



OpenPlant Forum

Engineering plants for bioproduction

23 - 26 July 2018

John Innes Centre, Norwich



@_OpenPlant
#OpenPlantForum

www.openplant.org

OPENPLANT FORUM 2018 PROGRAMME

Monday 23 July 2018

OPENPLANT FUND PITCHES

Interdisciplinary teams led by early career researchers from Cambridge and Norwich will pitch their OpenPlant Fund projects ideas to a panel of judges from both academia & industry. Projects should generate a tangible outcome of relevance to plant synthetic biology or cell-free synthetic biology or provide a training opportunity in synthetic biology. Project outcomes will be shared openly with the community through www.biomaker.org.

13:00 LUNCH

- 14:00 Introduction
- 14:10 Extending the type IIS toolkit for subcellular localisation in *Marchantia polymorpha*
- 14:20 Developing a frugal transcription factor relative affinity measurement pipeline (TRAMP)
- 14:30 Site-directed integration of transgenes into the nuclear genome of plants using CRISPR/Cpf1/ssDNA
- 14:40 Harnessing cytosine DNA hypomethylation to explore the potential for crop improvement in wheat
- 14:50 Receptor-like kinases at the initiation and maintenance of AM symbiosis
- 15:00 Visualising genetic circuits in space and time with paper-based cell-free translation
- 15:10 Optimising open enzyme purification using 3D-printing and automation

15:30 COFFEE

- 16:15 Judges decisions, comments and closing remarks
- 16:30 End

17:00 DRINKS RECEPTION

18:00 CURRICULUM HACKS

The field of Synthetic Biology is introducing breakthrough technologies for a wide range of practical challenges including diagnostics, environmental conservation, bioproduction, crop improvement and human health. As a result, biological engineering is promising to play a key role in the future well-being and economic development of sustainable societies in all parts of the world. To unlock this

potential, we will need skilled and interdisciplinary workforces to take new technologies forward. Curriculum Hacks will explore the development of practical, reusable models for interdisciplinary learning, and will showcase a range of activities developed for schools, universities, community labs and industry. Speakers include:

- Jim Haseloff, University of Cambridge
- Colette Matthewman, John Innes Centre
- Fernan Federici, Pontificia Universidad Católica de Chile
- Carol Ibe, University of Cambridge and JR Biotek Foundation
- Alexandra Jenkin, Gatsby Plant Science Education Programme

19:30 SHOWCASE AND BUFFET DINNER

The showcase will include a range of practical examples of tools, resources and methods for teaching about synthetic biology and related topics. Showcase includes:

Synthetic Biology for Schools
Science and Plants for Schools
Science Art Writing Trust
Cambridge Biomakespace
Accessible 3D Models of Molecules
Karen Ingram, Artist
Open Resources for Teaching Synthetic Biology in Low-Resource Settings (Lab 13
Practical Science Education Project, Kumasi Hive, Ghana)
Virtual Reality as a Tool for Teaching and Engagement, University of East Anglia
Content Mine
...and more...

CONNECTING VIA SOCIAL MEDIA

The OpenPlant Twitter account is @_OpenPlant and we will be tweeting on #OpenPlantForum. Photos of the event will be made available on Flickr.

Tuesday 24 July 2018

08:30 ARRIVAL AND COFFEE

09:00 WELCOME ADDRESS

Dale Sanders and Anne Osbourn, *John Innes Centre*

09:10 SESSION 1: REFACTORIZING REGULATION

Keynote: The potential for using engineered RNA binding proteins to control organelle gene expression

Ian Small, University of Western Australia

Natural colours from engineered plant cell cultures

Ingo Appelhagen, John Innes Centre

Riboswitches for regulating gene expression in microalgae

Gonzalo Mendoza Ochoa, University of Cambridge

Lessons from plant-infecting viruses and bacteria promoters

Yaomin Cai, Earlham Institute

El DNA Foundry: unleashing the power of automation for plant and microbial science

Jose Carrasco-Lopez, Earlham Institute

10:35 COFFEE

Sign up for lunchtime tours of the Earlham DNA Foundry, with manager Jose Carrasco-Lopez. The OpenPlant Hub is a meeting room that is bookable at the registration desk (for groups up to 20).

11:05 SESSION 2: SIMPLE TEST PLATFORMS

Engineering polysaccharide structures in plants

Paul Dupree, University of Cambridge

Making plant-based expression more accessible
Eva Thuenemann, John Innes Centre

Open tools in *Marchantia* for plant bioengineering
Susana Sauret-Gueto, University of Cambridge

Quantifying growth and gene expression patterns in gemmae of *Marchantia polymorpha*
Mihails Delmans, University of Cambridge

Domesticating the circadian clock in *Marchantia polymorpha* for synthetic biology approaches
Lukas Mueller, University of Cambridge

Pectin remodelling enzymes in early land plant development
Giulia Arsuffi, Sainsbury Laboratory, Cambridge University

12:35 LUNCH

Poster session (odd numbers): Please take the opportunity to peruse the posters and exhibits from OpenPlant researchers and visitors.

Join a tour of the Earlham DNA Foundry to see their state-of-the-art automation facilities.

