1 waste streams

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The chemolithoautotrophic bacterium Cupriavidus necator (previously Ralstonia eutropha), is an industrially relevant microbe that can convert CO2 and other C1 waste streams into biomass. Here, we engineered C. necator to overproduce the platform chemical isopropanol by taking advantage of its natural carbon flux to the storage polymer polyhydroxyalkanoate (PHA). Key PHA pathway genes in the genome were knocked out and replaced with isopropanol biosynthesis pathway genes, diverting carbon to isopropanol production. Isopropanol biosynthesis was further enhanced through additional plasmid overexpression of pathway genes. As part of this project, we developed a synthetic biology toolkit for C. necator that will also be used in other C1-utilising microbes.

A molecular Rosetta Stone to aid in storage and retrieval of digital data archived in DNA

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It’s estimated that the volume of data globally will reach 175 zettabytes by 2025, and mainstream storage media (such as magnetic tape) will no longer be adequate to store it all. DNA provides a promising alternative medium: it is nature’s storage molecule (storing genetic information) with high density and durability, and multiple research groups have created DNA storage systems both *in vitro* and *in vivo*. However, a major unsolved problem for long term data storage is digital obsolescence. The rapid progression of technology can result in a “digital black hole” where data becomes lost or unreadable over time due to unsupported file formats or changes in software or hardware. To combat digital obsolescence, long-term data storage systems must be robust to such changes: we need systems that are universal, format- and technology-independent, and the data itself must always be decipherable. To this end, we have designed a self-explanatory and self-contained DNA storage system to aid DNA archiving. This DNA storage system utilises a 3-bit RGB colour system that expresses combinations of red, green, and blue fluorescent proteins linked to an artificial genetic code where each codon represents a colour, creating a self-explanatory system for coding images. By specifying this relationship between the artificial genetic code and colours, a synthetic DNA sequence of codons, each specifying a coloured pixel, can be created. Thus, synthetic sequences can be used to specify rows of pixels and column order, yielding a matrix of coloured pixels that forms a 2D image. In turn, this 2D image can be used to encode format information of the stored data, creating a self-contained system. Our ‘molecular Rosetta Stone’ DNA storage system uses an inherently ‘crackable’ code and thus fulfils the requirements required for combating digital obsolescence. This is due to the universality of the visible light spectrum (colour) and the independence from a specific read/write technology (DNA can be sequenced by multiple methods), so our system can always be read and the relationship between codons and colour deciphered. We will present our proof-of-principle design and report on progress in developing this system.

Rumen Health Through Industry Collaboration: One Additive, Three Solutions – Methane, Acidosis, and Bloat

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Enteric methane emissions, ruminal acidosis, and frothy bloat are major challenges in ruminant production, impacting both sustainability and animal welfare. This study investigates a novel feed additive that is able to address all three issues simultaneously. Using *in vitro* rumen simulation systems, we assessed its impact under conditions that induce acidosis or bloat. The additive consistently reduced methane production without impairing fibre digestion or volatile fatty acid (VFA) profiles. In acidosis simulations, it prevented pH collapse—even though the additive acidifies water—suggesting a microbial rather than chemical mode of action. Under bloat-prone conditions, it reduced foam formation, indicating possible anti-frothing effects or altered microbial gas dynamics. Microbiome analyses revealed shifts in key taxa linked to methanogenesis, lactate accumulation, and mucopolysaccharide production.

Interestingly, we observed a transient spike in lactate following additive application that was rapidly converted into VFAs. To prevent a premature negative effect of the additive in grain-fed cattle, we aim to identify key genes involved in this conversion, focusing on Megasphaera—a known lactate-utilizing genus. Our goal is to engineer Megasphaera strains with improved oxygen tolerance and/or increased metabolic efficiency, supporting more robust rumen stability under stress conditions. This combined strategy offers a promising pathway to improve animal health, productivity, and environmental outcomes through a single, multifunctional additive.

Building a Precision Fermentation Platform in Yeast for Producing Dairy Proteins and Lipids

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Dairy plays a central role in global diets not just for its nutrition, but for the taste, texture, and functionality it brings to food. Yet producing dairy through animals comes with high environmental and ethical costs. While plant-based alternatives have gained traction, they often fall short of replicating the molecular precision of real dairy components. To truly replace dairy, we need a different approach one that recreates these molecules without the cow. Precision fermentation offers that possibility.

