Norwich
EngBio Day

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ABSTRACTS

Invited Speaker

The Environmental Biotechnology Innovation Centre: Using microbes to solve environmental problems

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The Environmental Biotechnology Innovation Centre (EBIC) brings together 9 leading universities across England, Wales and Scotland, including Bangor, East Anglia, Edinburgh, Essex, Glasgow, Heriot Watt Newcastle, Southampton forming a coordinated consortium for large-scale, long-term research and innovation programmes targeting complex environmental challenges. The Hub is structured to integrate academic excellence with strong industrial engagement across the water, waste, environmental, and biotechnology sectors, supported by extensive partner commitment. This ecosystem ensures that research is tightly aligned with real-world requirements, enabling rapid translation of scientific advances into deployable environmental solutions. EBIC focuses its core activities across three thematic areas:

1. next-generation biosensing for environmental monitoring,
2. bioremediation and bio-sequestration of pollutants, and
3. biologically enabled wastewater and waste treatment processes.

These themes are supported by cross-cutting capabilities in DNA engineering, biomolecular design, host and microbial consortia engineering, and data-driven system modelling and AI-assisted design-build-test-learn workflows. EBIC builds on and connects existing national capabilities and infrastructure, including the Edinburgh Genome Foundry, Earlham Biofoundry, Industrial Biotechnology Innovation Centre (IBioIC), National Biofilm Innovation Centre (NBIC), and UKCRIC water and wastewater research facilities, alongside major UK investments in engineering biology. This coordinated integration avoids duplication and strengthens the overall UK innovation landscape by maximising complementarity across institutions and platforms.

A key feature of EBIC is its commitment to talent development, interdisciplinary collaboration, and responsible innovation. It fosters an open research culture that prioritises diversity, early career researcher development, and long-term capacity building. Flexible interdisciplinary translational research funding mechanisms support high-risk, high-reward projects, technology development, and cross-sector collaboration, with dedicated support for early career researchers. Beyond technical innovation, EBIC actively engages with policy stakeholders, industry, and the public to ensure responsible development, regulatory alignment, and societal acceptability of engineering biology solutions. Through this integrated approach, EBIC aims to deliver measurable environmental impact while establishing a globally leading platform for sustainable biotechnology innovation.

Invited Speaker

Engineering synthetic plant artificial chromosomes (synPACs)

Dasha Cuthbert[1] , Melissa Salmon[1], Hélène Yvanne[1], Chinemerem Akamihe[1], Alan Houghton[2], Matthew Downie[2], Victoria Butler[2], Josh James[3], Lucy Heap[3], Katrin Geisler[3], Raymond Wan[3], Mark McCullough[3], Ewan Moody[3], Saad Hussain[3], Sergio Gutiérrez[3], Dia Ghose[3], Haotian Cui[3], Antony Hall[1], Conrad Nieduszynski[1], Carolina Grandellis[1], Anne Osbourn[2], David Seung[2], Patrick Cai[3].

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Synthetic genomics has transformed microbial engineering, enabling genome-scale design, construction, and testing. However, comparable technologies in plants remain limited by major bottlenecks, including genome-scale design, megabase-scale DNA assembly, high-capacity delivery into plant cells, and stable artificial chromosome formation. Our long-term ambition is to develop a robust and reproducible engineering ecosystem for synthetic plant artificial chromosomes, or synPACs, that will allow plants to be programmed at scale. We are establishing this platform directly in potato (Solanum tuberosum), one of the world's most important non-grain food crops and a key species for global food security. Potato provides both an important translational target and a challenging plant system in which to build technologies that could later be adapted to other crops. Our pipeline integrates computational chromosome design, hierarchical megabase-scale DNA assembly in yeast, delivery of synthetic DNA from yeast to plant cells, and functional testing of synthetic regulatory and trait modules.

Recent progress supports the feasibility of this strategy. We are developing a potato tissue atlas, applying genomic language models to identify motifs within regulatory elements, performing both in vitro and in vivo testing of putative regulatory elements, and designing and synthesising synthetic functional modules. We are also advancing Genome CAD software to visualise, design, and refine megabase-scale synthetic plant chromosomes. In parallel, we are developing an automated yeast DNA assembly workflow, which has supported the construction of early synPAC v1 and v2 assemblies. Finally, we have established robust spheroplasting and protoplasting methods for yeast and plant cells, respectively. Finally, we have demonstrated cytoplasmic yeast–plant cell fusion assays that use reporters to monitor these events. Together, these advances move us toward a scalable platform for engineering synthetic chromosomes in plants, with the potential to accelerate crop improvement, enable novel plant functions, and support more sustainable agriculture.

