Progress Report
July 2016

OpenPlant is a BBSRC/EPSRC Synthetic Biology Research Centre supported by the Synthetic Biology for Growth Programme
Introduction to the OpenPlant initiative

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. However, agricultural systems face global threats from new pathogens, climate change, soil degradation and restricted land and water use. Plants are genetically facile, and GM plants are currently grown on the >100 million hectare scale. Plant systems are ripe for synthetic biology, and any improvement in the ability to reprogram metabolic pathways or plant architecture will have far-reaching consequences. This part of the field is in its infancy and is wide open. We believe that there is a crucial need to accelerate the development of new tools and methods for plant synthetic biology, provide mechanisms for open exchange of resources, apply these standardised tools to world-leading projects in trait development, and facilitate interdisciplinary exchange, discussion, outreach and international development.

The OpenPlant initiative has been funded with three main aims:

1. to create a hub for interdisciplinary exchange between Cambridge and Norwich, between the fundamental and applied sciences, that will underpin advances in UK agriculture and bioproduction.
2. to establish systems for the open exchange of new plant tools and DNA components that will promote commercial innovation and international scientific exchange.
3. to explore the wider implications of the technology at local and global scales. This will bring together a wide range of engineers, scientists and policy developers to explore new technologies and possible models for sustainable agriculture, bioproduction and land use.

Implementation

The OpenPlant initiative supports two tiers of activities. First, we are developing and implementing open technologies that will underpin systematic approaches to bioengineering of plants. These include:

- A common syntax for plant DNA parts and hierarchical assembly of genetic circuits.
- Open DNA registries for sharing information, to join an international web of registries with plant specific DNA parts.
- Legal tools for open exchange of DNA parts and other reagents in biotechnology.
- Establishment of a moderated archive for publication of DNA part descriptions (synbioRxiv).
● Development of a major new lower plant system as a simple and facile chassis for Synthetic Biology, to enable high throughput screening and analysis at the cellular scale.
● New DNA parts for the control and quantitative imaging of genetic circuits in plants.
● Techniques for routine genome-scale engineering in plants.
● Software tools with improved performance for DNA part catalogues, automated assembly, modelling of synthetic gene circuits and cellular morphogenesis.

Second, the development of new tools is contributing to the engineering of new traits in plants, such as:
● Altered photosynthesis and leaf structure.
● Changes in plant carbohydrate content.
● Engineered pathways for the metabolic engineering of natural products.
● New forms of symbiosis and nitrogen fixation for crop plants.
● Methods for high level production of biomolecules by transient expression.

Current agricultural practices and cultivation of trees, crops and pastures are responsible for major pressures on natural environments and land use globally. The OpenPlant initiative brings together an exceptional collection of scientists, whose skills range from biophysics, chemistry and DNA assembly - to crop physiology and agronomy. In addition, we have recruited experts involved in conservation, entrepreneurship, law, policy development and the social sciences in Cambridge, Norwich and elsewhere – who have demonstrated an interest in tackling the technical aspects of surveying future technologies.

We believe that we are seeing the emergence of a new technology for the engineering of plant feedstocks that has the potential to radically alter agriculture and bioproduction. (i) Integrated and interdisciplinary approaches are required, and (ii) Synthetic Biology inspired methods are being applied rapidly to the wider plant field. OpenPlant has contributed a combination of organisation, support and direct sponsorship to a network of working groups, forums, small scale funding initiatives and research projects in order to promote these objectives. Such as:

**OpenPlant Fund**
A mini-finding scheme for inter-disciplinary and inter-institutional plant synthetic biology projects, including software, DNA synthesis, wet lab experiments, instrumentation, workshops and outreach.
http://openplant.org/fund/

**Synthetic Biology Strategic Research Initiative**
A strategic research initiative in Synthetic Biology has been established at the University of Cambridge. It provides a clearing house of information about synthetic biology research and activities in this field and can be found at:
http://www.synbio.cam.ac.uk

**Synthetic Biology Outreach in Cambridge**
There are a wide variety of regular open meetings like Cafe Synthetique, Science Makers and the SRI Forums - with a particular focus on building tools and interdisciplinary research across biology, engineering, computing, physical sciences and the humanities.
http://www.meetup.com/Cambridge-Synthetic-Biology-Meetup
We encourage anyone who is interested in participating in these groups to contact the organisers via the website addresses provided.

Biomakespace in Cambridge
A group of scientists, engineers, students and entrepreneurs are developing the new Cambridge Biomakespace - an innovation space for biology and biological engineering. This will be located in the historic, original MRC Laboratory of Molecular Biology building, supported by OpenPlant and the University of Cambridge Synthetic Biology Strategic Research Initiative.
http://biomake.space

OpenLabTools and Open Technology Week
Managed from the Cambridge University Engineering Department, this interdisciplinary effort promotes development of low-cost hardware and software tools for international development and laboratory use.
http://www.openlabtools.org

Interdisciplinary Cambridge-JIC iGEM teams
Cambridge, now in partnership with the John Innes Centre, has fielded teams of undergraduates from Biology, Engineering and the Physical Sciences in the international Genetically Engineered Machine competition since 2005.
http://igem.org/Main_Page

Cambridge University Synthetic Biology Society
The Cambridge University Synthetic Biology Society, CUSBS, is a new undergraduate society aiming to spread the word of Synthetic Biology. Founded by Cambridge-JIC iGEM team members, they are a growing team of students taking on practical bioengineering projects.
http://cusbs.soc.srcf.net

EUSynBioS: Society for Students and Postdocs in Synthetic Biology
Founded in Cambridge in 2014, the European Association of Students and Post-docs in Synthetic Biology (EUSynBioS) is an international student-led initiative to shape and foster the community of young synthetic biology researchers within Europe by providing a central resource for interaction and professional development.
http://www.eusynbios.org

SAW Trust
Based in Norwich, the SAW Trust runs an international programme for interdisciplinary outreach and education based on an innovative collision of science, art and writing in the classroom.
http://www.sawtrust.org

Centre for Global Equality
The Centre is a network of 50 organisations who work together to reduce global inequality by enhancing access to knowledge and fostering innovation.
http://centreforglobalequality.org
OpenPlant progress in year 1: summary

The OpenPlant initiative started in September 2014. Over the first year of the research programme we made notable progress in: (i) standards and infrastructure for DNA assembly in plants, (ii) development of a framework and methods for more open sharing of DNA parts, (iii) substantial advances in the development of Marchantia as a simple model for plant synthetic biology, (iv) development of improved capacity for automated metabolic analysis and (v) translation of these approaches to the HyperTrans system for rapid testing of metabolic circuits in plants. Major achievements from the first year of the project were:

**Foundational work**
1. Refurbishment and equipping of new OpenPlant laboratories in Cambridge and Norwich.
2. Establishment of a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al., 2015; RFC106).
3. Drafting of an Open Materials Transfer Agreement, a simple, standardised legal tool to enable sharing of materials and associated data on a more open basis.
4. Implementation of a “single-click” OSX-installable version of the JBEIR-ICE open source DNA registry and DNA manipulation software.
6. Characterisation of miR1157 and miR1162 precursors for use as synthetic gene regulators in *Chlamydomonas reinhardtii*.