We are developing a production platform using two industrial yeast species: one tailored for protein expression and the other for lipid biosynthesis. Our goal is to enable the efficient microbial production of functional dairy proteins and fats. To guide strain engineering, we have built genome-scale metabolic models (GSMMs) and gene regulatory networks (GRNs) for both hosts, allowing us to predict engineering strategies that can enhance yield.
In parallel, we have assembled a modular synthetic biology toolbox including promoters, secretion signals, CRISPR editing systems, and replicative origins designed to streamline strain construction. These tools are being applied within a Design–Build–Test–Learn (DBTL) framework to iteratively improve performance.

Initial constructs are being validated in shake flask cultures, and 10 L pilot-scale fermentation trials are underway to evaluate scalability. While dairy proteins and lipids are our initial focus, the platform is designed to be adaptable. We share this work as a platform for future collaboration in sustainable microbial biomanufacturing.

Slow release of hen egg white lysozyme from polyhydroxybutyrate particles

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Polyhydroxybutyrate (PHB) particles are a known slow-release mechanism for drug-delivery and bioremediation applications. These can be produced synthetically (synthetic particles; SPs) or recombinantly in a microbial host, such as Escherichia coli, termed bioparticles (BPs). Hen egg white lysozyme (HEWL) is an amphipathic protein with antimicrobial activity via the ability to cleave the β1-4 linkage in bacterial peptidoglycan. This study aims to determine if PHB particles act as an effective slow-release mechanism for HEWL to prevent the growth of the food-spoilage organism *Clostridium estertheticum*, and whether there are differences in the activity between SPs and BPs.

C. estertheticum is one of the main causative agents of blown pack spoilage (BPS) of vacuum-packed fresh meat products. A psychrophilic, anaerobic, spore-forming bacterium, *C. estertheticum* produces carbon dioxide giving the distinctive “blown-pack” appearance, accompanied by a mix of volatile compounds causing a putrid-cheesy aroma.

The enzymatic activity was tested by lysing *Micrococcus luteus*. It was noticed that BPs exhibited a higher lytic activity (691 units/g PHB) compared to SPs (182 units/g PHB). After multiple washes (10 cycles), the activity of the BPs still outperformed SPs with an activity of 138 units/g PHB ($p = 0.0019$) and 25.5 units/g PHB ($p = 0.013$), respectively. To account for differences in HEWL binding, the ζ-potential was measured. SPs showed a more negative ζ-potential (-37.8 mV) than BPs (-19.6 mV). Interestingly, adding HEWL to BPs did not affect their ζ-potential ($p = 0.06$); conversely, adding HEWL to SPs resulted in a less negative ζ-potential (-9 mV).

The study also analysed the effect of the particle with the highest activity against *M. luterus* (HEWL-loaded BPs) on the formation of BPS of meat spiked with *C. estertheticum*. It was observed that HEWL-loaded BPs were effective in preventing BPS. After 7 weeks of storage, the samples of HEWL-loaded BPs still showed slightly more than 60% no signs of BPS, whilst all inoculated control samples had blown.

In this study, BPs emerge as the more suitable candidate as a slow-release vehicle for HEWL in food packaging. Future research will investigate the mechanism that enables greater activity by BPs.

Characterisation of the Tailoring Steps of Nargenicin-like Macrolide Biosynthesis

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The nargenicin- and streptoseomycin-like macrolides are a family of antibiotics distinguished by a characteristic ether-bridged cis-decalin skeleton and potent, narrow-spectrum antibacterial activity. They exert their effect through inhibition of DnaE, the alpha subunit of DNA polymerase, an enzyme not currently targeted by clinically used antibiotics. Members of this class can be broadly classified into two groups based on specific structural features that appear to dictate their spectrum of activity. Nargenicin-like molecules primarily target Staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA), whereas streptoseomycin-like molecules act against microaerophilic bacteria such as *Neisseria* spp. and *Helicobacter pylori*. The identification of new members of the nargenicin/streptoseomycin family may uncover molecules with novel activity spectra or improved pharmacological properties.

Here, we used a genome-mining approach to identify previously uncharacterised nargenicin-like biosynthetic gene clusters, enabling the isolation and characterisation of several new members of the family. Using CRISPR knockouts, we identified the enzymes responsible for these alternative tailoring steps and overexpressed them in the original nargenicin producer to further derivatise the core scaffold. A deeper understanding of the biosynthesis of this class will support efforts to broaden the antibacterial spectrum of these promising compounds.