13:40 SESSION 3: PATHWAY ENGINEERING

Microbial biosynthesis of complex plant-derived natural products
Christina Smolke, Stanford University

Engineering triterpene production in *Nicotiana benthamiana* through transient expression
James Reed, John Innes Centre

Harnessing the global network of botanic gardens for metabolic engineering and synthetic biology
Sam Brockington, University of Cambridge

Designing a horizon scanning tool for bioengineered products
Dave Rejeski, Environmental Law Institute

15:30 COFFEE

16:00 SESSION 4: HARNESSING GLOBAL BIODIVERSITY PANEL

Jenni Rant, The Science Art Writing Trust
Dave Rejeski, Environmental Law Institute
Nicola Patron, Earlham Institute
Samuel Brockington, University of Cambridge

17:15 FINISH

18:00 DRINKS RECEPTION AT THE SAINSBURY CENTRE FOR VISUAL ARTS

The Robert and Lisa Sainsbury Collection will be available for your private viewing, as well as an exhibition of stained glass, Brian Clarke: The Art of Light (free of charge). More information on the collections at scva.ac.uk

19:00 CONFERENCE DINNER AT THE SAINSBURY CENTRE FOR VISUAL ARTS

Karen Ingram (Artist) and Nicola Patron (Earlham Institute) will run a short activity called "Postnatural Botany" during the dinner.

"Postnatural Botany" is a game inspired by the tradition of the Renaissance "Bestiary," in which explorers would verbally explain to artists the magnificent creatures they encountered on their journeys, when the artists themselves had not actually witnessed the sights. Players will take on the role of Explorer, Artist, or Regulator to consider how they communicate about new findings in science. You will receive whimsical guidelines in how you must strip down communication, listening, and interpretive skills in order to carefully consider "the curse of knowledge", bias, and the human relationship to discoveries in nature.

If you have not yet provided your menu choices for the conference dinner or would like to see if there are spaces available for the dinner, please contact the forum registration desk.

Wednesday 25 July 2018

08:30 ARRIVAL AND COFFEE

09:00 SESSION 5: TOOLS FOR METABOLIC ENGINEERING

Keynote: Synthetic biology tools for engineering metabolic pathways
Claudia Vickers, University of Queensland

Systematic analysis of diverse genomes reveals parallel genomic neighborhoods leading to divergent metabolic pathways in *Brassicaceae*
Zhenhua Liu, John Innes Centre

Systematic review of natural triterpene oxidation diversity provides focus to the search for new synthetic biology tools
Michael Stephenson, John Innes Centre

Engineering *Marchantia's* chloroplast
Eftychios Frangedakis, University of Cambridge

Modulating properties of antibodies using genetically encoded unnatural amino acids
Amit Sachdeva, University of East Anglia

10:30 COFFEE

Sign up for lunchtime tours of the Earlham DNA Foundry, with manager Jose Carrasco-Lopez. The OpenPlant Hub is a meeting room that is bookable at the registration desk (for groups up to 20).

11:00 SESSION 6: PLANT-BASED BIOPRODUCTION

Keynote: Moss-made pharmaceuticals: From bench to bedside
Ralf Reski, University of Freiburg

Designer algae - opportunities for predictable metabolic engineering in microalgae
Alison Smith, University of Cambridge

Improving *Nicotiana benthamiana* as bioproduction system for proteins and small molecules
Quentin Dudley, Earlham Institute

From *Catharanthus* to catnip: finding enzymes to optimise the production of anti-cancer compounds

Benjamin Lichman, John Innes Centre

Tomato as a biofactory for health-promoting compounds

Eugenio Butelli, John Innes Centre

12:30 LUNCH AND POSTER SESSION

Poster session (even numbers): Please take the opportunity to peruse the posters and exhibits from OpenPlant researchers and visitors.

Join a tour of the Earlham DNA Foundry to see their state-of-the-art automation facilities.

13:45 SESSION 7: SHARING AND TECHNO-SOCIAL PLATFORMS

Introduction

Jim Haseloff, University of Cambridge

Addgene, A unique nonprofit accelerating science

Joanne Kamens, Addgene

Expanding options for material transfer via the OpenMTA

Linda Kahl, BioBricks Foundation

bioRxiv: preprint opportunities for synthetic biology

Richard Sever, bioRxiv at Cold Spring Harbor Laboratory Press

14:35 PANEL

15:20 CLOSING REMARKS

15:30 COFFEE

16:00 MEETING OF THE SCIENCE ADVISORY BOARD

(SAB AND MANAGEMENT GROUP ONLY)

19:00 BBQ (BY INVITATION)

Thursday 26 July 2018

WORKING GROUP

NEW MODELS FOR DOCUMENTATION, DISTRIBUTION AND PUBLICATION BY BIOENGINEERS- WEB MASHUPS!

10:00 - 16:30

A day-long workshop focussed on characterisation and documentation of DNA Parts. The day will include technical discussion about new models for bio-technical publication based on user-generated content, including web-based platforms and ways of working that have emerged from the electronics and soft-ware industries.

Please contact colette.matthewman@jic.ac.uk if you are interested in joining this workshop.

A New Planet

Thomas Louveau, John Innes Centre



A dried plant extract in a round flask taken from behind on a dark background. The extraction is part of the purification process of new-to-nature metabolites produced by synthetic biology approaches.

CONFERENCE INFORMATION

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VENUE

John Innes Conference Centre
Norwich Research Park
Colney Lane,
Norwich, NR4 7UH
United Kingdom
Tel. +44 (0)1603 450 000

SPEAKER PRESENTATIONS

Please make sure that you upload your presentation to the computer at the latest in the break before your session. Presentation files should be in powerpoint or pdf format. If you would prefer to use a Mac, please bring your own laptop and make sure you have a VGA or HDMI adaptor.