**Trait Development**
7. Development of *Marchantia paleacea* as a new system for engineering actinomycorrhizal associations.
8. Generation of draft genome and transcriptome maps for *M. polymorpha* and *M. paleacea*.
9. Refactoring and use of the HyperTrans system for rapid testing of DNA circuits for terpene synthesis in *Nicotiana benthamiana*.

**Outreach and Responsible Innovation**
10. Funding of 16 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich - including hardware, wetware development and support for collaboration between OpenPlant and African scientists.
11. Support for a joint Cambridge-JIC iGEM2015 team in the Hardware Track.
12. Support for a Synbio Beta Activate event in Cambridge, to promote entrepreneurial interactions.
14. Delivery of two summer schools on Plant Synthetic Biology and CRISPR Technology in Plants, co-sponsored by ERA-SynBio and Plant Methods/GarNET, respectively.
15. Delivery of three Science, Art and Writing educational workshops, and two school outreach events.
16. Delivery of international workshops on IP, MTAs and Innovation.

These are described in more detail in the 2015 OpenPlant Annual Report.
OpenPlant progress in year 2: summary
So far, major achievements over the second year of the project have been:

**Foundational work**
1. Commissioning of advanced imaging and robotics equipment at the Cambridge OpenPlant laboratory.
2. Completion of the genome sequence and transcript map of the Cam-1 (male) and Cam-2 (female) isolates of *Marchantia polymorpha*. Data will be included in forthcoming publication of genome.
3. High resolution map of the time course of gene expression during sporeling germination and chloroplast differentiation.
5. Official acceptance of the common syntax for plant DNA parts as a new standard (Phytobricks) in the iGEM 2016 competition, and introduction of an award for plant synthetic biology.

**Trait Development**
9. Design and synthesis of an artificial protein scaffold library, built to the Phytobrick standard and verified by BiFC.
12. Identification of a large repertoire of carbohydrate active enzymes in *Euglena gracilis*.
13. Transformation of gene editing constructs into potato, to create digestion-resistant starches, and preliminary screening of transformed plantlets.
14. Gram-scale production of triterpenes for analysis and assay, using the HyperTrans system.
15. Production of the plant-derived iridoid alkaloid strictosidine in yeast.
17. Asteraceae P450 proteins as a toolkit for targeted modification of sesquiterpenes.
18. Development of the HyperTrans system for use in tomato.
19. Screening tomato introgression lines for regulators of monoterpene biosynthesis.
20. Yeast one-hybrid analysis for the identification of transcription factors that regulate triterpene metabolic gene clusters.
21. Characterisation of the gene targets of AtMYB12 and SIMYB12 in tomato, as an effective way of enhancing phenylpropanoid metabolism, for high levels of resveratrol and genistin production.
22. Construction of synthetic wide host range metabolons for ectopic production of dhurrin, a plant defence compound.
23. Construction and distribution of HyperTrans DNA vectors that are compatible with the Phytobrick standard.

24. Testing of the HyperTrans system in Marchantia and BY2 cells.

25. Consultation on the design of the LeafSystems high throughput production facility, due for completion in Q2 2017.

**Outreach and Responsible Innovation**

26. Funding of 14 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich. A summary of the funded projects is provided in the information pack, and include projects to deliver SynBio training in Africa, increase SynBio capacity in Africa, and produce resources for schools and universities in South America.

27. The Cambridge-JIC iGEM2015 team won a gold medal at the international Jamboree, with a project entitled “OpenScope” - a low cost, open source, 3D printed, fully automated microscope powered by a Raspberry Pi and customised software.


29. Responsible Innovation workshop with Kathy Liddell, Law Faculty, Cambridge

30. OpenPlant continues to collaborate with Linda Kahl, Biobricks Foundation, and an international IP working group to implement an Open Materials Transfer Agreement with the aim of improving freedom-to-operate by enabling international exchange of DNA parts. OpenPlant participated in the inaugural meeting of BioNet group at Asilomar and supports the development of an open technology platform for peer-to-peer exchange and provenance tracking of biomaterials (http://www.bionet.io).

31. Organised OpenPlant All Hands meeting for scientific exchange, Newmarket.

32. Participated in Open Technology for Biology workshop, Chile.

33. OpenTechnology Week events in Cambridge, including Technology for the Bottom Billion workshop and Makethon, coordinated with the Centre for Global Equality (http://centreforglobalequality.org).

34. Workshops on ethics and openness run at OpenPlant (March, 2016), outreach with the SAW trust (March, 2016), and BBSRC Media Training for OpenPlant (March 2016).

35. Outreach in schools: OpenPlant exhibit on schools day and continuing in family area during Latitude Festival (July 2016). Exhibit at 2-day Cambridge Science Festival (March 2016). Exhibit at Youth STEMM Awards mid-year conference, John Innes Centre (January 2016). SAW workshops in primary schools with scientists from OpenPlant (March 2015, January 2016). SynBio workshop at Inside Science, event at John Innes Centre for Year 10 pupils interested in science careers (November 2015)

36. Nineteen post-graduate students are participating in projects funded by the OpenPlant Fund. In addition, three PhD students being recruited directly to OpenPlant (Cambridge) this year.

37. Undergraduates have formed a student society for Synthetic Biology at the University of Cambridge (http://cusbs.soc.sr.cf.net).

38. Other students and postdocs at University of Cambridge and John Innes Centre are being recruited to OpenPlant, to share projects, resources and equipment, through the ROC Group - a self organised and highly effective group of junior researchers.
Schematic view of OpenPlant activities

Improved legal mechanisms and libraries for open exchange of DNA parts (brown), along with standardised and automated assembly and biological testing (grey) underpin faster development of new plant traits (green), and facilitate wider innovation, scientific leadership and international collaboration in the field (blue).
Management group

**David Baulcombe**
Prof. Sir David Baulcombe’s group was the first to identify small interfering (si)RNAs as the specificity determinant of RNA silencing and through their genetic analyses have identified many components of RNA silencing pathways. Relevant to this application the group has unravelled many aspects of the role of RNA silencing in virus defense and other aspects of genetic and epigenetic regulation. His work has been recognised through several awards including the 2008 Lasker Award for Basic Medical Science, the 2010 Wolf Prize for Agriculture and the 2012 Balzan Prize for Epigenetics.

**Dale Sanders**
Prof. Dale Sanders’ research investigates how plant cells respond to changes in their environment and how they store the nutrients they acquire. He is a leading authority on the mechanisms for the transport of chemical elements across cell membranes in plants. These mechanisms have key roles in the control of crucial crop traits such as nutritional value of foods, seed germination, response to drought and how plants cope with toxic compounds in the soil.