Metagenomic discovery of portable resistance modules for engineered immunotherapies

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Engineered immunotherapies have been transformative for hematological malignancies; however, their effectiveness against solid tumors remains limited. These therapies could potentially benefit from labelling with defensive genes that provide resistance to chemotherapy, enabling the co-administration of both treatment modalities. However, most forms of chemotherapy resistance that arise spontaneously in the clinic either affect other aspects of cellular function or are not easily transferrable to different cell types. To provide portable chemotherapy resistance genes, we have identified genes encoding enzymes capable of detoxifying a broad range of chemotherapeutics. Using a bespoke cloning strategy that places genes captured directly from soil metagenomic DNA immediately downstream of a strong *Escherichia coli* promoter, ribosome binding site, and hexahistidine tag, we have developed a robust pipeline for functional screening of chemotherapy resistance elements that provides access to promiscuous activities manifesting as weak and otherwise undetectable phenotypes. Expression of some of the encoded enzymes in human cells has increased chemotherapeutic IC_{50} values by up to 80-fold, demonstrating their potential to enhance cell survival under chemotherapy challenge. To further optimize their catalytic efficiency and substrate specificity, we are applying directed evolution with the goal of enhancing their protective capabilities in human cells. Ongoing characterization of these enzymes will provide insights into their mode of action and inform rational design strategies.

Echo Acoustic Liquid Handling and Next-Generation TnX Transposase-Based Technologies Enable Miniaturised, Automated NGS Library Preparation for Ultra-High-Throughput Sequencing

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Using Echo's rapid, contact-free acoustic dispensing, combined with ExpressPlex 2.0 chemistry and miniaturised reactions, we demonstrate economies of scale and ultra-high-throughput workflows for plasmid sequencing.

The degree of miniaturisation achieved was approximately one-fifth of the standard ExpressPlex reaction volume, enabling significant reagent cost savings. This approach is also applicable to amplicon sequencing and other construct-validation applications in protein engineering and synthetic biology.

Harnessing Protein Design to Target NUDIX Effectors

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In plant–pathogen interactions, pathogens deploy effector proteins to suppress the plant's immune system. However, these effectors can also be recognized by immunity receptors, triggering pathogen detection and activation of defence pathways. Recent advances in our structural understanding of how immunity receptors bind effectors, combined with the modularity of some receptors that have the potential to bind "any" effector, have spurred significant interest in immunity receptor engineering. Concurrently, major strides in computational approaches; including accurate protein structure prediction, modelling of protein–protein interactions, and de novo protein binder design, have further expanded our capacity to engineer these receptors and opened additional avenues for suppressing pathogen virulence. Here, I present our recent efforts in applying both experimental and computationally derived protein binders to target NUDIX effectors from pathogenic fungi.

Aptamer Biosensor for Smoke Taint Detection in Wine Grapes

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Bushfire smoke contamination represents a significant and growing challenge for Australian viticulture, with more than $400 million in grape losses reported over the past decade. As climate change increases the frequency and intensity of fire events, the industry urgently requires rapid and reliable tools to assess smoke exposure and reduce the financial risks of harvesting and processing affected grapes. Current laboratory-based methods, while accurate, are costly and limited by turnaround times, constraining their application for timely decision-making during harvest.

This project addresses this gap by developing an aptamer-based biosensor platform for the detection of phenolic glycosides, the molecular markers of smoke taint. Through a capture SELEX approach, we identified and characterised aptamer 6431 as a high-affinity molecular recognition element for syringol gentiobioside (SyrGG), one of the key phenolic glycosides associated with smoke taint. Binding analyses using isothermal titration calorimetry (ITC) revealed nanomolar affinity and remarkable specificity, and showed no detectable interaction with glucose. Given that sugars—mainly glucose and fructose—can constitute up to 18% (w/w) of fresh grapes, this absence of cross-reactivity underscores the aptamer's selectivity and robustness for SyrGG detection. Integration of aptamer 6431 into a portable assay is underway, with optimisation focused on identifying the most effective biosensing configuration. Lateral flow, electrochemical, and optical (fluorescent or colourimetric) formats are being evaluated to develop a rapid and reliable on-site test for vineyard applications.

By providing grape growers and winemakers with a portable, cost-effective diagnostic tool, this technology can support evidence-based decisions during bushfire events, strengthening the resilience of the wine sector.

Developing novel biosensors using AI and yeast

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Biosensors have the potential to revolutionize medical diagnostics, personal health and environmental contaminant monitoring, food safety, biodefense, and industrial processes. Biosensors are powerful tools for detecting target ligands, but their development is often hindered by the labor-intensive process of identifying specific binding pairs. Usually, biosensors require two ligand binding pairs which are built either by searching the literature for known domains or by performing protein engineering techniques. While nature has evolved many metabolites and protein binding domains, there are not enough known domains to generate biosensors for all desirable target molecules.

