OpenPlant Forum

Fast and frugal engineering with biology

24 - 26 July 2017

Downing College, Cambridge

@_OpenPlant
#OpenPlantForum

openplant.org
glsr.it/openplant2017

OPENPLANT FORUM 2017 PROGRAMME

Monday 24 July 2017

13:00 - 17:00 OPENPLANT FUND PITCHES

Interdisciplinary teams led by early career researchers from Cambridge and Norwich pitch their ideas for £5000 OpenPlant Fund projects to a panel of judges from academia, industry and NGOs. In this round, projects should generate a tangible outcome of relevance to synthetic biology or cell-free synthetic biology and all outcomes must be shared openly with the community.

13:00 LUNCH

14:00 Introduction

14:10 A cell-free sensor platform for the quantification of arsenic concentrations in drinking water

14:20 Cell-free diagnostics for the surveillance of livestock viruses

14:30 Development of Manufacturing Capability for Rare Sugar Nucleotides

14:40 Engineering of Chlamydomonas reinhardtii to produce betalain pigments and the use of riboswitches to direct metabolic flux

14:50 CGSENS: Visualization of CG methylation using a fluorescence protein biosensor

15:00 Design of synthetic plant and mammalian gene regulatory networks using nonparametric Bayesian approaches

15:10 GardenSeq Chasing the invisible diversity of microbial life forms in vegetable garden beds with a portable DNA-sequencer

15:20 Plug and play synthetic biology education resource

15:30 COFFEE

16:00 Single cell pollen meiosis screening in wheat

16:10 Harvesting the genetic value of interspecific wheat introgressions

16:20 Identifying nutrient-status dependent elements regulating the wheat transcriptional response to neighbours

16:30 Bench-top Controlled Environment Growth Chamber for Speed-Breeding and Crop Transformation

16:40 Closing Remarks (Judging Panel)

17:00 DRINKS RECEPTION AND POSTER/DEMO SESSION

An opportunity to peruse previous OpenPlant Fund projects and ongoing research with drinks and networking.

18:30 INDUSTRY FORUM: BUILDING A SUSTAINABLE GLOBAL BIOECONOMY

Talks from a range of synthetic biology companies about their journeys to setting up impactful ventures and the opportunities and challenges they foresee in establishing a sustainable bioeconomy with emerging fast and frugal biotechnologies. Topics may include the role that synthetic biology and industry could play in furthering the sustainable development goals, additional challenges to achieving impact in emerging and resource-constrained markets and the role of open IP as a commercial strategy. Speakers include:

- Deep Science Ventures
- Orr Yarkoni and Jim Ajioka, Colorifix
- Miha Pipan, Entomics

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.

GLISSER

We are using Glisser during OpenPlant Forum 2017 - an event technology designed to make presentations more interactive and useful. Slides will be shared directly to your phones or devices so you can download all presentations, and we'll also be using Glisser to let you text in questions, and vote in polls throughout the event.

There's no need to download anything. Just put this url: http://glsr.it/openplant2017 Into your internet browser on your device or phone.

Tuesday 25 July 2017

08:30 **ARRIVAL AND COFFEE**

09:00 WELCOME ADDRESS

Jim Haseloff, University of Cambridge

09:15 KEYNOTE TALKS: CELL-FREE EXPRESSION SYSTEMS FOR SYNTHETIC BIOLOGY

Development of Versatile and Cost-effective Cell-free Protein Synthesis Technology
Jim Swartz, Stanford

Rapid, Low-cost Tools for Human Health: Using Cell-free Synthetic Biology for Diagnostics and the Portable Manufacture of Therapeutics
Keith Pardee, University of Toronto

Establishing microfluidic cell-free systems for the rapid characterization of genetic networks
Sebastian Maerkl, EPFL

10:45 **COFFEE**

11:15 PROGRAMMING CELL-FREE SYSTEMS AND DESIGN FOR DIAGNOSTICS

Prototyping of cell-free protein synthesis in non-model bacterial systems
Simon Moore, Imperial College

Designing Diagnostics
James King and Tempest van Schaik, Science Practice

Poster Lightning Talks

12:15 **LUNCH AND POSTER SESSION**

Please take a chance to peruse the many posters from OpenPlant researchers and visitors. If you would like to hold informal meetings over lunch, the OpenPlant Hub is a 10-person meeting room that is bookable via the regsitration desk.