POSTERS AND LEAFLETS/LITERATURE

Poster boards, tables and velcro pads will be available for those who have registered to bring a poster or would like to put out leaflets/stickers. You are welcome to attend your poster at any time during the breaks, but please make sure you stand next to your poster during lunch on Tuesday 24 July if you are allocated an odd number and during lunch on Wednesday 25 July if you are allocated an even number.

WIFI

Eduroam is available onsite. Details of the Conference Centre Wi-Fi will be available at registration.

SOCIAL MEDIA

The OpenPlant Twitter account is @_OpenPlant and we will be tweeting on #OpenPlantForum. Please join the conversation. However, please respect the wishes of talk and poster presenters if they request no photos or sharing of their material on social media..

THE OPENPLANT HUB

A meeting room is available throughout the conference to be booked for informal or formal meetings (of up to 20 people). Sign up for a time slot at the Forum Registration Desk.

POWER

Access to power sockets will be limited during sessions so we advise that you bring your electronic equipment fully charged where possible and we will try to provide increased access during breaks and lunch.

PARKING

External delegates arriving by car and wishing to park on site must display a parking permit. If you do not have one, please ask at the forum registration desk.

PHOTOS/VIDEOS

Our on site photographer will be shooting photos at various points during the Forum. These may be used on the website and to advertise future events.

If you do not wish to appear in event photos, please make yourself known to the registration desk.

CONTACT US

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United Kingdom

Email: colette.matthewman@jic.ac.uk
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**POSTER
ABSTRACTS**

1. DECODING PLANT-INFECTING VIRUSES AND BACTERIA TO INFORM THE RATIONAL DESIGN OF SYNTHETIC REGULATORY ELEMENTS FOR PLANTS

Yaomin Cai and Nicola J. Patron
Earlham Institute, Norwich

Just a handful of promoters, mainly derived from plant-infecting viruses and bacteria, are used to drive constitutive expression in plants. However, the construction of complex and extensive synthetic pathways and networks requires an expanded set of regulatory elements that function predictably in given cell types. Here, we are studying how promoters from plant-infecting viruses and bacteria drive constitutive expression in multiple plant species and characterising their functional motifs and promoter architecture. We are using this information to inform the design of novel, synthetic minimal regulatory elements. We have established a rapid, experimental system for the comparison and functional characterisation of CREs in plants cells, characterised a cis-regulatory element (C-CRE) common to many viral and bacterial promoters and studied the effects of the of the relative positions of putative transcription factors binding sites (TFBS). We are now studying how changes in sequences affect TF binding affinity. The knowledge gained is being used for the rational design of new, synthetic promoters and to tailor existing promoters to function in new species.

2. MOLECULAR TOOLS FOR PRECISION GENOME ENGINEERING IN PLANTS

Oleg Raitskin¹, Christina Schudoma¹, Anthony P. West^{1,2}, and Nicola Patron¹

¹ Earlham Institute, Norwich

² Isogenica Ltd., Essex

RNA-guided Cas proteins from bacterial CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems for adaptive immunity have been used to induce targeted mutagenesis at endogenous loci in numerous plant species. In this process, a programmable ribonuclease complex generates double stranded breaks in genomic DNA. In the somatic cells found in mature tissues such as leaves, these breaks are preferentially repaired by non-homologous end-joining (NHEJ), which is sometimes imperfect. The efficiency of induced mutations varies between species and between targets. Further, mutations are observed at non-target loci and wild-type Cas9 requires a canonical 'NGG' motif at the target locus.

We have developed an expanded toolbox of molecular tools for RNA-guided Cas-mediated plant genome engineering: (A) To improve specificity and to increase the available targets at which double-strand breaks can be induced in plant genomes (B) To enable the integration of new DNA at known genetic loci and (C) To enable the production of engineered plants without the delivery of integration of DNA.

3. UNLEASHING THE POWER OF AUTOMATION FOR PLANT AND MICROBIAL SYNBIO

Jose A. Carrasco, Nicola J. Patron
Earlham Institute, Norwich

The Earlham Institute's Plant and Microbe DNA Foundry has been established to support the design, generation and exploitation of high value compounds and bio-actives obtained from plants and microorganism. To enable this, we have automated the fabrication of plasmid construct at high-throughput and low reaction volume. This allow us to scale up the projects increasing reliability and robustness and reducing the cost of experimentation. We use a modular setup that harnesses an automated freezer working in a 96-well-plate format, and a suite of liquid handling robots to array source plates, assemble larger constructs from standard DNA parts and perform the downstream microbiological workflow.

4. MOBIUS ASSEMBLY FOR PLANT SYSTEMS (MAPS)

Andreas I. Andreou, Jessica Nirkko, Marisol Ochoa-Villarreal, Naomi Nakayama
SynthSys Centre for Synthetic and Systems Biology, Institute of Molecular Plant Sciences, University of Edinburgh

Plant synthetic biology is a fast-evolving field which creates tools and methods to facilitate research and bioproduction in plant systems. We have been creating tools and protocols to devise specialized plant cell biofactories by navigating cell differentiation and its competency in the biosynthesis of specific compounds. We have developed Mobius Assembly for Plant Systems (MAPS), a versatile Golden Gate assembly system for the rapid and simple generation of DNA constructs. It uses small binary vectors and contains features to simplify combinatorial assemblies. Employing MAPS and the high-throughput protoplast expression system we established, we are characterizing a new library of short promoters and terminators for plant gene expression. Three chemically inducible systems are combined to form a sequential plant expression system, which can be used to activate multiple gene expression at different time points.