**Anne Osbourn**
Prof. Anne Osbourn investigates plant natural products - function, synthesis and mechanisms underpinning metabolic diversification. An important advance from the Osbourn laboratory has been the discovery of gene clusters for specialized metabolic pathways in plants, a finding that has opened up new opportunities for elucidation of new pathways and chemistries through genome mining and for the development of synthetic/refactored clusters for improved/high-value plant traits. She has also developed and co-ordinates the Science, Art and Writing (SAW) initiative, a cross-curricular science education programme for enabling engagement of scientists with society.

**Jim Haseloff**
Dr. Jim Haseloff and his lab engineered the first synthetic RNA enzymes with targeted substrate specificity, developed fluorescent proteins for plants, new misexpression systems in plants, new 3D microscopy and visualisation methods and computer models for plant morphogenesis. He has pioneered the application of Synthetic Biology approaches in plants, including new quantitative imaging techniques, genetic circuits for cell-cell communication, and adoption of lower plant systems for bioengineering.
Programme Coordinators

Colette Matthewman is the Norwich-based Project Manager for OpenPlant. With a research background in the plant sciences, she has a broad overview of OpenPlant research activities, and coordinates events, training, and outreach to build new synergies and increase the impact of the centre. She is a member of an OpenPlant working group exploring new IP solutions for biotechnology and is leading a project to develop resources for school pupils to learn about synthetic biology.

Jenny Molloy is the Cambridge-based Coordinator for OpenPlant and the University of Cambridge Synthetic Biology Strategic Initiative. Jenny is a molecular biologist by training and researched genetic control of mosquito populations while becoming increasingly interested in the role and impacts of open source in science. She enjoys being an enabler of open approaches and her role involves coordination of events and activities including the IP working group and OpenPlant Fund, through which the centre is developing new legal tools for sharing and a wide variety of innovative open technologies.

Principle Investigators

Jim Ajioka's lab works on large scale DNA assembly of synthetic circuits in Gram positive bacteria and protozoan biology. He leads a Wellcome Trust programme to build and employ novel biosensors, using Synthetic Biology techniques. Jim's lab is also funded by the EPSRC for foundational work such as generalised codon optimisation, robust switches and counters and big DNA manipulation. The lab's work on big DNA extends to the collaboration with the Haseloff lab on plant plastids.

Pietro Cicuta works on the interface of Physical and Life Sciences addressing various questions: (a) self-assembly and physical properties of lipid bilayers; (b) mechanisms of synchronisation of motile cilia, particularly the human airways; (c) physical mechanisms of regulating gene expression in bacteria. There is a strong synergy across this research, and indeed more widely between the disciplines of soft matter physics and biological physics, given by the experimental techniques and theoretical concepts. We develop optical tweezers, microrheology, advanced confocal microscopy and image analysis to address dynamics in cells.
Sarah O’Connor uses transcriptomic and genomic data to elucidate the alkaloid pathways of Madagascar Periwinkle, a medicinal plant that produces compounds that are used to treat a variety of cancers and other diseases. All plants synthesize thousands of complicated molecules that they use to protect themselves from predators, attract pollinators and communicate with other plants. Thousands of years ago, humans realized that many of these plant-derived molecules also have a powerful impact on human health and well-being. Advances in genomic and transcriptomic sequencing have rapidly advanced our understanding of the complex metabolic pathways that produce these high-value chemicals.

Paul Dupree studies plant cell wall polysaccharide synthesis, structure and function. These carbohydrates have important functions in the human diet, agriculture, bioenergy, paper and packaging and for building construction using timber. He has developed a range of innovative techniques for quantitative analysis of polysaccharides, such as PACE for studies of polysaccharide structures and enzyme activities, and DASH capillary electrophoresis of oligosaccharides using DNA sequencers. Having discovered a number of the enzymes that synthesise cell walls, he is now engineering plants to produce novel polysaccharide structures. This approach will generate plants with modified cell walls for improved material properties, and will enable production of high value plant products.

Rob Field has 30 years’ experience in glycobiology and associated (bio)chemistry. His interests lie in understanding and exploiting carbohydrate recognition, in the design of enzyme inhibitors as probes plant and microbial metabolism, and for the development of lectin-binding anti-adhesive agents to impact on cell adhesion by microbial pathogens (trypanosomes, Campylobacter, flu virus). These activities are underpinned by synergistic synthetic chemistry and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Lisa Hall’s main theme of research is heterogeneous analytical systems, with a primary but not exclusive focus on molecular sensors, the latter including both chemical and biological systems. The activities are concerned with interfacing these systems and/or principles of mechanism and action, with transduction technologies to achieve diagnostic devices and monitoring capability. This research is directed towards environmental, medical and industrial application, with the group pro-active in responding to and advising industry of existing capability and future direction.

Julian Hibberd’s research aims to understand how C4 photosynthesis operates and to provide insight into the molecular mechanisms driving its evolution. The group uses a mixture of wet-lab, computational and synthetic approaches to answer these questions. His work includes the demonstration that C3 plants possess characteristics of C4 photosynthesis, the identification of cis-elements that underpin the expression of multiple C4 genes in evolutionarily independent C4 lineages, and technologies to allow specific cell types to be marked and isolated in leaves of C3 species.
Christopher Howe has long experience with the biochemistry and molecular biology of photosynthetic bacteria and chloroplasts, with a particular emphasis on electron transfer reactions. His lab has pioneered the development of ‘biophotovoltaic’ technology – the direct production of electricity from photosynthetic microorganisms – which underpins his contribution to OpenPlant. He has also made influential contributions to our understanding of the evolution of chloroplast genomes in organisms ranging from plants to protists. He is a scientific advisor to two local companies working in microbial biotechnology.

James Locke examines gene regulation at the single cell level in order to gain a quantitative understanding of gene circuits. Single cell analysis has revealed that gene regulation can be surprisingly dynamic and heterogeneous. In James’ lab, they use single cell time-lapse microscopy, mathematical modelling and synthetic biology approaches to understand the design principles of dynamic gene regulation. They do this in simpler model systems such as cyanobacteria and B. subtilis, as well as in Arabidopsis.

George Lomonossoff is a project leader in the Department of Biological Chemistry JIC and is currently the president of the International Society for Plant Molecular Farming. He has worked on plant viruses and plant virus-derived expression systems for more than 30 years and developed the CPMV HyperTransTM system for which he was named BBSRC Innovator of the Year in 2012. This expression system is currently used by many academic institutions around the world. It has also been licensed to a number of commercial organisations, including Medicago Inc. (QC, Canada) who have used it to produce candidate influenza virus vaccines.

Cathie Martin uses genetics, biochemistry and molecular biology to investigate the basis of cellular specialisation in plants. This includes many aspects of metabolic specialisation, particularly phenylpropanoid metabolism and its regulation. She has used this to effectively engineer the production of polyphenol bioactives in crops, demonstrating health-promoting properties in preclinical studies. Her expertise on transcriptional regulators of metabolic pathways has been applied in a wide range of plant species, establishing effective plant production systems of natural products including natural colours and bioactives from Chinese medicinal plants.