This project focuses on the development of novel biosensors using high-throughput screening and AI techniques. We have developed the Simultaneous Yeast Display (SYD) technique, which leverages yeast mating to identify chemically induced dimerization (CID) systems for biosensor applications. By modifying yeast cells to mate only in the presence of specific ligands, we can screen libraries of protein-ligand pairs, efficiently discovering new binding pairs. In proof-of-concept experiments, we demonstrated successful interactions using high-affinity protein pairs such as cohesin-dockerin, as well as ligand-binding systems including Pro1a-Pro1b in the presence of progesterone.

To improve the limit of detection due to background noise, we further developed rationally designed libraries, incorporating AI-generated ligand-binding domains, and pivoted toward using a Yeast Three Hybrid (Y3H) system. Specifically, we applied AI tools to generate binding pairs against resolved structures, such as maltose-binding protein and cholic acid. This approach allowed for more targeted screening and enhanced the probability of identifying effective CID systems. This system, coupled with split-ubiquitin variations, provides a robust platform for developing biosensors tailored to different target molecules.

Physcomitrium patens chloroplasts as biofactories for future astronauts: tool building for chloroplast expression of useful bioproducts

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As we get closer to realising our dream of Mars colonisation, how to maintain a sustainable supply of food, medicine and other proteins essential for survival without reliance on Earth becomes the ever-important topic. Plants, particularly the model moss *Physcomitrium patens*, present potential as chassis for such production thanks to their ease in maintenance compared to mammalian and bacterial cell systems, and the promise of high yield through protein expression from the chloroplast genome, which encodes for the most abundant protein on Earth: RuBisCo. However, while ample tools have been developed for transient and stable expression from the nuclear genome of *Physcomitrium*, much work is required for expanding the toolkit for *Physcomitrium* chloroplast engineering, such as exploring reporters beyond the aadA spectinomycin expression cassette and other expression elements from promoters to terminators.

My project addresses this limitation by testing different genetic elements for *Physcomitrium* chloroplast expression, as well as improving the method of transformation based on our learnings from nuclear transformation. To start with, different reporters were targeted into the chloroplast genome from nuclear expression. These include different fluorescent and luminescent reporters, including UV-excitable fluorescent proteins and fluorescent protein fusion with the monomeric nanoluciferase (nanolanterns). Both native and heterologous transit peptides were tested and the relative expression level of the reporters with the transit peptides were analysed. These will set benchmarks to guide choices for chloroplast expression reporters, as well as provide tools for future experiments on utilising nuclear-encoded, chloroplast-targeted activators for inducible expression from the *Physcomitrium* chloroplast genome.

Developing synthetic RNA editing factors for the control of chloroplast gene expression

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In the race to meet global demands for sustainable bioproduction, chloroplast transformation is increasingly being recognised as a powerful tool, promising high yields of vital biomolecules such as pharmaceuticals or enzymes for industrial applications. However, precision control of chloroplast transgene expression remains a significant challenge, and consequently most chloroplast transformation projects result in constitutively expressed transgenes with limited ability to control the level, location, or timing of expression.

Here, we present an approach to achieving inducible chloroplast expression by harnessing RNA editing to create start codons (ACG-to-AUG editing) in a chloroplast mRNA, thus initiating translation. Our strategy utilises a synthetic RNA editing enzyme based on consensus sequences of plant pentatricopeptide repeat (PPR) proteins, which is able to specifically edit the ACG start codon of a reporter gene encoding a fluorescent protein in both bacteria and in transgenic *Nicotiana tabacum* chloroplasts and induce reporter protein expression. Further experimentation into optimal synthetic protein design, as well as application in vivo in the moss *Physcomitrium patens* is currently underway.

This strategy will ultimately enable dynamic, condition-dependent control of transgene expression within the chloroplast, providing a valuable tool for fine-tuning chloroplast-based bioproduction. The outcomes of this research will contribute to expanding the toolkit for controlling gene expression in organelles, as well as deepen understanding of the fundamental mechanisms underlying RNA editing and open the door to new applications in sustainable biotechnology.