5. Ψ-TRAP: MICROFLUIDIC PLATFORM FOR LONG-TERM PHENOTYPING OF SINGLE PLANT CELLS

Eric Thorand, Teuta Pilizota, Naomi Nakayama
SynthSys Centre for Synthetic and Systems Biology, University of Edinburgh

Synthetic biology strives to genetically engineer cells through the design-construct-test cycle. Our capability to test predicted functions can be enhanced by accurate and large-scale cell phenotyping platforms. Currently, the effects of genetic constructs in plant cells are often measured at the cell population level, which by averaging out characteristic cell to cell distribution of a given behaviour, can lead to misleading conclusions. In order to capture the stochasticity and heterogeneity of individual cell behaviours important for cell function, we need to characterise a population at the single cell level.

6. AMIPLANT; A WIKIDATA-ENABLED OPEN PLANT KNOWLEDGEBASE

Peter Murray-Rust¹, Charles Matthews¹, Giulia Arsuffi^{1,2}

¹ContentMine, Cambridge

²Sainsbury Laboratory, Cambridge

Hundreds of papers about plants are published every day, but there is no simple systematic way of searching and indexing them. We have developed a toolkit to automatically generate queries using Wikidata (the semantic version of Wikipedia - terms and facts), search remote databases. An example: we search for chemistry of Marchantiales (which include the Marchantia genus). We find all genera contained within this and generate an exhaustive detailed search automatically, search open repositories (EuropePMC, bioarxiv) and download papers.

We then use a set of local dictionaries to filter the downloads and prioritize the display and presentation. Dictionaries include plants, other species (e.g. animals), chemistry, plant diseases, geolocation, etc. We can use co-occurrence to find new correlations, such as between 2 species of plants, or plants and chemistry.

All software is Open Source and compatible with R or Jupyter Notebooks

7. OPEN TOOLS IN MARCHANTIA FOR PLANT BIOENGINEERING WORK

Susana Sauret-Gueto¹, Eftychios Frangedakis¹, Linda Silvestri¹, Mihails Delmans¹, Marius Rebmann¹, Marta Tomaselli¹, Jim Haseloff¹, Anthony West², Nicola Patron²

¹Department of Plant Sciences, University of Cambridge

²Earlham Institute, Norwich

At OpenPlant we are establishing Marchantia as a testbed for plant synthetic biology. The relative simplicity of genetic networks in Marchantia, combined with the growing set of genetic manipulation, culture and microscopy techniques, are set to make this primitive plant a major new system for analysis and engineering.

We are setting up standardised practices for DNA assembly following the common syntax and Loop type IIS cloning method, compiling a Marchantia DNA toolkit and establishing registries to facilitate standardisation and sharing. The constructs are compatible with the OpenMTA, and thus suitable for open distribution. The DNA toolkit includes parts for selection of successful transformants, quantitative imaging of multispectral markers and targeted mutagenesis with CRISPR/Cas9. We have also generated a collection of proximal promoters of all Marchantia transcription factors (TFs), to screen for tissue specific expression patterns.

OpenPlant aims at making these resources available, establishing a hub for exchange in the Plant Synthetic Biology community.

8. SYSTEMATIC CHARACTERISATION OF PROMOTER EXPRESSION PATTERNS IN WHOLE PLANTS COMBINED WITH SINGLE CELL RNA SEQUENCING TO DISSECT AND ENGINEER GENE REGULATORY NETWORKS IN MARCHANTIA POLYMORPHA

Marius Rebmann, Mihails Delmans, Susana Sauret-Gueto, Linda Silvestri, Jim Haseloff

Department of Plant Sciences, University of Cambridge

Marchantia polymorpha is emerging as a model species and bioengineering testbed for plant development, owing to its relative simplicity and lack of redundancy in gene regulatory networks, simple morphology that is directly accessible to microscopy and a rapidly growing molecular toolbox for genetic engineering. Expression patterns of fluorescent reporters from native promoters have been used extensively for dissecting regulatory networks of plant development. However, typically effects were studied at low throughput, one gene at a time. Machine learning algorithms are powerful new methods that may be able to learn complex interaction networks from automated high throughput microscopy data, inferring gene regulatory networks from expression patterns of hundreds of regulated promoters. Reliable expression patterns are critical for this approach. By systematically characterising expression of fluorescent reporters from the endogenous *Ef1a* and the exogenous 35S promoter in whole plants for > 40 lines through several generations and multiple stages of gemma development we demonstrate significant gene expression variability across lines, developmental stages and generations, consistent with extensive influence of local chromatin context on transgene expression. We tested four promising genetic insulator sequences with the aim of developing simple genetic tools for stabilising expression of fluorescent reporters. We also present a dataset of approximately 4 000 single cell transcriptomes from four-day old gemmalings. Leveraging the best of both approaches, we aim to combine time-lapse microscopy data, rich in spatial and temporal detail, with single cell transcriptomes providing a genome wide snapshot of gene activity, to dissect regulatory networks in developing gemmae.