14:15 TOOLS FOR FAST AND FRUGAL BIOENGINEERING

Loop Assembly
Bernardo Pollak, University of Cambridge

Improving the Plant Genome Engineering Toolbox
Nicola Patron, Earlham Institute

Developing an open access expression system to liberate transient expression of proteins in plants
Hadrien Peyret, John Innes Centre

Poster Lightning Talks

15:30 **COFFEE**

16:00 FAST, FRUGAL AND OPEN BIOTECHNOLOGY FOR DEVELOPMENT

Open source technologies for science and education in Latin America
Fernan Federici, Pontificia Universidad Católica de Chile

The agricultural Bio-economy in South Africa and potential for applying new genetic engineering technologies
Maneshree Jugmohan-Naidu, South African National Department of Science and Technology (DST)

Why IP won't deliver technology justice
Simon Trace, Oxford Policy Management

Cultivating innovative solutions to global challenges in the Cambridge ecosystem: challenges and opportunities
Lara Allen, Centre for Global Equality

Taking big ideas to a resource poor country - Zimbabwe
Fiona Robertson, University of Zimbabwe

A panel discussion will follow the talks

18:00 DRINKS RECEPTION

CONFERENCE DINNER

The OpenPlant Forum Gala Dinner will be held at 19:30 in the Main Hall at Downing College. Please note that only those who have registered can attend and only vegetarians will receive the vegetarian option. Tickets for people with special dietary requirements must be collected from the Forum Reception desk.

If you are not a speaker or funded by OpenPlant, please pay the £25 fee for the conference dinner by cash or cheque at the Forum Reception desk by 16:00 on Tue 25 July.

STARTER

Confit of Duck and Chicken with Pancetta Apricot, Char Grilled Sour Dough
(V) Celeriac Velouté, Black Truffle Focaccia

MAIN

Fillet of Scottish Salmon Fennel Purée, Crushed Potato, Braised Baby Fennel, Red Amaranth Cress, Lemon Oil
(V) Walnut Gnocchi Pepper ragout, salsify and mushroom soil

DESSERT

Trio of Desserts: Hazelnut and Chocolate Macaron, Madagascar Crème Brûlée, Lime Meringue Pie

Wine will be served at the table and coffee and chocolates will follow in the Grace Howard Room from 21:00. A cash bar will be available until 23:30.

21:00 SYNTHETIC BIOLOGY CURRICULA OF THE FUTURE: FOSTERING CREATIVITY AND INTERDISCIPLINARITY

- Jenni Rant, SAW Trust
- Jake Wintermute, CRI Paris
- Colette Matthewman, John Innes Centre
- Karen Ingram, Independent Artist
- Helene Steiner, Microsoft Research
- Biodesign Challenge Team, Royal College of Art

22:00 CASH BAR AVAILABLE UNTIL 23:30

Wednesday 26 July 2017

08:30 **ARRIVAL AND COFFEE**

09:00 KEYNOTE TALK: POST-TRANSCRIPTIONAL REGULATION IN MARCHANTIA

Mario Arteaga, Universidad Veracruzana

09:30 ADVANCES IN TOOLS FOR ALGAL SYNTHETIC BIOLOGY

A synthetic biology approach to harness the regulatory potential of miRNAs in the green alga Chlamydomonas reinhardtii
Francisco Navarro, Plant Sciences, University of Cambridge

Using Synthetic Biology To Engineer Medicinal Algae
Payam Mehrshahi, Plant Sciences, University of Cambridge

Turning sugar to gold: uncovering enzymatic tools in microalga for β-1,3-glucan synthesis
Sue Kuhaudomlarp, John Innes Centre

10:30 **COFFEE**

11:00 CONSTRUCTING A SHAREABLE TOOLKIT FOR RAPID PLANT MODEL SYSTEMS

Open tools for liverwort analysis and engineering
Susana Sauret-Gueto, University of Cambridge

A new piece to an old puzzle: investigating plant-microbe interactions in early land plants
Philip Carella, University of Cambridge

Reengineering the Marchantia Polymorpha chloroplast genome, one base at a time
Orr Yarkoni, University of Cambridge

12:30 **LUNCH AND POSTER SESSION**

13:45 METABOLIC ENGINEERING FOR NOVEL CHEMICALS AND THERAPEUTICS

CRISPR/Cas9 Mediated Gene Silencing for L-DOPA production in Beetroot
Noam Chayut, John Innes Centre

Wednesday 26 July 2017 (continued)

13:45 METABOLIC ENGINEERING FOR NOVEL CHEMICALS AND THERAPEUTICS (CONTINUED)

Catnip: the molecular and evolutionary origin of nepetalactone biosynthesis
Benjamin Lichman, John Innes Centre

Utilising transient expression towards the preparative production of triterpenes: A tool for medicinal chemistry?
Michael Stephenson, John Innes Centre

14:45 REFLECTIONS FROM PARTICIPANTS

Creating a cell-free lab for students
Clare Hayes, University of Warwick

Science and Plants for Schools
Dan Jenkins, Science and Plants for Schools

15:15 CLOSING REMARKS

Anne Osbourn, John Innes Centre

15:30 **COFFEE AND NETWORKING**

16:00 **END OF CONFERENCE**

16:45 PUNTING & BBQ

The OpenPlant postdocs have arranged a punting trip (16:45) and BBQ (18:00 at the Sainsbury Laboratory in the Botanic Gardens. A taxi/minibus back to Norwich will be provided at 20:00. ***Pre-registration is required and places are limited***.