Giles Oldroyd is a leading investigator in plant-symbiotic interactions, with a particular focus on the signalling processes that allow the establishment of nitrogen-fixing and arbuscular mycorrhizal associations. His work has provided the genetic underpinnings to understand the symbiosis signalling pathway that allows rhizobial recognition in legumes and mycorrhizal associations in most plants. He leads an international programme funded by the Bill and Melinda Gates Foundation and the BBSRC that is attempting to engineer cereal recognition of rhizobial bacteria as the first step towards engineering nitrogen-fixing cereals.
Nicola Patron is a Group Leader in Synthetic Biology at the Earlham Institute. Her work aims to develop technologies to engineer photosynthetic organisms for the biosynthesis of materials and therapeutics and to improve plants for increased production and nutritive value. Her broader interests are in understanding the function of DNA sequences and the mechanisms and consequences of gene transfer. As a SynBio LEAP fellow Nicola was recognized as an emerging leader in synthetic biology with a desire to ensure that synthetic biology has positive social impact; she is interested in the complex questions of ownership and intellectual property that surround genetic sequences and biomolecules.

Sebastian Schornack studies processes enabling the colonisation and discrimination of filamentous pathogenic and beneficial microbes with plants. He uses genetics, cell biology, biochemistry and genomics to identify such processes in monocot, dicot and early land plants through mutants, association genetics or the identification of interaction partners of microbial effector proteins. He is credited with the discovery of DNA base-specific TAL effector repeat-binding in promoter elements of target host genes. This led to generation of customised TAL based transcription modulators and nucleases with unrivalled DNA binding specificity that are now being widely exploited in animals and plants.

Alison Smith (JIC) studies starch and sucrose metabolism. Her recent work is on starch degradation in Arabidopsis leaves at night, the control of flux through this pathway and its relationship to carbon availability and growth. Her lab also studies pathways of starch metabolism in crops, including potato and wheat. A major current interest is the relationship between starch synthesis and grain yield in wheat.

Alison G Smith (CAM) focuses on metabolism of plants and algae, particularly biosynthetic pathways for high value products such as vitamins, pigments and lipids. She has been developing tools for improved genetic manipulation of microalgae, in particular by generating regulatory genetic circuits using vitamin-responsive promoters and riboswitches. By taking a synthetic biology approach to generate standard parts and workflows for optimal transgene expression, the aim is to establish microalgae as suitable platforms for industrial biotechnology production. In addition, she has established the Algal Innovation Centre in Cambridge that allows scale up of algal cultivation.

Alex Webb’s lab is investigating how plants measure time by studying the circadian clock. They identify how the circadian clock provides benefits to plants to maximize their growth and productivity. As part of these studies they discovered that the regulation of photosynthesis, carbon metabolism and growth are regulated by the circadian clock. They use molecular genetic, transcriptomic, imaging and physiological techniques to understand circadian mechanisms. They also develop new engineering approaches for systems biology in collaboration with Engineers. They are collaborating with Bayer to convert our biological discoveries in to real world solutions for crop improvement.
OpenPlant
Postdoctoral Researchers
The iridoids are a family of monoterpenes found in a wide range of plant species. Iridoid compounds have been shown to have bioactive properties such as anticancer and antimicrobial activities. Iridoid biosynthesis also contributes one half of the monoterpane indole alkaloid scaffold, a large diverse family of compounds which include the anticancer agents vincristine and vinblastine, and widely recognised compounds such as quinine and strychnine. I am currently focussed on two aspects of iridoid biosynthesis: biochemical characterisation and enzyme discovery. I am attempting to understand the full biochemical details of key early steps in iridoid biosynthesis, including substrate channelling and protein-protein interactions. Progress in this area will impact on the metabolic engineering of microbes or plants for iridoid/alkaloid production—yields of valuable products can be increased through a full understanding of the natural plant systems. The second area of focus is enzyme discovery. By obtaining enzymes responsible for the chemical diversity of the iridoids and alkaloids, we will be able to produce a wide variety of compounds in metabotically engineered host organisms. I am looking for novel iridoid biosynthesis enzymes in the mint family (Lamiaceae) and am currently on the hunt for the enzyme involved in nepetalactone biosynthesis, the key bioactive ingredient in catnip and catmint (Nepeta sp).

I am currently undertaking training in molecular evolution and phylogenetics with the aim of taking the principles of evolution into synthetic biology. I hope that this will reveal new methods of optimising and editing synthetic biology systems and devices. I am also involved in outreach in schools, where I talk to students about scientific research including the exciting developments in synthetic biology.

**Figure 1.** Key early steps include hydroxylation of geraniol, then double oxidation of 8-hydroxygeraniol by an alcohol dehydrogenase (ADH). The key cyclisation reaction to nepetalactol is catalysed by iridoid synthase (ISY). Iridoids are commonly combined with other pathways: with sugars to form glycosides and with tryptamine to form monoterpane indole alkaloids.
I am jointly hosted by the labs of Lisa Hall (Chemical Engineering and Biotechnology) and Jim Haseloff (Plant Science) as an interdisciplinary fellow part funded through OpenPlant. My background training is as a physicist, with a specific emphasis on optics and microfabrication. I undertook a PhD in Macquarie University (Sydney, Australia) where I developed microphotonic circuits using a 3D laser printing technique. My postdoctoral research continued in Toshiba's Cambridge Research Labs where I worked on advanced manufacturing techniques for semiconductor quantum dots.

As a part of OpenPlant I am passionate about using optical analytical tools to study the production of secondary metabolites in specialised plant tissues. Specifically, the oil bodies of the Liverwort, Marchantia polymorpha, are potentially rich reservoirs of bio-active compounds. Using Raman microscopy, a label-free, non-destructive spectroscopy technique it is possible to study metabolic processes in real-time. As this is non-destructive it can be performed in situ and therefore both spatial and temporal information can be obtained. My hope is to correlate this data with information available using other approaches such as Matrix Assisted Laser Deposition Ionisation Mass Spectroscopy (MALDI), Gas Chromatography Mass Spectrometry (GC-MS), fluorescence microscopy and other high resolution analytical approaches. In future this could be then adapted to studies of transgenic plant species as an additional tool to study metabolic pathways. Additional model species can also be explored, for instance Nicotiana benthamiana, and potentially crop plants. I am keen to engage with teams operating in the area of natural product chemistry, metabolic engineering or teams focused on alternative analytical approaches.

During the week of 21-25 June the UK’s first Bio-Hackathon was hosted in the Department of Plant Science (Cambridge). Thanks, in part, to a grant provided by the University of Cambridge Synthetic Biology Strategic Research Initiative. This event brought together a diverse interdisciplinary group of 50 participants from across the UK and the world. Teams focused on “bioware” by incorporating hardware, software and wet lab tools. One team developed a 3D printed microfluidic prototyping tool, another built a comparison software tool for DNA synthesis pricing.