9. AIR CHAMBER DEVELOPMENT IN MARCHANTIA POLYMORPHA

Marta Tomaselli, Eftychios Frangedakis, Susana Sauret-Gueto and Jim Haseloff

Department of Plant Sciences, University of Cambridge

Marchantia polymorpha is considered to be a living relative of early land plants. *M. polymorpha* is an emerging plant model system, with a small genome, low gene redundancy, and a set of highly efficient molecular tools for genome manipulation. *Marchantia* thalli are covered with epidermal structures, called air chambers, that partially resemble stomata. Each air chamber is composed of an intercellular space filled with chloroplast-rich filamentous cells and they connect to the environment through an open pore which allows gas exchange. Air-chamber development is still not well understood. Their formation is a repetitive process, which progresses from the notch area, towards the centre of the thallus. Nevertheless, no accurate description, correlating landmarks of air chamber development to a quantitative description of their formation, is available. The aim of my PhD is to provide a better understanding of *Marchantia* air-chamber development by: a) generating an accurate map of air-chamber formation using high-resolution confocal microscopy and b) investigating the role of genes potentially involved in air-chamber patterning, focusing on gene family involved

in patterning in higher-plants. Mutants generated through CRISPR/Cas9 genome editing combined with the developmental map, will provide a deeper insight to the process of air chamber formation.

10. BENTHAMIANA AS BIOFOUNDRY

Visual: Karen Ingram (www.kareningram.com)

Process and Research: The Patron Group, The Earlham Institute (www.patronlab.org)

Benthamiana as Biofoundry is an artistic interpretation by Karen Ingram inspired by the basic research conducted by The Patron Group at the Earlham Institute in Norwich, UK. The Patron Group explores the plant *Nicotiana benthamiana* as a platform for sustainable biomanufacturing of useful chemical compounds. This work requires basic research into the regulation of gene expression and understanding how cells respond and adapt to the presence of foreign molecules.

Amid the hype of emerging biotechnologies, the notion of basic research is one that's difficult to convey "Why are you doing that?" "What's it used for?" Through in-depth discussions, Ingram and Patron seek to probe the "surreal becoming the real" as well as the notion of "all research is applied someday". Chirality, gene expression, imperfections and plant life as a palette are also explored.

This work was originally produced for and published as part of 'Convergent Visions' Faesthetic #15. In partnership with SXSW for the SXSW Art Program 2018, Ingram invited and curated the contributions of an international panel of artists who were asked to create works that explore the future of design, health & wellness, and how human-created technologies impact the earth and society.

11. IMPROVING NICOTIANA BENTHAMIANA AS BIOPRODUCTION SYSTEM FOR PROTEINS AND SMALL MOLECULES

Quentin M. Dudley^{1,2}, Sarah E. O'Connor², Nicola J. Patron¹

¹Earlham Institute, Norwich Research Park, Norwich, Norfolk NR4 7UH

²John Innes Centre, Norwich Research Park, Norwich, Norfolk NR4 7UH

The wild tobacco relative *Nicotiana benthamiana* is a commonly used plant for manufacturing proteins, for synthesising complex metabolites such as fragrances or medicines, and for testing unknown metabolic enzymes. It can be stably transformed and is particularly useful as a transient expression chassis where heterologous proteins or enzyme pathways can be generated in just a few days. However, small molecules produced in *N. benthamiana* are often over-glycosylated, oxidized/reduced, acylated or modified with glutathione and hydrophobic proteins are often degraded by native proteases or coexpressed with deleterious peroxidases.

We hypothesize that *N. benthamiana* upregulates expression of a variety of enzymes to detoxify foreign molecules. Therefore, to improve *N. benthamiana* as a bioproduction platform, we are using CRISPR-Cas genome engineering to deactivate native enzymes that likely make unwanted modifications to the target metabolite or protein. As a proof-of-concept, we have

demonstrated activity of SpCas9 nuclease to make targeted double-strand breaks (producing insertion/deletions) that inactivate the peroxidase NbPOX1. Additionally, we have used transcriptomic analysis to generate a candidate list of further enzyme targets for inactivation.

12. VIRUSES IN MOTION: STUDYING VIRAL DYNAMICS USING AN INSECT VIRUS AND CRYO-ELECTRON MICROSCOPY

Roger Castells-Graells¹, John E. Johnson², George P. Lomonosoff¹

¹Department of Biological Chemistry, John Innes Centre, Norwich, UK

²Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, USA

The transient expression of viral coat proteins results in the synthesis of virus-like particles (VLPs) that mimic the structure of the original virus but lack the infectious genome. We are engineering these VLPs to generate new bionanotechnological tools, such as vaccines and nanomachines, and to understand viral protein dynamics for future applications.

13. TRANSIENT EXPRESSION FOR THE PREPARATIVE PRODUCTION OF TRITERPENES IN NICOTIANA BENTHAMIANA

James Reed¹, Michael J. Stephenson¹, Bastiaan Brouwer¹, Paul Brett¹, Maria A. O'Connell², Anne Osbourn^{1*}

¹ John Innes Centre, Norwich

² University of East Anglia, Norwich

The triterpenes are an important and diverse family of natural products. Despite this observed diversity all triterpene alcohols are believed to be derived from the same linear precursor known trivially as 2,3-oxidosqualene. Transient expression of biosynthetic enzymes in *Nicotiana benthamiana* leaf can divert the endogenous supply of 2,3-oxidosqualene towards the production of heterologous products. We have optimised this system for the preparative production of high-value triterpenes, and demonstrated its utility by producing gram-scale quantities of product. Through the combination of unnatural pairings of tailoring enzymes, we were able to produce a small library of structurally related analogues, including novel compounds, and assay the effects of these structural variations on medicinally relevant biological activity. This work highlights the potential of the platform as a tool for medicinal chemistry; allowing convenient preparative access to difficult to synthesize compounds for the systematic study of structure-activity relationships.