Punting Location: Scudamore's Punting Station, Mill Lane, Cambridge CB2 1RS
BBQ Location: Sainsbury Laboratory, Cambridge University, Bateman St, Cambridge CB2 1LR

Contact: Susana Sauret-Gueto (ss2359@cam.ac.uk)

CONFERENCE INFORMATION

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VENUE

Howard Theatre, Downing College, Regent Street, Cambridge CB2 1DQ
Porters' Lodge Tel: 01223 334800

ACCOMMODATION

For those being accommodated by OpenPlant, you will be staying in Downing College, Regent Street, Cambridge, CB2 1DQ. Please check-in via the Porters Lodge. Accommodation includes breakfast and wifi is available via eduroam or guest logins obtained from the Porter's Lodge.

Payment: Nothing is payable on check-out.

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. Ask at the registration desk.

WIFI

UniCam and eduroam networks are available onsite, temporary logins will be issued to visitors on check-in or via the Forum Registration Desk.

SOCIAL MEDIA

The OpenPlant Twitter account is @_OpenPlant and we will be tweeting on #OpenPlantForum. The conference will also be on Glisser at http://glsr.it/openplant2017 - view presentations, ask and vote on questions and more. 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 ten-person meeting room is available throughout the conference to be booked in 30 min slots for informal or formal meetings that gather small groups around a particular topic. Sign up for a 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

Parking is not available onsite without prior arrangement and is very limited in the City Centre.

PHOTOS/VIDEOS

Photos and video will be shot during the event. A camera crew from Little Dragon Films will also be on-site filming one to one interviews with selected participants as part of an on-going project on open material transfer agreements.

If you do not wish to appear in event photos, please collect a lanyard from the registration desk and you will be removed from images posted by the organisers.

CONTACT US

Dr Jenny Molloy
Department of Plant Sciences
University of Cambridge
Downing Street
Cambridge
CB2 3EA

Email: synbio@hermes.cam.ac.uk
Tel: +44 (0)1223 766546 (ext 66546)

Conference Urgent Number: +44 7787 445 330
The Downing College Events Team and Porters Lodge can also help with urgent queries or emergencies.

POSTER ABSTRACTS

SCIENCE SERVED THREE WAYS – PUBLIC ENGAGEMENT WITH THE SAW TRUST

Jenni Rant[1] and Emma McKechnie-Welsch[2,3]
[1]The SAW Trust
[2] John Innes Centre, Norwich
[3] Intern with the OpenPlant Synthetic Biology Research Centre and The SAW Trust

The Science Art and Writing (SAW) Trust (registered charity no. 1113386) specialises in helping researchers improve and exercise key professional development skills through participation in enriching engagement experiences with communities. Working across the disciplines with professional artists and writers, and using intriguing scientific images, our workshops invite people to explore science through practical activities, poetry and the visual arts. 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.

Visit our exhibition at the OpenPlant Forum to see some of our newest activities including the first Global Gardens workshop for adults, Synthetic biology at Latitude Festival, meet DNA Dave the robot and see the outputs from our latest SAW projects in schools designed and delivered by OpenPlant intern Emma.

CELL-FREE PAPER BASED DIAGNOSTICS

Laura Mitchell[1], Raghd Rostom[2], Chun Man Chow[3], Emily Groves[4] and Simon Wiegrebe[5]
[1] Department of Chemistry, University of Cambridge
[2]Wellcome Trust Sanger Institute & University of Cambridge
[3] Department of Chemical Engineering and Biotechnology, University of Cambridge
[4] Department of Medicine, University of Cambridge
[5] Department of Economics, University of Cambridge

Harnessing recent advances in synthetic biology and paper microfluidics, cell-free paper based diagnostics offer a platform for low cost, in-field tests with a very wide range of possible specificities. Synthetic gene networks can be designed to generate quantifiable outputs, such as chromoproteins, in the presence of specific input signals like heavy metals or viral RNA sequences. These can be freeze-dried onto paper, along with the cellular machinery used for gene transcription and translation. When rehydrated, rapid determination of the presence/absence of a substance of interest can be made. With a simple visible readout, little or no laboratory experience or infrastructure is required.

We are an interdisciplinary group of students who have been investigating a number of potential applications for this technology, and have developed an assessment cycle to assist in this evaluation. We identify three particular end uses for which the technology could have enormous social and economic benefits for farmers, communities and whole economies across the developing world. Come and visit our poster to talk to us about this work; our findings from recent travel to Kenya and South Africa, and our plans going forward as OpenDiagnostics.