Xeno Nucleic Acid Joining Ligases for Non-Natural Nucleic Acid Synthesis

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DNA ligases are a class of enzymes that join pieces of double-stranded DNA by forming new bonds in the phosphodiester backbone. The DNA is double-stranded due to Watson and Crick base-pairing rules between naturally occurring bases A-T and G-C. However, scientists have designed additional unnatural base pairs (UBPs) that still follow these Watson-Crick rules but have different hydrogen bonding patterns that expand the genetic alphabet. One example is the eight-letter 'Hachimoji' system which includes the two natural base pairs and two synthetic base pairs commonly known as S-B and P-Z.

The ability of DNA ligases to join nucleic acid backbones containing UBPs has not been widely investigated, however ligases have potential use in creating aptamers containing UBPs and can also join an ever-expanding toolbox required for cloning UBP-DNA. Eventually, it may be possible to construct entire genomes with UBPs that could be made into proteins; however, this will require compatible enzymes.

We have previously demonstrated that DNA ligases can join DNA containing UBPs. In addition, we have determined reaction variables that can lead to higher activity with UBP substrates and found some conditions where complete ligation was observed. Here we describe our ongoing work to develop DNA ligase enzymes suitable for use in molecular biological and synthetic workflows with XNA. To do this, we will continue to optimize the ligation reaction with different types of substrates containing UBPs as well as structurally characterizing and creating mutants of our in-house panel of ligases.

SynBio from lab to field - Edited eukaryotic translation initiation factors confer resistance against maize lethal necrosis

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Maize lethal necrosis (MLN), which is caused by maize chlorotic mottle virus along with a potyvirus, has threatened the food security of smallholders in sub-Saharan Africa. Mutations in eukaryotic translation initiation factors (eIFs), which also facilitate virus genome translation, are known to confer variable resistance against viruses. Following phylogenetic analysis, we selected two eIF4E proteins from maize as the most likely candidates to facilitate MLN infection. A knockout (KO) of each of the corresponding genes in elite but MLN-susceptible maize lines conferred only partial protection. Our inability to knockout both the genes together suggested that at least one was required for survival. When we edited (ED) the eIF4E genes in Mini Maize, however, the plants with the eif4e1-KO became highly resistant, whereas those with the eif4e2-KO remained susceptible. Neither of the causal viruses could be detected in the MLN-inoculated eif4e1-KO plants. The eIF4E2 cDNA in Mini Maize lacked the entire 4th exon, causing a 22-amino acid in-frame deletion, which shortened the protein to 198 amino acids. When we introduced mutations in the 4th exon of the eIF4E2 gene in two elite, MLN-susceptible lines pre-edited for an eif4e1-KO, we obtained as strong resistance against MLN as in eif4e1-KO Mini Maize. The MLN-inoculated lines with eif4e1-KO/eIF4E2-exon-4ED performed as well as the uninoculated wild-type lines. We demonstrate that the C-terminal 38 amino acids of eIF4E2 are dispensable for normal plant growth but are required for the multiplication of MLN viruses. Our discovery has wide applications across plant species for developing virus-resistant varieties.

Generating Tools to Probe Human Peroxiredoxin 2 Oligomeric State

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Peroxiredoxins are a ubiquitous family of thiol peroxidases which catalyse reduction of hydrogen peroxide (H_2O_2) to water in cellular compartments. They play an important role in cell signalling, protect macromolecules from extensive oxidative damage caused by high levels of H_2O_2, and are implicated in ageing and various diseases. The catalytic mechanism of peroxiredoxins has been well characterised; however, their oligomeric state within cells remains uncertain. Peroxiredoxins have been proposed to dynamically switch between a dimer and a decameric ring composed of five dimers. The factors influencing this switch remain undetermined and could be a combination of the protein's oxidation state, concentration, environmental pH, or post-translational modifications.

Our aim is to develop tools to track the oligomeric state of human peroxiredoxin 2 (Prx2) in the cytoplasm of living cells. Our first approach to developing suitable tools is the design of novel protein binders in silico using BindCraft, a pipeline leveraging AlphaFold2, MPNN, and PyRosetta. We have used this pipeline to design proteins targeting either the Prx2 dimer or decamer and then refined our selection of binders using Boltz-1 and AlphaFold3 for a preliminary assessment of likely specificity. Selected binders will be expressed in *E. coli* and binding to Prx2 dimeric and decameric states assessed with size exclusion chromatography (SEC). Our second approach is selecting Prx2 oligomeric-state-specific nanobodies from a synthetic yeast surface display library. The enriched nanobodies were expressed in *E. coli* and their binding against either the Prx2 dimer or decamer was assessed using SEC. Immunoprecipitation experiments in living cells will then be used to analyse the ability of the nanobodies to specifically pull down Prx2. Together, these tools will help us to further our understanding of the dynamics of the Prx2 dimer and decamer within living cells.