14. A SYSTEMATIC ANALYSIS OF DYNAMIC BIOSYNTHETIC GENE CLUSTERS REVEALS EXPANSION OF METABOLIC DIVERSIFICATION IN BRASSICACEAE

Zhenhua Liu¹, Hernando Suarez Duran², Michael Stephenson¹, Marnix Medema², Anne Osbourn¹

¹ John Innes Centre, Norwich

² Wageningen University, Wageningen, Netherlands

A wide variety of important plant specialized metabolites, such as the antimicrobial triterpene avenacin in oat, the anticancer alkaloid noscapine in opium poppy, and the insect repellent cyanogenic glycoside dhurrin in sorghum, are synthesized by enzymatic pathways encoded by

biosynthetic gene clusters (BGCs). Genome-mining approaches can now predict thousands of putative BGCs in plants, yet the function and evolutionary history of most BGCs are unknown. How and why plants have evolved BGCs is still a mystery. These questions are difficult to answer due to the species-specific appearance and complex composition of BGCs. Here we have carried out a systematic analysis of triterpene BGCs across the Brassicaceae family. Multiple analyses, including customized comparative genomics, phylogenetic analysis and functional characterization demonstrates that these clusters are highly dynamic and can assemble independently in different Brassicaceae lineages. Evolutionary clues further suggest that superficially similar genomic architectures have evolved repeatedly in closely related species, giving rise to the production of different metabolites. Comparative genomics and neighbourhood associations thus are effective tools to identify species-specific pathways and reveal the underlying evolutionary mechanisms of metabolic diversification. This study not only provides a template for understanding the evolution and the function of the largely untapped plant BGCs, but also reveals the astonishing capability of plants to synthesize their hallmark specialized metabolites.

15. A SYNTHETIC BIOLOGY APPROACH TO THE PRODUCTION OF PLANT TERPENOIDS IN MICROALGAE

Stefan Grossfurthner, Payam Mershani, Alison Smith

University of Cambridge, Cambridge

Plants produce a wide variety of secondary metabolites, including a large group of compounds known as terpenoids. In addition to their biological functions, terpenoids often have commercial value as drugs, fragrances, commodity chemicals, and fuels. Extracting these natural products from plant material is often costly and inefficient - an alternative approach is to transfer the metabolic pathway for biosynthesis of the desired terpenoid compound from the host plant to a heterologous host that is better suited for industrial production. Microalgae possess a unique combination of traits that are attractive from both economic and environmental perspectives, including their capacity for phototrophic growth on waste streams. This potential has not been developed extensively and successful demonstrations of metabolic engineering in algae are relatively limited. We are taking a synthetic biology approach to implement pathways for biosynthesis of plant sesquiterpenoids and diterpenoids in the model green alga *Chlamydomonas reinhardtii*. In particular, we aim to establish how compartmentation of these heterologous pathways within the algal chloroplast can be engineered to optimise production of the target compound by introducing transgenes into the nuclear and chloroplast genomes of the host.

16. RIBOSWITCHES FOR REGULATING GENE EXPRESSION IN MICROALGAE

Gonzalo Mendoza-Ochoa, Alison Smith

University of Cambridge, Cambridge

Chlamydomonas reinhardtii is a well-studied, fast-growing alga that uses light and low-cost nutrients to grow. For these reasons, it is considered an attractive host organism for sustainable biotechnology. However, to reach its full potential as a biotechnological host, the repertoire of genetic tools to engineer this organism needs to be expanded. My work is

focused on the thiamine-pyrophosphate (TPP) riboswitch as a tool to control gene expression. Riboswitches are highly-structured cis-acting elements and their role is to regulate expression of certain transcripts involved in cell metabolism. When the riboswitch binds to its specific ligand (e.g. TPP) it changes conformation. As a consequence, the riboswitch interferes with transcription, mRNA stability or translation of its transcript. Our lab has demonstrated that the TPP riboswitch of *Chlamydomonas* can be used to switch OFF expression of reporter genes in this organism, as thiamine is added to the culture media. In continuation of this work, first I will systematically characterize the native riboswitches of *Chlamydomonas* using high throughput techniques. Then, with the data generated, I will create 1) an ON system that is induced, instead of repressed, by TPP and 2) novel TPP riboswitches for controlling gene expression in other plant organisms. Inducible/repressible systems have direct and valuable applications, including expression of trans genes that are detrimental to cell growth, or in functional studies of essential genes. As a result, we believe that the tools and knowledge generated will be important for the plant sciences community.

17. COLOUR BIO-FACTORIES: TOWARDS SCALE-UP PRODUCTION OF ANTHOCYANINS IN PLANT CELL CULTURES

Ingo Appelhagen¹, Anders Keim Wulff-Vester², Anne-Kathrine Hvoslef-Eide², Julia Russell¹, Anne Oertel^{4,5}, Stefan Martens^{3,4}, Hans-Peter Mock⁵, Andrea Matros⁵, Cathie Martin¹