The winner team built a tool called “Alpha-Brick” which is a drag and drop tool for assembling bio-bricks and plugs directly into Transcriptic (a cloud laboratory) allowing immediate order of an assembled part.

Photo: Prototype microfluidic rapid 3D printed circuit fabricated during the Bio-Hackathon.
Plants produce a wide variety of specialised metabolites. These molecules play key roles in the interaction of plants with their biotic and abiotic environment. In addition to their ecological functions, plant-derived specialised metabolites are major sources of pharmaceuticals and other high-value compounds.

Recently, it was discovered that the genes for the biosynthesis of several major classes of these compounds are physically co-localised in so called ‘gene clusters’ in plant genomes. Such clustering of non-homologous genes contrasts the expected arrangement of genes in eukaryotic genomes. The co-localisation of functionally-related genes enables the formation of fundamentally different mechanisms of gene regulation in comparison to the control of dispersed genes. The purpose of this project is to improve our understanding of the transcriptional control of plant metabolic gene clusters. The focus within OpenPlant will be on chromatin related regulatory processes that govern the expression of gene clusters. By chromatin immunoprecipitation, chromosome conformation analyses and genome engineering we aim to characterise the chromatin environment at gene clusters and its impact on cluster regulation. The findings of this project will open up new opportunities for the discovery and engineering of metabolic pathways using genetic and chemical approaches. They will also underpin synthetic biology-based approaches aimed at refactoring of plant metabolic gene clusters and the development of synthetic traits.

Figure 1: A metabolic gene cluster and the different steps in a specialised metabolic pathway are shown. Nützmann & Osbourn. Current Opinion in Biotechnology 2014, 26:91–99.
My project involves optimization of CRISPR/Cas9 methodology of genome editing in plants. CRISPR/Cas9 is a method of choice to perform genome engineering. There are however significant limitations which prevent broader implementation of this technology in plants.

These limitations include variable efficiency of editing at different targets, off target activity, inefficient inheritance of the created mutations, ability to edit simultaneously several targets, limited selection of targets/PAM repertoire and the need to segregate Cas9 and sgRNA from the created mutations. Numerous configurations of CRISPR/Cas9 designed to address these limitations had been published. Our aim is to establish a uniform testbed and toolkit, where many of these configurations are tested under the same conditions and their editing efficiency and off target activity will be assessed. In order to minimize variability in transgenic expression we established editing essay in plant protoplasts.

Our experimental design includes transforming protoplasts from the same harvest with different configurations of CRISPR/Cas9, including Cas9 variants which specifically edit NGG, NGAG, NCGG and NNGGGT PAMs, Cpf1s which recognise TTTN PAM, and SpCas9 variants with reduced off target activity, and assessing frequency of indels and double stranded breaks activity employing DNA capture assays and Next Generation Sequencing. Currently we gained experience in efficient extraction and transformation of the protoplasts from different plant species using our CRISPR/Cas9 constructs and we are establishing high throughput protoplast transformation methodology using automatic dispenser. In the next step we will attempt to regenerate plants from the edited protoplasts. We also trying to find the ways to perform successful CRISPR/Cas9 assisted targeted repair of gene of interest. We follow the two-step strategy: transforming the plants with “landing pad” with subsequent insertion of the repair template. Successful insertion of the repair template should restore the herbicide resistance and facilitate selection of the plants with successful repair.

I participate in the proposal for Open Plant funding titled “Establishing Low Cost Microfluidic System for Single Cell Analysis” (Dr. Steven Burgess is a principal applicant). The aim of the project is to establish cost-effective microfluidic device for single cell sorting and analysis. Significant reduction of the cost comparatively to the commercially available systems is achieved by producing some of the parts of the device such as microscope and syringe part with 3D printing technology and utilizing open source materials and repositories. Among various applications for this device will be sorting the transformed protoplasts according to the cell size and strength of the fluorescence of the transgene, and cost-effective miniaturizing and automatizing Golden Gate cloning assembly reactions.
Ivan Reyna-Llorens

**Group Leader** - Prof. Julian M. Hibberd
**Department** - Department of Plant Sciences, University of Cambridge

My research involves using synthetic biology and evolution for improving agricultural traits, more specifically to improve photosynthesis. As the world population continues to expand, it is predicted that crop yields will have to increase by 50% over the next 35 years. Traditional breeding programs cannot keep pace with this current population growth rate. Plant biomass is produced by carbon dioxide (CO2) fixed by the enzyme Rubisco during photosynthesis.

This process known as C3 photosynthesis can be very inefficient as Rubisco also interacts with Oxygen (O2) in a wasteful process known as photorespiration. In order to increase yields, photorespiration should be reduced considerably. Fortunately, some plants have evolved such mechanism already. C4 photosynthesis results from a series of anatomical and biochemical modifications in the leaf that lead to photosynthesis being compartmentalized between mesophyll and bundle sheath cells. This division of labour generates a CO2 enriched environment where photorespiration is effectively abolished. C4 plants therefore produce more yield and use water and nitrogen more efficiently. The fact that C4 photosynthesis has evolved independently in more than 60 lineages allows us to think it is possible to engineer C4 photosynthesis in C3 plants. In order to engineer this trait, cell specific genetic circuits need to be developed. Unfortunately there is a limited number of genetic parts driving cell specificity in leaves. My main objective in OpenPlant is to generate a library of leaf specific motifs that can be used to drive the expression of both nuclear and plastid encoded genes in specific compartments and specific cells of leaves.

Together with colleagues in the Department of Plant Sciences, Department of Chemistry and the Department of Physics I am part of an OpenPlant fund project that aims to use microfluidics for high-throughput analysis of genetic parts. We hope to generate a whole toolbox of parts that are useful to rewire different traits.

Photo: Tobacco protoplasts under fluorescence microscope. Protoplasts in green are cells expressing GFP while red cells are showing the auto-fluorescence caused by chlorophyll.
Michael Stephenson

**Group Leader** – Anne Osbourn  
**Department** – Metabolic Biology, John Innes Centre

I am a chemist, with a background in natural product total synthesis, medicinal chemistry and pharmacy. In the Osbourn group we are interested in plant secondary metabolites and this places us at the very interface between biology and chemistry. I bring skills in small molecule extraction, purification and structural characterisation, which strengthens the group’s ability to functionally characterise biosynthetic enzymes through transient expression in plant and yeast platforms.

This aids the exploration of potential gene clustering in the evolution of plant biosynthetic pathways, and the use of these enzymes towards a synthetic biology approach to the preparative production of high value chemicals. I am also personally interested in utilising this growing tool kit of enzymes to introduce useful handles for further modification of plant secondary metabolites by synthetic chemistry, with a view to screen for medicinal relevant biological activity. I also have a keen interest in public outreach. I have been involved in supervising work experience placements and school visits, and was part of a team representing OpenPlant at this year’s Latitude festival in Suffolk.