Invited Speaker

GlycoCell: engineering glycoconjugate biomanufacturer platforms for health

Ryan Griffiths[1], John Heap[2] and **Nathalie Juge[1]**

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Glycans are essential molecules of wide structural and functional diversity, contributing to structure and storage, cell-cell communication, cellular interactions, protein function, physiological and developmental processes.

For example, pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus gordonii* and *Streptococcus pneumoniae* have an accessory secretion pathway dedicated to the secretion and glycosylation of serine rich repeat proteins (SRPPs) implicated in adhesion, aggregation, biofilm formation, immune modulation and virulence. We previously reported aSec machinery in *Limosilactobacillus reuteri* strains, showing strain-specific glycosylation of SRRPs.

The aim of the GlycoCell Engineering Biology Mission Hub, a UK-wide collaborative hub led by University of Nottingham, is to develop and apply biomanufacturing platforms to produce glycans, glycoconjugates, and glycoproteins in microorganisms.

GlycoCell develops and integrates cutting-edge technologies such as the GlycoForge platform facility which combines high-throughput automation, new design principles, data-driven refinement of combinatorial libraries, and custom software for guided design and hands-off construction to rapidly engineer cells to make new glycans and glycoconjugates. These include engineering *Saccharomyces* yeast cells able to mimic the glycosylation patterns of fungal pathogens, *Pichia* yeast cells to produce humanised glycans, *E. coli* to produce glycan variants and glycoconjugate vaccines for streptococcal diseases, and *Bacillus subtilis* to produce O-glycoproteins from major pathogens.

Invited Speaker

ELEMENTAL - Engineering Biology for Critical Metals Recovery

Martin Warren[1]

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Abstract TBC

Selected Speaker

Engineering accessory secretion systems for the production of glycoconjugates in Bacillus subtilis

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The accessory secretion (aSec) system is a protein export pathway present in Gram-positive bacteria dedicated to the secretion and glycosylation of large multi-repeat adhesins called serine rich repeat proteins (SRRPs) [1]. Typically aSec consists of the translocation machinery (SecA2, SecY2, Asp4), chaperones (Asp1, Asp2, Asp3) and a variable number of glycosyltransferases (GTs) that O-glycosylate the secretory target SRRP [2].

The versatility of the aSec glycosylation system prompted us to explore this pathway for O-glycoconjugate manufacturing in Bacillus subtilis. We transferred the aSec pathway from Limosilactobacillus reuteri ATCC 53608 strain into B. subtilis 168, resulting in glycosylation and secretion of LrSRRP53608 as confirmed by MALDI-TOF analysis, but protein yield was low. Screening of alternate genome integration sites for heterologous aSec production revealed that genes encoding extracellular secreted proteins (mpr, apr, amy) were equally suitable, but led to similar protein yield. Accessory Sec pathways from pathogenic Streptococci have been cloned and transferred en bloc for heterologous expression in B. subtilis and preliminary data showed glycosylation of the recombinant SRRPs. We are applying the Start-Stop assembly of aSec operons to generate a range of modified O-glycoconjugates and increase yield. Briefly, aSec genes were cloned in level 1 vectors containing individual expression units with a B. subtilis promoter library. These were ligated into in-house developed level 3 E. coli/B. subtilis shuttle vectors, transformed directly into B. subtilis by electroporation, and screening is underway.

It is expected that this engineering biology approach will open novel avenues for producing next generation glycoconjugate vaccines or adjuvant technologies.

[1] Latousakis D, MacKenzie DA, Telatin A, Juge N. Serine-rich repeat proteins from gut microbes. Gut Microbes. 2020;11(1):102-117. [2] Latousakis D, Nepravishta R, Rejzek M, Wegmann U, Le Gall G, Kavanaugh D, Colquhoun IJ, Frese S, MacKenzie DA, Walter J, Angulo J, Field RA, Juge N. Serine-rich repeat protein adhesins from Lactobacillus reuteri display strain specific glycosylation profiles. Glycobiology. 2019;29(1):45-58.

Selected Speaker

Scaling Protein-Protein Interaction Screening via Automated Yeast Two-Hybrid and Acoustic Liquid Handling

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Protein-protein interactions (PPIs) are a fundamental aspect of molecular biology underpinning the function of biological systems. Identification and validation of PPIs is therefore vital for understanding and engineering such systems. Predictive machine learning models such as AlphaFold have proved highly useful for predicting PPIs, yet the lack of scalable, experimental validation means very few of their output predicted interactions are confirmed. This highlights a need for high-throughput methods of PPI validation to benchmark these models.