USE OF PPR PROTEINS AS MOLECULAR TOOLS FOR CHLAMYDOMONAS REINHARDTII

Aleix Gorchs Rovira[1], Saul Purton[2] and Alison Smith[1,3]
[1]University of Cambridge
[2]University College London
[3]OpenPlant Synthetic Biology Research Centre

The chloroplast of Chlamydomonas reinhardtii is a highly intricate and complex system. The number of genes encoded in the chloroplast of this eukaryotic alga is small compared to its nuclear genome. However, these chloroplastic genes are tightly regulated with their translation and RNA processing depending mainly on nuclear-encoded proteins. Penta- or tetratricopeptide repeat proteins (PPR/TPR proteins) play a significant role in chloroplast gene regulation. TPR proteins are mainly involved in mediating protein-protein interaction whereas PPR proteins are thought to interact directly with specific mRNAs. These PPR proteins can be directly implied in processes such as intron removal or 5'UTR stabilisation.

These proteins have potential for biotechnological purposes such as gene regulation of exogenous genes in the chloroplast. Previous work on NAC2, a TPR protein stabilising the 5'UTR of psbD, regulated by the THI4 riboswitch has led to the creation of a vitamin-dependent regulated genetic circuit between the chloroplast and the nucleus of this algae (Ramundo et al. 2013). Further improvement of this system as well as the addition in the genetic circuit of METE-regulated MRL1, a PPR protein stabilising the upstream region of rbcL, will lead to the creation of a thiamine and B12 dependent double switch to tightly regulate the expression of exogenous genes placed in the chloroplast. However, it is important also to establish a system that would allow to study the effect of these nuclear-encoded regulators. Therefore, establishment of fluorescent proteins as reporters for the chloroplast in C. reinhardtii must be pursued as well as the consolidation of easy and fast chloroplast transformation protocols such as electroporation.

EXPANDING THE PLANT GENOME ENGINEERING TOOLBOX

Oleg Raitskin[1,2], Anthony West[1], Nathalia Volpi[1,3] and Nicola J Patron[1,2]
[1]The Earlham Institute, Norwich
[2] OpenPlant Synthetic Biology Research Centre
[3] Department of Plant Biology, Institute of Biology, University of Campinas (UNICAMP), Brazil

Molecular tools adapted from the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) loci that confer adaptive immunity in bacteria and archaea have adapted for genome engineering in eukaryotes. RNA-guided Cas (CRISPR Associated) proteins have been used to induce targeted mutagenesis at endogenous loci in numerous plant species. However, the efficiency of editing varies between species and between targets, mutations are often observed at non-target loci and use of wild-type Cas9 limits engineering to target loci containing a canonical NGG motif. We have developed an expanded toolbox of molecular tools for RNA-guided Cas-mediated plant genome engineering to improve specificity and to increase the number of available of potential target sites in plant genomes. To compare and quantify efficiency and specificity we coupled automated DNA assembly to a transient workflow using protoplasts and Illumina MiSeq targeted resequencing. This workflow is

amenable to automation and offers potential as a rapid, high-throughput screening platform for plants.

WIKIFACTMINE: MINING THE OPEN BIOSCIENCE LITERATURE FOR PHYTOCHEMICAL DATA

Peter Murray-Rust[1], Jenny Molloy[2,3] and Tom Arrow[1]
1 ContentMine Ltd, (non-profit) Future Business Centre, Kings Hedges Road, Cambridge, UK
2 Department of Plant Sciences, University of Cambridge
3 OpenPlant Synthetic Biology Research Centre

WikiFactMine is a Wikimedia project [1] with ContentMine [2] to extract all open factual information from the bioscience and index against Wikidata [3] (which has 30 million entries of data/metadata). We have developed an Open Toolkit [4]:

- getpapers: which daily downloads the complete Open bioscience literature (many hundreds), primarily through EuropePubMedCentral.
- Wikidata [4] dictionaries; currently: genes (human and otherwise), species, genera, drugs, phytochemicals, diseases (human), geo-locations, organizations.
- AMI/Norma; creates semantic documents from the literature indexed against Wikidata
- Fatemeh; uses the literature to suggest new Wikidata entries.

We have run these tools for several months and have ca 3 million facts captured in the Zenodo [5] repository and viewable through FactVis [6]. We have a special section for phytochemistry where these facts can be used to link plant species and location to chemicals. For example we have scanned the output for a range of terpenoids, many of which have been reported as having medicinal or insecticidal properties and allows us to map phytochemical reports onto dates and locations.