¹ John Innes Centre, Norwich

² Norwegian University of Life Sciences, Norway

³ Edmund Mach Foundation, Italy

⁴ TransMIT GmbH, Germany

⁵ Leibniz Institute of Plant Genetics and Crop Plant Research, Germany

Anthocyanins are widely distributed, glycosylated, water-soluble plant pigments, which give many fruits and flowers red, purple or blue colouration. Their beneficial effects in a dietary context have encouraged increasing use of anthocyanins as natural colourants in the food and cosmetic industries. However, the limited commercial availability and diversity of anthocyanins have initiated searches for alternative sources of these natural colourants. In plants, high-level production of secondary metabolites, such as anthocyanins, can be achieved by engineering of regulatory genes as well as genes encoding biosynthetic enzymes. We have used tobacco lines which constitutively produce high levels of cyanidin 3-O-rutinoside, delphinidin 3-O-rutinoside or a novel anthocyanin, acylated cyanidin 3-O-(coumaroyl) rutinoside to generate cell suspension cultures. The cell lines are stable in their production rates and superior to conventional plant cell cultures. Scale-up of anthocyanin production in bioreactors has been demonstrated. The cell cultures also have proven to be a valuable production system for ¹³C-labelled anthocyanins. Our method for anthocyanin production is transferable to other plant species, such as *Arabidopsis thaliana*, demonstrating the potential of this approach for making a wide range of highly-decorated anthocyanins. The tobacco cell cultures represent a customisable and sustainable alternative to conventional anthocyanin production platforms and have considerable potential for use in industrial and medical applications of anthocyanins.

18. NOVEL CYCLASES FROM NEPETA CATALYSING STEREOSELECTIVE FORMATION OF IRIDOIDS

Benjamin R. Lichman, Mohamed O Kamileen, Gabriel R Titchiner, Gerhard Saalbach, Clare Stevenson, David M Lawson, Sarah E. O'Connor
John Innes Centre, Norwich

Iridoids are a large family of natural products with roles in plant defence and insect interactions. Numerous iridoids have been examined for their medicinal potential. The iridoid pathway contributes to the monoterpene indole alkaloids (MIAs), compounds with clinical applications including anticancer (vincristine) and antimalaria (quinine). Due to the bioactivity and limited accessibility of these compounds, there is growing interest in the reconstitution and manipulation of the iridoid and MIA pathways in heterologous hosts such as *Saccharomyces cerevisiae*. However, these systems have been limited by side-products and low yields. We have discovered novel enzymes from catmint (*Nepeta mussinii*) which catalyse the stereoselective formation of the iridoid scaffold—the ‘signature’ step in the pathway. These enzymes promise to improve the yields of synthetic biology approaches to iridoid and MIA production.

19. BRANCHING OF CONIFER XYLAN AND ITS INTERACTION WITH CELLULOSE MICROFIBRIL IN SPRUCE WOOD

Jan Lyczakowski¹, Oliver Terrett¹, Weibing Yang², Xiaolan Yu¹, Dinu Iuga³, Steven Brown³, Marta Busse-Wicher¹, Ray Dupree³ and Paul Dupree¹

¹ University of Cambridge, Cambridge

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³ University of Warwick, Coventry

Relatively little is known about the biosynthesis and molecular architecture of conifer wood. Softwood is mainly composed of cellulose, galactoglucomannan, xylan and lignin. We have recently demonstrated that in hardwoods, xylan interacts with the cellulose fibril as a two-fold screw, and that GUX (GlucUronic acid substitution of Xylan)-dependent xylan branching is critical for biomass recalcitrance. Here we investigated the decoration of softwood xylan by GUX enzymes and studied the interaction of xylan with cellulose in spruce wood. Using *in vitro* and *in planta* assays we demonstrate that two distinct conifer GUX enzymes are active glucuronosyltransferases. Interestingly, these enzymes have different specific activities with one adding evenly spaced GlcA branches and the other one adding consecutive GlcA decorations. Two-dimensional solid state NMR analysis of never-dried ¹³C-enriched spruce wood reveals that the majority of conifer xylan is cellulose-bound in a two-fold screw conformation. We speculate that the evenly spaced GlcA branches allow xylan-cellulose interaction whereas the consecutive decorations added by the second enzyme are likely to inhibit it. This work is the first to investigate the molecular architecture of native spruce wood using 2D ¹³C-¹³C solid state NMR and to characterise conifer enzymes likely to determine the recalcitrance of softwood to processing. In addition to having a potential for improving the efficiency of softwood processing, our work is a proof of concept for *in vivo* and *in vitro* biosynthesis of novel polysaccharide structures with potential industrial application.

20. ESTABLISHMENT OF A NEW CARBOHYDRATE PHOSPHORYLASE FAMILY ACTING ON -1,3-GLUCAN AND ITS BIOCATALYTIC APPLICATION IN OLIGOSACCHARIDE SYNTHESIS

Sakonwan Kuhaudomlarp¹, Nicola J. Patron², Bernard Henrissat³, Martin Rejzek¹, Gerhard Saalbach¹, Robert A. Field¹

¹ John Innes Centre, Norwich

² Earlham Institute, Norwich

³ CNRS, France

β -1,3-glucan is composed of glucose residues linked by β -1,3-glycosidic bonds. The molecule is present prominently as a storage molecule called paramylon in *Euglena gracilis*. β -1,3-glucan has been shown to have various benefits to human health, such as stimulating human immune response and may also be useful as a conjugate for drug and vaccine delivery.

β -1,3-glucan phosphorylases are proposed to be involved in paramylon metabolism in the aforementioned microalgae. We detected the activity of the enzymes in partially purified *Euglena* protein extracts. We recently discovered sequence candidates of these enzymes by analyzing the transcriptome of microalgae such as *Euglena gracilis* and other microorganisms. One of the candidates (EgP1) and its bacterial orthologue (Pro_7066) were successfully expressed as recombinant proteins in *E. coli*. In vitro characterisation of EgP1 and Pro_7066 showed that the enzymes are β -1,3-glucan phosphorylases, which can use glucose and longer β -1,3-gluco-oligosaccharide as its acceptor and α -glucose-1-phosphate as its donor to produce β -1,3-glucan product. As a proof of concept, we combined the use of Pro_7066 with sucrose phosphorylase in a one-pot enzymatic reaction (Figure 1) comprising of sucrose and inorganic phosphate as sole substrates, which allowed production of β -1,3-gluco-oligosaccharides. Several hundreds of GH149 orthologues were identified and can be mapped to polysaccharide utilization loci (PULs), implying a role for GH149 in glucan degradation. This work represents one of the first concerted efforts to understand glucan biochemistry, and particularly phosphorylases, in the microalgae and important implication of the phosphorylase as part of PII s.