In response to this need, we are developing a high throughput, semi-automated pipeline for screening PPIs by utilising the Earlham Biofoundry's automation platforms. We are upscaling the classical Yeast Two-Hybrid method for PPI screening, beginning with semi-automation of the yeast co-transformation process via the Opentron Flex liquid handler.

Transformation is followed by dispensing of the transformed yeast onto selective media via the Echo 650 acoustic liquid handler, which utilizes soundwaves to transfer minute volumes, down to 2.5 nanolitres, of fluid between plates. Using this method, we have validated a pair of plant-fungal positive controls: the Oryza sativa NLR Pikm-HMA with the Magnaporthe oryzae avirulence effectors AVR-PikD and AVR-Mgk1. We are working to define two additional positive control pairs of human isoforms for the application of this pipeline to validating PPI between human proteins.

The pipeline has the potential to accelerate the PPI-screening process, providing a powerful tool for molecular and engineering biology and supporting advances in sustainable agricultural and health research.

Selected Speaker

FIGHTBLIGHT: Bioengineering plant immune receptors for durable potato blight resistance

Andrés Posbeyikian, **Maián Garro**[1], Daniel Lüdke, AmirAli Toghani, Adeline Harant, Sophien Kamoun

[1] The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

Plant pathogens evolve effectors proteins to target host factors and overcome immunity, threatening global food security. Plant immunity relies on intracellular NLR receptors, including NLRs with integrated domains (NLR-IDs) used as “bait/decoy” to detect pathogen effectors. Because IDs are modular, they can be swapped to reprogram receptor specificity, as we previously demonstrated with PIKOBODIES, NLR-nanobody fusions built on the rice Pik receptor pair (Kourelis et al., 2023, PMID: 36862785).

FIGHTBLIGHT extends this strategy to potato late blight caused by *Phytophthora infestans*. As proof of concept, we are engineering potato NLR-IDs into redeployable scaffolds by replacing naturally occurring C-terminal IDs with alternative effector recognition modules. Focusing on the R1 family of Solanaceae NLRs, whose members carry C-terminal HMA domains, we identified *S. demissum* R1 and R1-Adr from *Solanum andreanum* as scaffolds that trigger cell death upon recognition of the *P. infestans* effector AVR1.

We are now testing C-terminal swaps with alternative binding modules for new effector recognition specificities, establishing R1 and R1-Adr as reprogrammable scaffolds. In parallel, we identified substitutions in the conserved MHD motif that render R1-Adr constitutively active, broadening our understanding of R1 activation and enabling heterologous study of alternative scaffolds in *N. benthamiana*. Ultimately, we aim to lay the foundation for cisgenic late blight resistance using potato-derived sequences as IDs to engineer durable, broad-spectrum recognition of conserved *P. infestans* effectors.

Selected Speaker

Developing a standardised cloning and transformation system to generate markerless bacterial mutants for a broad range of species and environmental applications

Amanda Hopes[1], Harry Maguire[1], David Lea-Smith[1]

[1] University of East Anglia, Norwich Research Park, Norwich, UK

The Environmental Biotechnology Innovation Centre (EBIC) is a nine-university engineering biology hub focused on applying microbial systems to solve environmental challenges. These include bioremediation, metal recovery, biotechnology and biofuel production.

As the main EBIC laboratory for generating constructs and bacterial mutants, we work with over 40 species from a range of taxa and environments. Therefore, we need a high throughput, standardised cloning system and transformation method for a broad range of species.

Using synthetic biology, we have created and tested Golden Gate compatible constructs. Our cloning strategy uses suicide plasmids and includes multiple options for antibiotics, origins of replication for cloning, and promoters to drive sacB to generate unmarked strains. Although our constructs are conjugation compatible, we primarily use electroporation and have developed a standardised transformation protocol that tests different parameters and conditions to establish a system for each species.

We are trialling the POSSUM kit to speed up and establish transformation, and to determine suitable origins of replication for transient marked strains.
Plasmid construction with our backbones is highly efficient with ~90% success rate and is compatible with high throughput production. We are testing our method across a range of species and have created unmarked mutants for Gram negative, Gram positive and acid-fast bacteria. We are currently using our strategy to knockout genes, test ‘landing pads’ for gene insertion, and introduce heterologous gene cassettes and barcodes for multiple species and projects across EBIC.

This system will rapidly accelerate bacterial strain generation for biotechnology and understanding environmental and biogeochemical processes.