Impact of cell wall polysaccharide modifications on the performance of Pichia pastoris: novel mutants with enhanced fitness and functionality for bioproduction applications

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Pichia pastoris, known as *Komagataella phaffii*, is a widely utilized host for heterologous protein expression and biotransformation. Despite the numerous strategies developed to optimize the chassis host GS115, the potential impact of changes in cell wall polysaccharides on the fitness and performance of *P. pastoris* remains largely unexplored. This study aims to investigate how alterations in cell wall polysaccharides affect the fitness and function of *P. pastoris*, contributing to a better understanding of its overall capabilities. Two novel mutants of GS115 chassis, H001 and H002, were established by inactivating the PAS_chr1-3_0225 and PAS_chr1-3_0661 genes involved in β-glucan biosynthesis. In comparison to GS115, both modified hosts exhibited a looser cell surface and larger cell size, accompanied by faster growth rates and higher carbon-to-biomass conversion ratios. When utilizing glucose, glycerol, and methanol as exclusive carbon sources, the carbon-to-biomass conversion rates of H001 surpassed GS115 by 10.00%, 9.23%, and 33.33%, respectively. Similarly, H002 exhibited even higher increases of 32.50%, 12.31%, and 53.33% in carbon-to-biomass conversion compared to GS115 under the same carbon sources. Both chassis displayed elevated expression levels of green fluorescent protein (GFP) and human epidermal growth factor (hegf). Compared to GS115/pGAPZ A-gfp, H002/pGAPZ A-gfp showed a 57.64% higher GFP expression, while H002/pPICZα A-hegf produced 66.76% more hegf. Additionally, both mutant hosts exhibited enhanced biosynthesis efficiencies of S-adenosyl-L-methionine and ergothioneine. H001/pGAPZ A-sam2 synthesized 21.28% more SAM at 1.14 g/L compared to GS115/pGAPZ A-sam2, and H001/pGAPZ A-egt1E obtained 45.41% more ERG at 75.85 mg/L. The improved performance of H001 and H002 was likely attributed to increased supplies of NADPH and ATP. Specifically, H001 and H002 exhibited 5.00-fold and 1.55-fold higher ATP levels under glycerol, and 6.64- and 1.47-times higher ATP levels under methanol, respectively, compared to GS115. Comparative lipidomic analysis also indicated that the mutations generated richer unsaturated lipids on cell wall, leading to resilience to oxidative damage. Two novel *P. pastoris* chassis hosts with impaired β-1,3-D-glucan biosynthesis were developed, showcasing enhanced performances in terms of growth rate, protein expression, and catalytic capabilities. These hosts exhibit the potential to serve as attractive alternatives to *P. pastoris* GS115 for various bioproduction applications.

Accelerating the Design-Build-Test-Learn Cycle: AI-Driven Real-Time Biomass Prediction for Synthetic Biology

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The Design-Build-Test-Learn (DBTL) cycle is fundamental to synthetic biology, yet the "Test" phase remains a significant bottleneck, particularly in characterizing engineered microbial strains. Traditional methods for quantifying cell growth, such as dry cell weight (DCW) analysis, are offline, labour-intensive, and introduce significant delays, hindering high-throughput screening and dynamic process optimization. This disconnects between the rapid pace of genetic design and the slow pace of physical characterization impedes the efficient engineering of biological systems. Here, we present a machine learning-based framework for real-time, non-invasive prediction of cellular biomass in bioreactors. Our approach leverages data from standard, ubiquitous sensors (e.g., pH, dissolved oxygen, turbidity, CO2) to model and predict biomass concentrations instantaneously. We established this methodology using *Komagataella phaffii*, a workhorse chassis organism in synthetic biology, by conducting controlled fermentations to generate a robust training dataset of sensor readings paired with offline DCW measurements. Multiple ML models, including boosting algorithms and neural networks, were trained and validated to identify the most accurate and generalizable predictive model.

This work delivers a validated, open-source tool that empowers researchers to monitor the performance of engineered strains in real-time, dramatically accelerating the DBTL cycle. By providing immediate feedback on cellular growth dynamics, our model facilitates rapid optimization of fermentation conditions, improves the scalability of bioprocesses, and advances the industrial translation of synthetic biology. This data-driven approach represents a critical step towards creating "smart" bioreactors that can intelligently guide and control the production of novel bioproducts, from therapeutic proteins to sustainable biofuels.