**Bottom left:** Vacuum infiltration process designed to allow efficient scaling up of transient expression experiments facilitating the preparative extraction of products. **Top right:** shows fluorescence imaging of the leafs of a plant infiltrated with an A. tumefaciens strain containing a GFP expression construct, giving a qualitative indication of infiltration coverage using this procedure. **Bottom right:** β-amyrin. Taken by Andrew Davis (JIC Bioimaging Department).
Plants can be used as a production platform for high-value products such as vaccines, enzymes and metabolites, thereby providing a potentially fast and cost-effective alternative to other cell culture techniques. Developed within the Lomonossoff group, HyperTrans (HT) is a technology for rapid, high-level transient expression of proteins in plants. One key application of HT in the Lomonossoff group has been the production of virus-like particles for use as vaccines, scaffolds for nanotechnology and in fundamental research of virus assembly.

Virus-like particles (VLPs) consist of viral structural proteins which assemble into a particle resembling the virus but devoid of the viral genome and therefore unable to replicate. Different VLPs consisting of multiple copies of one, two or four different structural proteins have been successfully produced using the HT system and shown to be morphologically and immunologically representative of the virus. In recent years, a number of emerging diseases have been caused by enveloped viruses such as Zika virus and Chikungunya virus. Such complex virus structures can make the development of efficient vaccines and diagnostic reagents difficult and costly. In my OpenPlant project, we are working on developing strategies for the production of enveloped VLPs in plants. I am also working on modifying a large non-enveloped VLP to allow accommodation of cargo proteins on the inside of the particle.

In addition to my research project, I have been involved in the planning stages for a new translational facility (Leaf Systems International) which will enable scale-up of plant-based production of proteins and metabolites at the Norwich Research Park.

I have also participated in various outreach activities, such as a TV interview for regional news, the Great British Bioscience Festival, JIC’s Speed Science event as well as a work experience day for school children, amongst others.

Photo: High level expression of green fluorescent protein using HT technology. By Andrew Davies (JIC Bioimaging Department).
I am applying the genome editing tools to generate novel, commercially or nutritionally valuable glucans in model crop species. The primary objective of my OpenPlant project is to generate potatoes that contain digestion-resistant starches with two major nutritional benefits: reduced calorie intake from consumption of chips, crisps and other potato-based foods and increased supply of complex carbohydrates to the microbiota of the lower gut that reduces risk of several diseases including colorectal cancer and type II diabetes.

More specifically, the project involves knocking out the gene(s) of starch branching enzymes I and/or II using crispr-CAS9 method thereby increasing the ratio of amylose to amylopectin (linear to branched starch chains) in tubers without significantly compromising the starch yield. The engineered starch will be less accessible to starch degrading enzymes, thus more resistant to digestion.

**Photo above:** Transgenic potato plant grown in tissue culture (A) and glass house (B)

**Photo below:** DNA sequencing chromatogram of potato starch branching enzyme I gene obtained from mutant line. Editing takes place at nucleotide 154 (indicated by black arrow) where mutant form of the gene (minor peaks) misses the corresponding nucleotide “T” resulting in premature stop codon.
I’ve been involved in Synthetic Biology for better part of the last decade. My PhD work at Newcastle University focused on facilitating bio-electronic interface via engineered pathways as part of a larger collaborative grant to create a bio-robotic hybrid device. My more recent work at the University of Cambridge was on developing a field-use whole-cell Arsenic Biosensor for deployment in South Asia (www.arsenicbiosensor.org).

I worked with Jim Ajioka and Jonathan Openshaw on a science/arts collaborative project that came to be known as Syn City. The idea was to create dynamic, living sculptures using modified E. coli such that all the “paint” was living. Jonathan designed 3D printed structures of which we made moulds to cast Agar with an integrated 3D printed mesh skeleton. The modified bacteria could then be deposited on the structure, which developed colour over time. www.syncity.co.uk.

I’m relatively new at working with plants and the opportunity to reengineer the Marchantia polymorpha plastid as part of the Open Plant initiative is a great point of transition into this sphere. The main focus of my contribution to Open Plant is to reconstruct the entire 121kb plastid genome in a way that makes it easier to manipulate, facilitating future work on plastid transformation in M. polymorpha and, in time, other plants. I am also working together with Haydn King from the Ajioka Lab on creating a codon optimised reporter toolkit for use in the M. polymorpha plastid, consisting of a 13 fluorescent reporters across a wide spectrum ranging from near UV to near infrared. The codon optimisation platform should also become a useful tool for future work on plastid manipulation, in Marchantia and beyond.
OpenPlant Research Technician

Group Leader - Jim Haseloff
Department – Department of Plant Sciences, University of Cambridge

Research Technician for the Haseloff group since March 2016, I work closely with Susana Sauret-Gueto, Research Lab Manager, to ensure the smooth running of the lab and I am responsible for Marchantia polymorpha tissue culture.

I am working on the standardisation of existing protocols for the propagation, transformation and short and long term storage solutions, including cryopreservation.

This work will enable and facilitate the high-throughput screenings of Marchantia lines, such as the Enhancer Trap lines; a project on which several lab members collaborate. A summer student has joined us for a period of 8 weeks to work on this project and I am helping with her supervision and to provide laboratory training.

Susana Sauret-Gueto

OpenPlant Research Manager

Group Leader - Jim Haseloff
Department – Department of Plant Sciences, University of Cambridge

I joined the Haseloff Lab last October as OpenPlant Research Manager. My work revolves around the OpenPlant goal of developing foundation- al technologies for engineering the model plant Marchantia polymorpha. To effectively combine biology and engineering, Synthetic Biology needs appropriate technological platforms together with changes in the way the scientific community engages. My work is related to both topics, and I am thrilled about all the challenges ahead!
To start with, I am establishing modular pipelines that can scale from small projects to high-throughput experiments. For example, we are developing automation platforms for protocols like DNA assembly using the recently acquired Echo550 acoustic liquid handler. Alongside, we are standardising protocols in relation to Marchantia propagation, transformation and storage. For the screening and characterisation of the generated Marchantia lines, I am also developing imaging pipelines with a view to quantitatively characterise DNA parts and genetic circuits.

We are establishing an imaging hub at Plant Sciences, including a new Keyence digital microscope for real-time 3D reconstruction of Marchantia plants. We are very well equipped with a series of new fluorescent microscopes with different resolution capabilities, for example a new Leica stereo microscope with fluorescence as well as a new top of the range Leica SP8 confocal microscope. I am also coordinating a summer workshop on LithographX, open source and open development software for 2D/3D image processing and visualisation.

While we build upon infrastructures and protocols, we need to develop frameworks to facilitate the sharing and reuse of the knowledge produced between all OpenPlant labs in Cambridge and Norwich. To succeed, this needs to be driven by an open community, and I am very fortunate to have around me a group of researchers who recognise the mutual gains of standardisation and are ready to embrace it at the practical level. I established regular meetings for Researchers related to OpenPlant in Cambridge (ROC), both from CU and SLUC, to discuss on OpenPlant-related issues, as well as, of course, common research interests.