Selected Speaker

Engineering Biology for the Recovery of Critical Metals

Justin M. Bradley[1] Michael W. Hopkins[1] Callum Eke[1] Fraser MacMillan[1] and Nick E. Le Brun[1]

[1] Centre for Molecular and Structural Biology, School of Chemistry Pharmacy and Pharmacology, University of East Anglia, Norwich Research Park, Norwich, UK

Economic and geopolitical pressures have led to interest in developing a circular economy in technology critical metals. The ELEMENTAL consortium, which includes researchers from UEA, seeks to apply engineering biology to bioremediation these metals. Our work is focussed on producing strains of Escherichia coli that over-express ferritins engineered to contain high affinity binding motifs for the metals of interest, the ultimate goal being to develop a self-replicating system for the selective recovery of metals from waste streams.

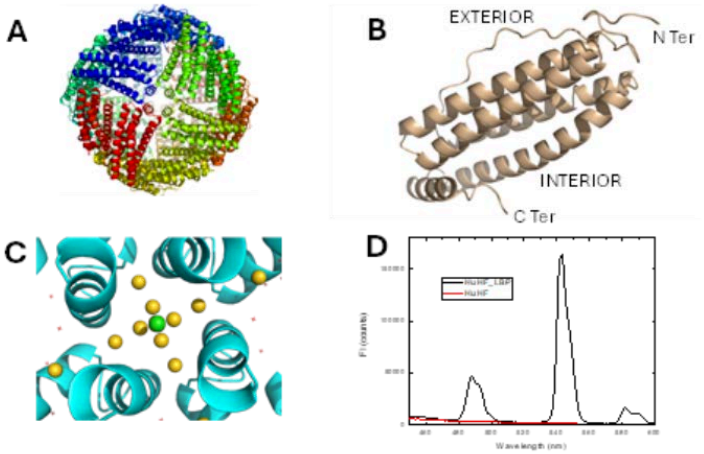

Figure 1. (A) the 24-meric ferritin cage assembly viewed along the channel at the 4-fold symmetry axis (B) A single ferritin peptide showing the position of the N- and C- termini relative to the interior and exterior of the protein cage (C) Structural model derived from X-ray diffraction data showing gold atoms bound at the 4-fold channel of an engineered ferritin (D) emission spectrum of Tb^{3+} ions bound to wild type human H-ferritin (red) and human H-ferritin with a lanthanide binding peptide (LBP) C-terminal extension (black)

Ferritins are hollow protein cages containing channels connecting the interior to bulk solution[1]. Extensions to the C-terminus of the peptide sequence protrude into the interior of the protein at the base of a channel with 4-fold symmetry (Figure 1). Here we report the structural and spectroscopic characterisation of ferritins with various metal binding peptide sequences[2,3] appended to their C-termini, together with assessments of the ability of the purified proteins to sequester metals from buffered solution and of engineered E. coli strains to sequester metals from growth media.

[1] E. C. Theil, Ferritin Protein Nanocages Use Ion Channels, Catalytic Sites and Nucleation Channels to Manage Iron/Oxygen Chemistry, Curr. Opin. Chem. Biol, 2011, 2, 304-311. [2] T. Hatanaka et al, The Origins of Binding Specificity of a Lanthanide Binding Peptide, Sci. Rep., 2020, 10, 19468. [3] J. I. B. Janairo, Sequence Rules for Gold Binding Peptides, RSC Adv., 2023, 31, 21146-21152

Selected Speaker

Yeast Does it Better: Yeast-Plant fusion as a Vector for Megabase Scale DNA Delivery

Dr Matt Downie[1], Dr Daria Cuthbert[2], Dr Victoria Butler[1], Dr Conrad Nieduszynski[2], and Dr David Seung[1].

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Biolistics and agrobacterium-mediated transformation have dominated plant genetic modification for the past four decades. However, both are limited by scale of delivery (~100-150 kb) and random integration into the genome. Synthetic Artificial Plant Chromosomes (SynPAC) are an encouraging new approach to engineer complex traits in plants by delivering long DNA sequences without interference with native genome.

The SynPAC project aims to deliver a 1 Mbp neo-chromosome to potato (S. tuberosum) carrying an array of complex and novel traits. My work focuses on the delivery of large DNA molecules from yeast, where they are assembled, to the plant protoplast.