[1] https://meta.wikimedia.org/wiki/Grants:Project/ContentMine/WikiFactMine/Midpoint
[2] http://contentmine.org
[3] http://wikidata.org/
[4] http://github.com/ContentMine
[5] https://zenodo.org/communities/contentmine/
[6] https://tarrow.github.io/factvis/#

IMPROVING A CUSTOM-BUILT LIGHT SHEET MICROSCOPE FOR DEVELOPMENTAL STUDIES

Stephanie Höhn[1], Pierre Haas[1] and Karen Lee[2]
[1] Department of Applied Mathematics and Theoretical Physics, University of Cambridge
[2] John Innes Centre, Norwich

Light sheet fluorescence microscopy (LSFM) is the state-of-the-art technique to study developmental processes in vivo. LSFM causes less photo-damage than confocal microscopy enabling longer time-lapse recordings. We had previously built a LSFM setup in the Goldstein group. The purpose of this project is to improve our setup and the quality of the generated LSFM data.

Optical sectioning is achieved by moving the sample through a light sheet and thereby

creating z-stacks. In our previous setup images were recorded by a single camera. Due to light absorption and scattering the images of the sample half facing away from the camera showed a significant loss in image quality. In order to correct for this loss we have added a second camera and detection arm opposing the first one and covering the second half of the sample. This improved setup is doubling the thickness of a sample for which we can acquire useful fluorescence data. This significantly increases the variety of future applications including studies on the morphogenesis of entire embryos in the multicellular micro-alga Volvox and the development of feeding structures of the aquatic carnivorous plant Bladderwort.

TIMBER SYNTHETIC BIOLOGY STRATEGIES TO IMPROVE BIOFUEL PRODUCTION AND GENERATE DESIGNER POLYSACCHARIDES.

Jan J Lyczakowski[1,3]*, Krzysztof Wicher[2], Oliver Terrett[1], Marta Busse-Wicher[1,3]* and Paul Dupree[1,3]*.
[1] Department of Biochemistry, University of Cambridge
[2] The Gurdon Institute, University of Cambridge
[3] OpenPlant Synthetic Biology Research Centre
* Equally contributing corresponding authors

Plant biomass, such as straw and timber, is a renewable bio-composite matter made from an intricate assembly of various polysaccharides and phenolic compounds. As a sustainable material, biomass can be used to create solutions to some of humanities greatest challenges. These include providing carbon-neutral energy and materials for a growing population. Here we describe how knowledge of basic biological processes combined with synthetic biology approaches can help us generate designer polysaccharide structures which not only can influence the properties of the biomass but may also be high value products themselves. Specific removal of one substitution of a polysaccharide component of biomass from a model plant Arabidopsis thaliana enabled us to more than double the simple sugar and ethanol yields after enzymatic saccharification and fermentation. Remarkably, the discovered modification of polysaccharide structure has no negative impact on plant growth. By mining large transcriptomic datasets we have identified putative enzymes responsible for the yield-limiting modifications in conifers. Using N. benthamiana over-expression with the pEAQ-HT vector and in plantae analysis we have demonstrated that the conifer enzyme is able to confer the specific glycosyltransferase activity both in vitro and in vivo. Heterologous expression of a conifer enzyme in A. thaliana lead to significant changes in polysaccharide structure. Therefore this work not only identifies an excellent conifer mutagenesis targets to improve conversion of softwood to biofuels but also is a proof of concept for in plantae synthesis of designer polysaccharides.

ARTIFICIAL CELLS FOR TISSUE MIMICS

Emma L. Talbot[1], Pietro Cicuta[2,3] and Jim Haseloff[1,3]
[1] Department of Plant Sciences, University of Cambridge
[2] Department of Physics, Cavendish Laboratory, University of Cambridge
[3] OpenPlant Synthetic Biology Research Centre

Compartmentalised lipid assemblies are ideal structures for artificial cells, enabling synthetic biological processes to take place within an enclosing membrane. The assembly of these artificial cells into a network which is responsive to external stimuli would provide an artificial

tissue mimic, and offers exciting opportunities for compartmentalised reactions across an array with well-defined structure. However, forming ordered networks requires regulation of the assembly of well-defined building blocks, for which control over membrane curvature and patterning poses a significant challenge. A synergy between synthetic biology and microfluidics-based bilayer-assembly will be used to overcome this problem and form artificial cell building blocks of pre-defined geometrical shape and membrane patterning, with encapsulated reaction species. This approach will enable complex chain reactions to be explored for bio-sensing applications in cell mimics with large numbers of compartments. The controlled patterning will be used to connect building blocks in an ordered array, mimicking a tissue, with an extended pattern over long length-scales that can be linked to membrane-controlled processes.