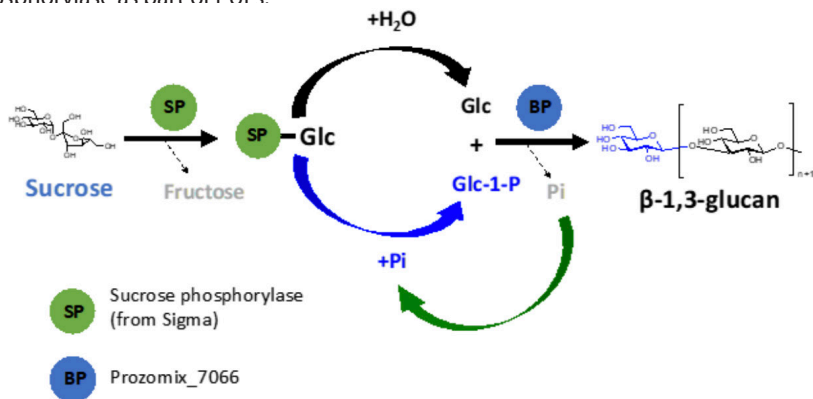


Figure 1. One pot enzymatic reaction using two enzymes; sucrose phosphorylase (SP) and -1,3-glucan phosphorylase (Pro_7066), to produce β -1,3-glucan.

21. POSTERS BY KAREN INGRAM

Bacterial Photography, 2013

Karen Ingram, Natalie Kuldell

The schematic that was created based on observing Natalie Kuldell's Bacterial Photography lab at MIT in 2013

BioBuilder: Eau That Smell, 2016

Karen Ingram, Natalie Kuldell, Katie Hart, Rachel Bernstein

Poster designed for "BioBuilder: Synthetic Biology in the Lab" published by O'Reilly, 2016

"Unicorn Mosaic" SXSW, 2013

Karen Ingram

Unicorn Mosaic made from genetically engineered fluorescent K12 bacteria, crowdsourced from participants at SXSW Create, 2013. A Cut/Paste/Grow project, with Wythe Marschall, Dan Grushkin, Grace Baxter, Joergan Geerds, and 200 SXSW 2013 attendees

SHOWCASE ABSTRACTS

Table of literature

OpenPlant and partners

OpenPlant and partners have produced a variety of thematic publications over the past couple of years. Please visit the table by the registration desk to browse the literature and take home copies of reports that you are interested in.

Leaf Expression Systems

Dr Nicholas Holton, Dr Franziska Kellner, Sarah Smith, Albor Alonso

Leaf Expression Systems is a new contract development business specialising in the plant-based expression and production of proteins, metabolites and complex natural products for research and bio-medical applications using a proprietary, transient expression technology, Hypertrans®.

The Science, Art and Writing (SAW) Trust

Jenny Rant, Samantha Stebbings, Shannon Woodhouse

The Science, Art and Writing (SAW) Trust is an international science education programme that breaks down the traditional barriers between science and the arts. SAW projects use themes and images from science as a starting point for scientific experimentation, art and creative writing and in doing so, stimulate creativity and scientific curiosity. Our work with the OpenPlant community is enabling scientists to take a multidisciplinary approach to introducing synthetic biology to the public and is providing a platform to exchange ideas.

ContentMine Ltd

Giulia Arsuffi, Peter Murray-Rust

We will showcase a different way to look at the scientific literature. We have used text mining to download, annotate and sort hundreds of papers about Marchantia. Our workflow results in an interactive table which provides a snapshot of a paper's content based on topics of broad biological interest as well as researcher-specific areas.

Grobotic Systems Smart Plant Growth Chamber

Alexis Moschopoulos, Richard Banks

Grobotic Systems is developing an affordable smart plant growth chamber for plant science research, with the aim of making precision controlled environments more accessible to plant scientists around the world. We are designing our system to offer the user unprecedented control, reliability and reproducibility. We will release an open hardware API so that users can design and build hardware and software add-ons, and will offer an online platform for users to share their data and designs with the wider community. Come and see our alpha prototype!

ABOUT OPENPLANT

OpenPlant is a joint initiative between the University of Cambridge, John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme.

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems offers even greater potential benefits. Plants are already cultivated globally at low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food.

There is urgent need to improve our ability to reprogram crop metabolism and plant architecture in the face of global threats from new pathogens, climate change, soil degradation, restricted land use, salinity and drought. The next generation of DNA tools for “smart” breeding of crop systems should be shared - to promote global innovation and equitable access to sustainable bioeconomies.

OpenPlant is:

- developing new tools and methods for plant synthetic biology,
- providing mechanisms for open sharing of standardised resources,
- applying these tools to world-leading projects in trait development, and
- facilitating interdisciplinary exchange, outreach and international development.

The initiative promotes interdisciplinary exchange, open technologies and responsible innovation for improvement of sustainable agriculture and conservation.



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