In parallel, myself and Daria developed and optimised protocols for protoplasting (potato and tobacco) and spheroplasting (yeast), respectively. By digesting these polysaccharide cell walls these dissimilar cells become amenable to fusion. Successful SynPAC delivery requires cytoplasmic and nuclear fusion. To detect these two distinct stages, we developed and tested genetic markers. Strong yeast expression of mScarlet along with 35S GFP(+IV2 intron) proved an effective combination. With required material established, we have developed a PEG-mediated yeast-plant fusion protocol.

To optimise this protocol, we explored parameters such as fusion time, mixing intensity, cell ratio and temperature. Further, we experimented with minimised reactions to allow more high-throughput study. To date, we have established a robust protocol for cytoplasmic fusion, but nuclear fusion remains elusive. Development of new reporters to permanently tag fused cells and report the location of the delivered nuclei are promising avenues to find optimal conditions for DNA delivery to the plant nucleus.

Selected Speaker

Integrase-based synthetic biology tool for studying plant development

Roza Bilas[1], and Sarah Guiziou[1].

[1] Earlham Institute, Norwich Research Park, Norwich, UK

Synthetic biology enables the development of programmable systems capable of recording, tracing, and manipulating cellular behaviours in living plant tissues. Integrase-based technologies provide a promising framework for generating stable cellular memory and investigating developmental processes with high spatial and temporal precision.

This work focuses on the development of synthetic biology platforms for studying root development in plants using site-specific serine integrases. Four complementary areas are explored.

First, fluorescent flow cytometry assays in Arabidopsis protoplasts are established for rapid optimisation and quantitative characterisation of recombinase constructs, switching efficiencies, and synthetic circuit architectures.

Second, integrase-based lineage tracing systems are developed to reconstruct developmental relationships between lateral root cells and capture cellular differentiation trajectories.

Third, history-dependent gene expression recording systems are designed to convert transient promoter activity into stable and heritable cellular memory through irreversible recombination events.

Finally, mosaic cell-marking strategies are implemented to study individual cells within their native tissue context and investigate interactions between marked cells and neighbouring cell populations during development.

Together, these approaches establish integrase-based synthetic biology tools as versatile platforms for investigating plant developmental processes and provide a foundation for future programmable engineering of adaptive root traits.

Selected Speaker

The TraDIS We Know - Bioengineering and Bioremediation

Heather Felgate[1,2], Leanne Sims[1,2], Natalie Byrd[3], Martin Warren[1,2], Ian Charles[1,2], Mark Webber[1,2]

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[3] The University of Manchester, Manchester, UK

The use of bioremediation is becoming increasingly attractive as an option to “clean up” polluted sites. Globally there are multitudes of contaminated wasteland containing radionuclides, such as Uranium, as well as industrial waste and E waste full of toxic and Rare Earth Elements (REE).

ELEMENTAL is an engineering biology mission hub comprising of nine collaborative institutes. One of the aims within the hub is to create large transposon mutant libraries in microorganisms which are able to reduce REE and other metals naturally and engineer new strains with improved ability to bioremediate selected metals.

At the Quadram Institute we have created two large TraDIS-*Xpress* libraries in *Shewanella oneidensis* in aerobic and anaerobic conditions. Comparison of the two shows large differences in gene essentiality and mutant content depending on how each was created. Using these libraries, in collaboration with Manchester we have been able to identify genes that could be potentially used in the detoxification of radionuclide contaminated lands in different conditions.

In conclusion, the creation of these two mutant libraries has shown that more consideration should be taken when creating large mutant libraries as the ‘fingerprint’ of mutant content is condition specific.

Selected Speaker

Horizontal gene transfer

Jacob Malone[1]

[1] John Innes Centre, Norwich Research Park, Norwich, UK

Horizontal gene transfer (HGT) is a fundamental process in bacterial evolution and is typically driven by mobile genetic elements, with conjugative plasmids one of the most significant sources of bacterial HGT. In addition to functional traits such as antibiotic resistance, plasmids also frequently encode homologs of bacterial regulators that can dramatically change the expression of chromosomally encoded bacterial traits, a process known as plasmid-chromosome crosstalk (PCC).

We recently characterised two PCC regulators from the megaplasmid pQBR103 that subvert ecologically relevant pathways in the soil bacterium Pseudomonas fluorescens. These plasmid-borne regulators appear similar to host proteins, but act in strikingly different ways, subverting host regulation and behaviour to benefit plasmid transmission. For example, RsmQ is a homolog of the bacterial translational regulator RsmA that disrupts the host Gac/Rsm pathway, influencing chemotaxis, metabolism and plasmid conjugation rate. Meanwhile, the plasmid-borne ParB-mimic ParQ functions as a transcriptional regulator of bacterial motility and chemotaxis. The RsmQ and ParQ regulons interact with one another and with the host cyclic-di-GMP network, with deletions in specific diguanylate cyclase genes recovering motility defects associated with plasmid carriage. pQBR103 apparently contains multiple putative PCC regulatory proteins, whose function is under active investigation in my lab.