FREQUENCY DOUBLING IN THE CYANOBACTERIAL CIRCADIAN CLOCK

Bruno M.C. Martins[1,2], Arijit K. Das[1], Liliana Antunes[1], Chao Ye[1] and James C.W. Locke[1,2]
[1] Sainsbury Laboratory, University of Cambridge
[2] OpenPlant Synthetic Biology Research Centre

Organisms use oscillatory circuits to control rhythmic gene expression and synchronise cellular processes. Circadian clocks, in particular, generate 24 hour rhythms in anticipation to daily cycles of sunlight. However, the clock can also generate shorter period oscillations. It remains unclear how clocks interact with other circuits to generate such frequencies, and what dynamics arise from those interactions.

We used single-cell time-lapse microscopy and mathematical modelling to study the coupling of the clock to a circuit that controls expression of the gene psbAI in the cyanobacterium S. elongatus. Genes regulated by the clock typically peak once a day, either at dawn or at dusk. However, when under low light, we observed frequency doubling in the expression of psbAI, i.e., it peaks twice a day. We also observed two peaks in single-cell growth rates, suggesting frequency doubling can affect the global state of the cell. Using an iteration of theory and experiment, we determined the network design principles underlying the dynamics of frequency doubling. Next, we applied genetic and environmental perturbations and found these dynamics can be modulated: we can generate either single-peak or two-peak expression, and modify the main frequency of the output.

Rational design of oscillators is a goal of synthetic biology, but natural systems are already endowed with reliable oscillators in the form of circadian clocks. Understanding how to harness clocks to generate specific (non-circadian) frequencies, and how to systematically integrate clocks with other pathways may give us powerful tools, enabling the assembly of complex synthetic circuits.

ENGINEERING CELL WALL POLYSACCHARIDES BY EXPRESSING SPECIFIC GLYCOSYLTRANSFERASE ACTIVITIES IN ARABIDOPSIS THALIANA

Henry Temple[1,2], Louis Wilson[1,2], Jan Lyczakowski[1,2], Li Yu[1] and Paul Dupree[1,2]
[1] Department of Biochemistry, University of Cambridge
[2] OpenPlant Synthetic Biology Research Centre

Plant Cell Walls represent the most abundant renewable source of polysaccharides on the planet. Despite this great abundance and importance only a small fraction of this biomass is currently used. Although there is an on-going interest in the use of cell walls polysaccharides, we are just starting to understand their biosynthesis in plant cells. Synthesis of polysaccharides occurs mainly through the activity of Glycosyltransferase (GT) enzymes which transfer an activated sugar in the form of a nucleotide-sugar onto a specific growing polysaccharide acceptor. After cellulose, hemicelluloses are the second most abundant polysaccharides present in nature. Unlike cellulose synthesis, hemicellulose synthesis take place in the Golgi apparatus. In this project, we are interested in identifying and characterising different Golgi localised GTs participating in the synthesis of hemicelluloses mannan and xylan. In addition, we aim to use these tools to modify hemicelluloses in a specific manner. Using the remarkably versatile GoldenGate cloning, we were able to obtain a large library of constructs to express several GT activities under tissue specific promoters. Furthermore, in order to obtain biochemical evidence of GTs activities we have used N.benthamiana heterologous expression with the pEAQ-HT vector system. We tested in vitro activities using specific polysaccharides acceptors and coupled it to oligosaccharides finger-printing using Polysaccharides Analysis using Carbohydrate Electrophoresis (PACE). Employing the strategy presented in this work, we are able to identify novel GT specific activities and engineer hemicellulose polysaccharides synthesis in a desired way. The next step is to evaluate the consequences of these modifications on the utility of these novel polysaccharides and on the properties of plant cell walls.

PRODUCT PURIFICATION IN THE ADVANCED BIOPROCESSING CENTRE, BRUNEL UNIVERSITY

Jonathan Huddleston, Peter Hewitson, Ian Garrard and Svetlana Ignatova.
The Advanced Bioprocessing Centre, Brunel University London

The Advanced Bioprocessing Centre (ABC) at Brunel University London is specialising in continuous bioprocessing and downstream processing (DSP) to solve challenging separation problems, and working closely with industry and academia across the world. All members of the team have industrial backgrounds, which brings a unique perspective to solving scientific problems. The ABC is currently the only centre in the world applying liquid flow processes as a platform separation technology. The ABC core technologies are continuous liquid-liquid extraction in dynamic regime often referred to as counter-current chromatography (CCC) or/and centrifugal partition chromatography (CPC); it also has high performance liquid chromatography (HPLC) and membrane filtration. The CCC planetary centrifuges works on a principle of a fluctuating centrifugal force providing thousands of mixing/settling steps and enabling density fractionation of various (bio)particles. The ABC facilities houses all scales of CCC processing equipment from analytical (5ml) for method development to pilot scale (18L)

for the DSP of synthetic and natural target compounds for clinical trials/research.
The ABC main research streams include but are not limited to:

- Natural product purification including generating material for initial fingerprinting, together with material difficult for solid phase chromatography or membranes (adsorption/blockage)
- Purification of biopharmaceuticals from complex matrixes (from fermentation broth containing particulates, sticky antifoaming agents or plant/animal tissues)
- Potential blood plasma purification as the technology can be used as a concentrator
- Continuous bioreactor/separator

METABOLIC GENE CLUSTERS – CO-REGULATION OF NEIGHBOURING GENES

Hans-Wilhelm Nützmann[1,2], Nan Yu[1], James MacDonald[3], Vinod Kumar[1], Paul Freemont[3] and Anne Osbourn[1,2]
[1] John Innes Centre, Norwich
[2] OpenPlant Synthetic Biology Research Centre
[3] Imperial College, London

Historically, it has been assumed that in eukaryotes the expression of one gene is neither influenced by nor does it influence the expression of genomic neighbours. While prokaryotes have well described multi-cistronic transcripts, in eukaryotes, such structures are the exceptions that prove the rule. However, diverse examples for co-localised functionally related genes have been identified in eukaryotes that resemble organised clusters in prokaryotes. Here, we employ metabolic gene clusters in plants to shed light at the molecular processes that govern the coordinate expression of neighbouring genes. Metabolic gene clusters are constituted by adjacent non-sequence related genes that encode the different catalytic steps required for the synthesis of bioactive specialised metabolites. We show that these clusters are delineated by distinct chromatin signatures and localised in genomic areas of intense local three-dimensional interactions. With our research we aim to improve models to predict the effects of neighbouring genes on transgene expression and the design principles of synthetic multi-gene cassettes.

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

Yao-Min Cai[1] and Nicola Patron[1,2]
[1] The Earlham Institute, Norwich
[2] OpenPlant Synthetic Biology Research Centre

A handful of promoters derived from plant-infecting viruses and bacteria are widely 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. In a different study, we are developing externally-tuneable, orthogonal regulatory systems that do not respond to endogenous cellular signals, but the utility and simplicity of single sequences such as CaMV 35s are widely demonstrated, particularly in transient plant bioproduction.

Here, we are studying how promoters from plant-infecting viruses and bacteria drive constitutive expression in multiple plant species. Potential transcription factor (TF) binding sites were identified using the MEME Suite Software (Multiple Em for Motif Elicitation) and the Arabidopsis Cistrome Database. We then analysed the expression networks of the corresponding TFs finding that, while each promoter uses a largely unique set of TFs, TFs from the development and pathogen response networks are highly employed. We also discovered a common motif, present across almost all viral and bacterial promoters for which functional validation is ongoing.

We are using these data to inform the design of a suite of synthetic minimal promoters with various expression levels for biotechnology, to confirm transcription-factor/binding site interactions, and to understand how architecture (motif identity, location, proximity and potential nucleosome inhabitation) affects the intrinsic properties of promoters. We are also comparing the architecture of constitutive plant-infecting viral and bacterial promoters to endogenous constitutive promoters.

ENGINEERING SYNTHETIC PENTATRICOPEPTIDE REPEAT (PPR) PROTEINS AS ON AND OFF SWITCHES FOR CHLOROPLAST PROTEIN TRANSLATION

Suvi Honkanen and Ian Small
ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia

Plant chloroplasts are increasingly used as a platform for producing high-value biomolecules such as therapeutic proteins and vaccine antigens. Chloroplast gene expression is primarily regulated on a translational level, yet we currently lack a mechanism by which chloroplast transgene translation could effectively be switched on and off in multicellular plants. I aim to develop synthetic pentatricopeptide repeat (PPR) protein based PhytoBricks that function as targeted 'on' and 'off' switches for chloroplast protein translation. These novel switches will allow effective control over translation of transgenes introduced into the chloroplast genome without interfering with the translation of native chloroplast proteins. As the PPR proteins will be expressed from the nuclear genome, they will provide means of linking nuclear gene regulatory networks to organellar protein translation. Two complementary approaches will be utilised to generate PPR protein based translational switches, which will first be tested in the liverwort M. polymorpha. Using these switches in different combinations will eventually allow the construction of most of the standard logic gates for the implementation of complex control circuits based on multiple input signals. In the longer term, the PhytoBricks developed here are expected to be adaptable to a wide range of genetic systems to control gene expression in many biotechnological applications.