I’d like to emphasise that all this work on lab infrastructures and workflows, standardisation and sharing, is being established along numerous research lines. In my case, I am fascinated by growth and development, and I am bringing my previous scientific background to projects related to mapping cell lineages during Marchantia gemmation development, modeling gemmae growth, and the induction of localised gene expression in space and time to engineer morphogenesis and metabolism. Currently, I am mainly involved in the lab project on Enhancer Trap (ET) lines.

These lines report the activity of endogenous regulatory sequences and each ET line expression pattern can be visualised throughout gemmae development. We are searching for lines that will tag specific cell lineages and reveal developmental transitions, which in addition, can later be used to genetically manipulate particular cell types.

I also participate in an OpenPlant Fund project on single RNA molecule imaging (Susan Duncan from John Innes Centre is the principal applicant).
The OpenPlant Fund supports innovative, open and interdisciplinary projects relevant to plant synthetic biology and aims to facilitate exchange between The University of Cambridge and institutes on the Norwich Research Park for the development of open technologies and responsible innovation in the context of synthetic biology.

Projects receive £4000 over six months, with an additional £1000 for follow-on work after reporting on their progress. Successful projects have included biological experiments, DNA part characterisation, protocol development, software, hardware, outreach, education and policy workshops. Within this diversity, all projects share a set of characteristics:

- A tangible outcome that can be readily shared.
- Promoting interdisciplinary working and exchange.
- Relevance to synthetic biology and providing a valuable contribution to the current field.
- Addressing focus areas of the OpenPlant initiative e.g. open technologies and responsible innovation.

In the first two years of OpenPlant, 29 projects were awarded funding, representing collaborations between 90 researchers across all sites, plus external partners.

29 projects
9 Hardware
11 Biology
7 Outreach, Policy and Exchange
3 Software

50% led by postdocs

>26 organisations involved in project teams

Find out more at http://openplant.org/fund/
The Big Algal Open Experiment
Dr Paolo Bombelli (Biochemistry, University of Cambridge), Dr Brenda Parker (Biochemical Engineering, UCL), Dr James Lawrence (Biochemical Engineering, UCL), Marc Jones (PhD student in Computational and Systems Biology, John Innes Centre)

Algae are amazing: they recycle over half of the carbon dioxide we exhale, and form the basis of many food chains, yet we still understand very little about how they grow. In future, we may wish to cultivate algae for food, fuel, or to clean up wastewater so we need to understand more about their biology!

With this in mind, we have set up the Big Open Algae Experiment to help us enhance our knowledge by performing the biggest parallel algae experiment in history. We are inviting universities and citizen scientists to participate in an open-source data collection experiment on outdoor microalgal growth. Up and down the UK, we’ll be running experiments using a bioreactor we have designed and asking people to submit their recordings of how well the algae are growing. Following and recording the algal growth will be easy and fun. This thank to a smart-phone app: the Alg-app. The Alg-app will enable everyone having access to a smartphone to get involved. During the OpenPlant Fund project, bioreactors, the website and app were constructed (http://bigalgae.com/about) and we plan to run the experiment with schools and universities later in 2016.

Responsible Innovation and Open innovation with Large BioResources: Goals, Challenges and Proposals
Dr Kathy Liddell (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), Dr John Liddicoat (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), Dr Rob Doubleday (Centre for Science and Policy), Dr Nicola Patron (Earlham Institute).

On 28 January 2016, the Centre for Law, Medicine and Life Sciences together with the Centre for Science and Policy, and OpenPlant hosted a workshop on responsible and open innovation with large bio-resources. The central question the workshop tackled was: whether, and to what extent, policies of openness are appropriate for successful innovation with bio-resources in synthetic biology and genomics. Closely related to this question was: how does one implement openness effectively in bio-resources intellectual property policies? Discussions were stimulating and highlighted the different approaches taken by the two fields.

Strengthening synthetic biology capacity in Kenya through bioinformatics training
Richard Smith-Unna (CU), Dr Vicky Schneider (Earlham Institute), Dr Jelena Aleksic (TReND), Richard Pilling (Intel)

From 30th November to 5th December 2015, 37 students from nine African countries attended our course, held at ICIPE in Nairobi, Kenya. The course involved six days of theory and practical work, starting from the principles of Unix and programming, through to advanced scientific programming and visualisation. Towards the end of the week students worked on specific analysis methods in various areas of genetics and genomics, with a special focus session on synthetic biology. An ongoing student-led study group, coordinated online, will help the students keep the momentum from the course going. The course is booked to repeat next year.

Setting up an open synthetic biology lab in Abuja, Nigeria
Richard Smith-Unna (CU), Dr Chinyere Okoro (Sanger Institute), Dr Ibukun Akinrinade (University of Bingham, Nigeria), Dr Jelena Aleksic (TReND), Dr Vicky Schneider (Earlham Institute)

As part of developing a synthetic biology lab in Bingham University, Abuja, We have been able to successfully collect equipment donations sufficient to set up a running molecular biology lab in Bingham University, Nigeria. These facilities were acquired from kind donations from Institutes in Switzerland and the UK. The shipment to Nigeria is underway. In addition, a laboratory space has been allocated in the University to set up the equipment. Preparations for the workshop is now in top gear as logistics are being arranged and course materials are being prepared. The anticipated date for the workshop is August, 2016.
Co-lab OpenPlant - interdisciplinary workshops of science art and design (ongoing)

Dr Paolo Bombelli (Department of Biochemistry, University of Cambridge), Dr Paloma Portela Torres (UCL), Lena Asai (Goldsmiths, London), Juan Manuel Garcia Arcos (CRI, Paris), Ke Fang (CRI, Paris)

Co-lab OpenPlant is a series of three workshops and a hackathon event with the objectives of creating new ideas around plant synthetic biology applications and fostering further collaboration by establishing links between designers, artists and scientists. Our workshop is a place where artists, designers, and scientists meet to initiate collaboration. We aggregate designers to learn science. We encourage scientists to value and learn artistic approach and design thinking. We will run three workshops in Norwich and Cambridge followed by a final making workshop in Cambridge to realise the ideas that arise.

Synthetic Biology for Schools: A multidisciplinary approach (ongoing)

Dr Jenni Rant (The SAW Trust), Dr Tim Rudge (Universidad Catolica, Chile), Tim Marzullo (Backyard Brains, Inc), Juan Keymer (Universidad Catolica, Chile), Nadia Radosz (John Innes Centre), Dr Colette Matthewman (John Innes Centre), Samantha Fox (John Innes Centre), Lawrence Pearce (John Innes Centre), Dr Nicola Patron (Earlham Institute), Dr Fernán Federici (University of Cambridge/Universidad Catolica, Chile), Lalitha Sundaram (Department of Pathology, University of Cambridge), Dr Steven Burgess (Department of Plant Sciences, University of Cambridge), Dr Ben Miller (School of Biological Sciences, University of East Anglia)

The synthetic biology community in Norwich and Cambridge are working on several ideas for developing educational materials, tools and practicals to bring multidisciplinary science and synthetic biology into schools. To increase their overall impact, we propose to create a complete package of activities, supporting information and hardware that can be successfully used in schools to introduce synthetic biology with a focus on plant chassis, and to provide learning opportunities across a wide range of disciplines. Our intention within the scope of this project is to target the resources for local schools, but subsequently we can look for national and international opportunities for dissemination.