The intersection of plasmid and bacterial signalling driven by PCC regulators promotes plant colonisation, sessility and biofilm formation, traits that in turn support community transmission of the pQBR103 plasmid. Together, these findings expand our knowledge of the regulatory mechanisms underpinning HGT, and have broad implications for our understanding of how plasmids influence microbial communities.

Poster

MSc in Biotechnology & Engineering Biology at UEA

Richard Bowater[1]

[1]School of Biological Sciences, University of East Anglia, Norwich Research Park, UK

The School of Biological Sciences at the University of East Anglia (UEA) has long-established, outstanding research and teaching expertise in biochemistry, microbiology, and molecular sciences. Strong links across the Norwich Research Park provide access to cutting-edge research and expertise. The MSc in Biotechnology and Engineering Biology covers fundamental theories of these fast-evolving topics while developing valuable transferable skills. The 1-year course provides comprehensive training in how biotechnological and engineering biology approaches can address global challenges, with a focus on sustainability and practical applications. A major focus of the course is a 5-6 month research project that can be undertaken in research groups based at UEA or our partners. Two start points (September and January) provide flexibility for students to enrol at a time that is optimal for them. From September 2026 students will have the option to add an integrated placement year, which will deliver valuable UK industry experience as part of a 2-year MSc degree. The presentation will provide details about how staff from Norwich-based research institutes and local companies can engage with the course.

Poster

Protein Engineering Strategies of the Decahaem Cytochrome MtrC from *Shewanella oneidensis MR-1* for the Bioremediation of Copper, Silver, and Gold

Harry Lang[1], Nick Le Brun[1], Julea Butt[1], and Tom Clarke[1]

[1]University of East Anglia, Norwich Research Park, UK

The deca-haem cytochrome MtrC, found in *Shewanella oneidensis* MR-1, is a key component of the organism's extracellular respiratory machinery. Under anoxic conditions, *S. oneidensis* transfers electrons derived from reduced quinones via quinol dehydrogenases through the MtrCAB complex, delivering them to extracellular electron acceptors, typically metal oxide species. This unique extracellular electron transfer capability has attracted significant research interest, particularly for applications in the bioremediation and recovery of valuable and rare metals from waste electrical and electronic equipment (WEEE).

This study explores the protein engineering of MtrC through the fusion of metal-binding peptides to its C-terminus, a region located in close proximity to the tenth and terminal haem group within the electron transfer chain. This strategic positioning is intended to couple electron transfer directly to engineered metal-binding sites, facilitating the sequestration of metal ions and promoting their subsequent reduction. In vitro, this system enables controlled investigation of interactions with bound metal species for a proof-of-concept model for in vivo, while in vivo it leverages the native metabolic pathways of *S. oneidensis* to drive reductive processes. These approaches aim to enable the nucleation and formation of metallic nanoparticles from sequestered ions at the engineered peptide interface.

Poster

Engineering integrase-based circuits in *Bacillus subtilis* for programmable and resilient plant-microbe interactions

Elena Garcia-Perez[1], Sarah Guiziou[1]

[1] Earlham Institute, Norwich Research Park, Norwich, UK

Climate change is increasingly threatening crop productivity and global food security, highlighting the need for sustainable strategies to enhance plant resilience. Plant Growth-Promoting Rhizobacteria (PGPR), such as *Bacillus subtilis*, can support plant growth, nutrient uptake and stress tolerance.

However, their practical application is often limited by poor persistence and lack of controllability in the rhizosphere. Here, we explore the use of synthetic biology to engineer programmable genetic circuits in *B. subtilis* that respond to environmental or plant-derived signals. Our approach is based on serine integrase systems as irreversible genetic “memory switches”, enabling permanent DNA rearrangements in response to transient inputs.

We have developed a modular toolkit for chromosomal integration and circuit assembly, including Golden Gate-compatible vectors targeting neutral loci. In parallel, we are constructing and characterizing a library of integrases under constitutive and inducible promoters. Recent work has focused on tuning the activity of the Bxb1 integrase using IPTG-inducible systems. While functional switching of a reporter cassette can be achieved, we observe significant basal activity of Bxb1, highlighting the challenge of leakiness in inducible systems.