ROLE OF THE AUXIN-PECTIN RELATIONSHIP IN THE EARLY LAND PLANT MARCHANTIA POLYMORPHA

Giulia Arsuffi and Siobhan Braybrook
The Sainsbury Laboratory, University of Cambridge

The cell wall is a dual-function interface that can act in a permissive or restrictive manner

depending on the context. The complexity of its structure and composition is what gives it this high degree of adaptability. In a similar fashion, the phytohormone auxin displays opposite effects on growth depending on the tissue under examination. Pectin de-esterification in muro also causes differential growth phenotypes across tissues. In the shoot apical meristem of Arabidopsis, auxin and pectin de-esterification are closely interconnected, as pectin de-esterification is necessary for auxin-induced organ formation.

However, the high complexity of angiosperm gene networks and cell wall composition make it difficult to elucidate how the relationship between auxin and pectin biochemistry changes the cell wall and, in turn, growth. The early land plant model Marchantia polymorpha benefits from simpler auxin and pectin gene networks as well as a more elementary cell wall composition. Together with its sequenced haploid genome and ease of transformation, these factors make it easier to study the auxin-pectin link. Systemic chemical inhibition of pectin de-esterification phenocopied the effects of high concentrations of exogenous auxin when this was applied to Marchantia gemmae. In addition, 4 out of the 14 pectinesterase genes were found to be differentially expressed in fast-growing meristematic areas compared to areas which had ceased growth. In order to isolate plants with defective pectin de-esterification and observe its effects on growth, cell wall mechanical properties and auxin distribution, we are creating CRISPR pectinesterase mutant lines. By connecting growth tracking, auxin distribution, pectin biochemistry and wall mechanics data, we plan to exploit Marchantia as a powerful model for plant developmental mechanics.

SPLITTALE - A TALE BASED TWO-COMPONENT SYSTEM IN PLANTA

Tom Schreiber, Anja Prange and Alain Tissier
Leibniz-Institute of Plant Biochemistry, Department of Cell and Metabolic Biology, Halle (Saale), Germany

Transcription activator-like effectors (TALEs) are bacterial proteins which act as transcriptional activators in planta. Due to their modular DNA-binding domain TALEs can be designed to target any desired DNA sequence and hence, in principle, can be used to activate any target gene. In order to broaden the applicability of TALEs we generated a splitTALE two-component system that is composed of the TALE-DNA-binding (DB) domain and the TALE activation domain (AD). BD and AD are fused to interacting protein domains which allows reconstitution of the TALE and induction of target genes if both components are present. The splitTALE system exhibits no background activity and can be used as an on-off AND gate system that does not depend on additional signals or subcellular localization. Here, the splitTALE system and its characteristics will be presented.

GENERATING VIRUS-LIKE PARTICLES FOR BIONANOTECHNOLOGICAL APPLICATIONS

Roger Castells-Graells[1], Keith Saunders[1] and George Lomonossoff[1,2]
[1] John Innes Centre, Norwich
[2] OpenPlant Synthetic Biology Research Centre

A key property of single-stranded RNA viruses is to multiply and produce high yields of virus particles that encapsidate the viral genome. The transient expression of viral coat proteins induces the synthesis of virus-like particles (VLPs) that lack the infectious genome. This

technology, which is now well established, can be used to produce high yields of VLPs that have a morphology essentially identical to that virus from which they have been derived. We use synthetic biology to engineer these VLPs in order to generate new bionanotechnological tools, such as nanomachines, and to understand viral dynamics for future applications.

Roger Castells-Graells will also be exhibiting a range of 3D printed protein and virus structures produced as part of an OpenPlant Fund project called 'Accessible 3D Models of Molecules'.

MICRODROPLET ENCAPSULATION AND SORTING OF GENETIC PARTS IN PLANTS

Ziyi Yu[1], Christian R. Boehm[2], Steven J. Burgess[2] and Ivan Reyna-Llorens[2,3]
[1] Department of Chemistry, University of Cambridge
[2] Department of Plant Sciences, University of Cambridge
[3] OpenPlant Synthetic Biology Research Centre

A current limitation for plant synthetic biology involves the screening of genetic parts on a high-throughput scale. Current validation techniques require fusing sequences to reporter genes and analysing gene expression in planta. This results in testing one circuit per individual plant, either through transient or stable genetic transformation. Applying these techniques to whole regulatory element libraries or to a considerable number of genetic circuits can be time consuming and it is not feasible at a laboratory scale. Here, we developed a high-throughput screening method for the analysis of plant genetic circuits. Our method uses a PDMS microfluidic device for the encapsulation and sorting of individual plant cells. As a result, we successfully managed to isolate and encapsulate protoplasts from the model species, Marchantia polymorpha and Arabidopsis thaliana. Moreover, we have used this device to assess the activity of a heat inducible promoter in M. polymorpha protoplasts. Using this approach we can accelerate the analysis of novel genetic circuits as well as our understanding on gene regulation in plants.

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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OPENPLANT FORUM 2018
23-25 JULY AT THE
JOHN INNES CONFERENCE CENTRE,
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