To address this, we are systematically modulating expression through promoter and RBS strength variation, as well as protein destabilization tags. Circuit performance is evaluated using fluorescence reporters and flow cytometry, enabling single-cell analysis of switching dynamics.

Ultimately, this framework will be extended from reporter systems to functional genes relevant for plant growth promotion, enabling the development of programmable, self-regulated microbial treatments for sustainable agriculture.

Poster

A conserved structural logic underlies sensor–helper NLR communication in the NRC immune receptor network

AmirAli Toghani[1*], Maián Garro[1*], Raoul Frijters[2], Sophien Kamoun[1], Mauricio P. Contreras[1,3]

1. The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, UK.

2. Rijk Zwaan Breeding B.V., Department of Biotechnology, Fijnaart, The Netherlands

3. Present address: Department of Plant Biochemistry, Center for Plant Molecular Biology (ZMBP), Eberhard Karls University, Tübingen, Germany.

*** Authors contributed equally.**

NLR immune receptor networks consist of expanded disease resistance proteins (sensor NLRs) that signal via core executors of immunity known as helper NLRs. Although some sensor NLRs are thought to activate their cognate helpers via an activation-and-release mechanism, the structural basis of sensor-helper communication is poorly understood.

Here, we identify and validate sensor-helper NLR interfaces that are critical for immune activation. Using AlphaFold 3 we predicted a high confidence model between the virus resistance protein Rx and its helper NLR NRC2. We validated the interfaces by loss and gain-of-function mutagenesis, including reconstituting a critical salt bridge through reciprocal mutations.

We showed that these interfaces are conserved across the NRC network of asterid plants despite over 120 million years of divergence and validated the sensor-NRC interfaces within the common lettuce network. Structure-guided bioengineering of a lettuce sensor NLR enabled expansion of its NRC helper compatibility profile.

These results are consistent with the activation and release model and point to predicting and bioengineering sensor-helper specificity in economically important crop species.

Poster

Engineering synthetic plant artificial chromosomes (synPACs) – tools for regulatory element screening

Melissa Salmon[1], Helene Yvanne[2], Davide Annese[1], Chinemerem Akamihe[1], Conrad Nieduszynski[2], Anthony Hall[2], Carolina Grandellis[1]

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[2] Earlham Institute, Norwich Research Park, Norwich, UK

Plants have huge potential to solve food insecurity and mitigate the climate change crisis. Conventional plant breeding methods cannot meet the growing demand for secure and sustainable food systems therefore radical innovations are needed to develop resilient crops more swiftly. Advances in synthetic genomics have enabled the construction of synthetic genomes in bacteria and yeast, and applying these breakthroughs to plants is the next logical step.

As part of a multidisciplinary project team between the University of Manchester, John Innes Centre and Earlham Institute, we aim to develop a pipeline for the routine generation and testing of synthetic plant artificial chromosomes (synPACs) in potato (*Solanum tuberosum*). Engineering synthetic genetic pathways requires characterised regulatory elements capable of modulating expression levels and providing spatial and temporal control. To build a regulatory element toolkit, we developed a dual reporter system to test a combinatorial library of tissue specific potato regulatory elements identified from existing RNAseq datasets.

Elements were synthesised as MoClo compatible golden gate parts and assembled using automated high-throughput DNA assembly workflows followed by testing in potato (cv Désirée) leaf protoplasts for ratiometric luminescence analysis. This allows regulatory elements to be ranked by expression strength and tissue specificity of selected elements to be confirmed by in-planta validation. The fully characterised toolkit will be used in gene cluster design for tissue specific expression of traits (such as fungal resistance or nutritional benefits) on the synPAC.

These screening tools are one step on the way to enable the rapid and efficient programming of plants with improved, or entirely novel functions and will pave the way to deploy this engineering pipeline to other plant species.

Poster

Sentinel bacteria: engineering *Pseudomonas fluorescens* biosensors to sense and record soil health

Louis Perrin[1], Sarah Guiziou[1]

[1] Earlham Institute, Norwich Research Park, Norwich, UK

Soil health is critical for agricultural productivity but remains difficult to measure because many soil functions are driven by dynamic microbial processes and transient chemical signals. Traditional chemical analyses provide only static measurements and often miss biologically relevant activity.

Microbial whole-cell biosensors offer a promising alternative, but they mostly rely on fluorescent outputs that are difficult to detect in opaque soil environments. In this project, we are developing a modular synthetic biology workflow to engineer biosensors in *Pseudomonas fluorescens*. Environmental signals are coupled to integrase-based genetic memory systems, enabling transient signals to be permanently recorded in DNA and later recovered by sequencing in multiplex settings, allowing for complex detection circuits.

Current work focuses on optimising DNA assembly, integrase efficiency, and genomic integration strategies with the development of Golden Gate compatible Tn7 insertion plasmids, and homologous recombination plasmids. We are also developing transparent soil-like systems to observe biosensor behaviour in complex environments. Ultimately, this work aims to enable scalable and deployable living diagnostics capable of monitoring soil microbial activity and environmental changes in agricultural ecosystems over time.

Poster

Validation of SynPAC deliverable traits by stable *Agrobacterium*-mediated transformation in *Solanum tuberosum* (potato)

Victoria Butler[1], Alan Houghton[1], Hélène Yvanne[2], Melissa Salmon[2], David Seung[1]

[1] John Innes Centre, Norwich Research Park, Norwich, UK

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Synthetic plants are a new and exciting avenue for crop improvement and poses a major advance in the field of plant biology. Our project, as part of the ARIA Synthetic Plants Program, aims to build and deliver a synthetic plant artificial chromosome (SynPAC) of ³1 Mb in potato plants (cv. Desiree).

We have proposed the delivery of three separate gene clusters on the SynPAC which aims to improve potato nutrition and yield, as well as deliver a complex metabolic trait by targeted gene expression. The nutritional cluster aims to increase potato tuber dietary fibre, while the yield cluster will be targeted to increasing source and sink strength of photosynthetic and tuberous tissues, respectively. The complex trait cluster, composed of the avenacin biosynthetic gene cluster, will be used to demonstrate the delivery and production of a novel metabolite in potato, requiring 12 genes for synthesis.

The assembly of the full SynPAC containing these three clusters will be performed in yeast, followed by fusion-mediated delivery from yeast to potato cells. Prior to assembly of the various gene parts in yeast, validation of each gene part and their combinatorial effects *via* stable *Agrobacterium*-mediated transformation in potato will be necessary to understand the functionality of each gene cluster prior to assembly on the SynPAC.

Furthermore, we are validating the strength and tissue specificity of numerous promoters from potato and other plant species to determine their suitability as tissue specific regulatory elements which can be used on the SynPAC.

Poster

High throughput Semi Automated Potato Tuber Protoplast Isolation

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Plants have huge potential to solve food insecurity and mitigate the climate change crisis. Conventional plant breeding methods cannot meet the growing demand for secure and sustainable food systems therefore radical innovations are needed to develop resilient crops more swiftly.

Advances in synthetic genomics have enabled the construction of synthetic genomes in bacteria and yeast, and applying these breakthroughs to plants is the next logical step. As part of a multidisciplinary team, the Earlham Biofoundry aims to develop a pipeline for the routine generation and testing of synthetic plant artificial chromosomes (synPACs) in potato (Solanum tuberosum).

Engineering synthetic genetic pathways requires characterised regulatory elements capable of modulating expression levels and providing spatial and temporal control. The project aims to develop an automated platform for high throughput testing of DNA elements in vitro grown minituber protoplasts (cv Désirée).

Using the dual reporter system developed by the Earlham Biofoundry, a combinatorial library of potato candidate regulatory elements identified from a potato tissue atlas built by the Hall group (EI) will be tested using the developed protocol. In addition, these results will then be correlated with in planta assays to understand protoplasts behaviour compared with the whole plant. These screening tools are one step on the way to enable the rapid and efficient programming of plants with improved, or entirely novel functions and will pave the way to deploy this engineering pipeline to other plant species.

Poster

Engineering synthetic plant artificial chromosomes (synPACs) – a Biofoundry automation prospective

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Advances in synthetic genomics have already facilitated the design and construction of bacterial1,2 and yeast3 genomes demonstrating the feasibility of synthesising entire synthetic genomes.

Applying these breakthroughs to plants will allow the application of engineering biology in plants at a previously inaccessible speed and scale and help bridge the gap between microbial and plant systems.

As part of a multidisciplinary team, we aim to develop a pipeline for the generation and testing of synthetic plant artificial chromosomes (synPACs) in potato (Solanum tuberosum). Critically, while automation in Synthetic Biology is rapidly gaining ground in microbial and mammalian cell cultures, plant synthetic biology still lacks behind in this sector.

Within this framework the Earlham Biofoundry aims to develop tools to advance automation in the field of Synthetic Biology and expand the screening capabilities available to plant Scientists by providing expertise and automation platforms keeping in mind accessibility and laboratory requirements.