Workshop on Genetic resources in the age of the Nagoya Protocol and gene/ genome synthesis (ongoing)

Professor Jim Haseloff (University of Cambridge), Dr Dominic Berry (University of Edinburgh), Dr Deborah Scott (University of Edinburgh)

The ongoing improvement of gene and whole genome sequencing and synthesis technologies presents possibilities of new practices, and demands discussion and debate in light of the long history of global bioresource management. Our workshop in November 2016 will The proposed workshop acts as a venue for collecting information on current developments, sharing views, highlighting potential areas of concern, and establishing grounds upon which to build better understanding of the interactions between and implications of the Nagoya Protocol and gene synthesis for collection, circulation, and use of genetic resources.

Hardware

Plant electrophysiology was popular with OpenPlant Fund teams, with venus fly traps being the most obvious choice for early testing of prototypes.
plants. We have developed a tool prototype for measuring plant electrical signal coupled with radio modules for long-distance data collection. This prototype (estimated cost £40) can sense and transmit signals from Venus flytrap responding to tactile inputs (see this video for demonstration). The tool can distinguish the action potential from other disturbances.

**Whiskeroscope: rodent whisker inspired sensor for use in analysis of plant tissue structure**

Jan Lyczakowski (Department of Biochemistry, University of Cambridge), Abhimanyu Singh (Independent, previously Department of Engineering, University of Cambridge), Christie Nel (Independent, previously Stellenbosch University)

Understanding mechanical properties of plant biomass is crucial for multiple industries, e.g. building construction and production of lignocellulosic biofuels. Current methods to analyse mechanical properties of biomass are slow and provide little accuracy. We have developed a novel sensor to evaluate stiffness of plant stems. The device is inspired by rodent whiskers and relies on two inputs, obtained using thin steel rod, to quantify stiffness. The instrument successfully discriminated between materials with unlike mechanical properties (steel and foam) and differently aged stem samples from willow. Whiskeroscope was also applied to study *Arabidopsis thaliana* stems with altered composition of cell walls.

**Open Labware for plant electrophysiology**

Dr Carlos A. Lugo (EBI, previously The Sainsbury Laboratory), Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), Christian R. Boehm (Department of Plant Sciences, University of Cambridge), Guru Vignesh Radhakrishnan (John Innes Centre), Dr Marielle Vigouroux (John Innes Centre)

In order to investigate electrical responses to mechanical and other external stimuli, our project consisted of replicating an open source Arduino shield which receives, amplifies and transmits “ECG”s from plant tissues into a computer or other circuits. We harnessed the electrical signals to trigger responses in a) other plants, b) other circuits. The resultant board’s schematics and other experimental tools such as manipulators and signal transducers are published on a dedicated project page including files for producing boards and 3D printed parts. A number of kits are available to give away to schools and labs interested in the system.

**Building a low-cost desktop plant experiment box (ongoing)**

Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), Dr Marielle Vigouroux (John Innes Centre), Dr Carlos Lugo (EBI)

Doing experiments in plant biology is a difficult task. Experimental conditions are difficult to control and often the impact of even slight variations of environmental conditions is difficult to predict. Commercial solutions to control the environment are available but quite expensive and normally are optimised for plant growth but not for running experiments. We want to build small independent “experimental boxes” which are optimised for in-vivo recording of single plant/single plate growth under different environmental conditions and subject to different stimuli. The boxes will be small in size (around 50x50x60 cm), cheap (estimate material cost <£1000 each) and flexible in features thanks to a modular design. The boxes will be under PC control and allow multiple experiment to run in parallel and in sync.

**Environmental sensor networks based on plant electrical signalling (ongoing)**

Sakonwan Kuhaudomlarp (John Innes Centre), Dr Pakpoom Subsoontorn (Department of Plant Sciences, University of Cambridge), Dr Kyle Lopin (Naresuan University, Thailand), Dr Settha Tangkawanit (Naresuan University, Thailand)

Tools for sensing and recording plant electrical signals could open up promising applications in agriculture and environmental engineering. Nonetheless, existing setups for monitoring plant electrophysiology often require the uses of cumbersome, expensive and specialised equipment and one would prefer to have a network of low-cost measurement tools that can function robustly in the field, capture overall electrical activities of multiple plants. Previously, our team have prototyped a plant electrical signal amplifier coupled with a radio module. Here we plan to improve upon our first prototypes, specifically, to expand detection bandwidth, to increase sampling rates, further reduce the cost and test device performance in wider range of plant species.

Jan Lyczakowski (Department of Biochemistry, University of Cambridge) displaying the Whiskeroscope prototype and early results at Cambridge Science Festival.
Plant electro-mechanics: improving low-cost plant electrophysiology for research and education (ongoing)
Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), Dr Marielle Vigouroux (John Innes Centre), Dr Carlos Lugo (EBI), Guru Vignesh Radhakrishnan, (John Innes Centre)

We prototyped a very low-cost plant electro-physiology sensor and would like to continue with further development of monitoring and data gathering capabilities of the shields, image analysis, signal long time monitoring. We will also couple the manipulators with a motor system web-application which can be used from desktop or mobile devices. All outputs will be fully open source.

Establishing 3D Printed Microfluidics for Molecular Biology Workflows (ongoing)
Steven Burgess (Department of Plant Sciences, University of Cambridge), Tom Meany (Department of Plant Sciences, University of Cambridge), Richard Bowman (Department of Physics, University of Cambridge), Oleg Raitskin (Earlham Institute), Neil Pearson (Earlham Institute)

With synthesis of DNA becoming cheaper, and plasmid construction automated, the testing of biological parts is becoming a bottleneck in the design-build-test cycle. Analysis of single cells offers a procedure for rapid screening of parts and this has been facilitated by advances in microfluidics. The downside of these approaches is that they tend to rely on expensive, specialist equipment, meaning they are out of reach to most molecular biology laboratories. However, developments in 3D printing, coupled to open-source design repositories, offer the potential to address this issue. By utilising expertise in Cambridge and the NBI (Norwich Biosciences Institutes), the aim of this proposal is to integrate available open-source or low-cost commercially available components, to produce a cheap, modular microfluidic setup for single cell analysis.

Universal precise large area colony scanning stage with measurement and selection tool integration (ongoing)
Tobias Wenzel (Department of Physics, University of Cambridge), Luka Mustafa (Institute IRNAS Race), Ji Zhou (Earlham Institute), Nick Pullen (John Innes Centre), Neil Pearson, (Earlham Institute)

Plant or microbial cultivation and monitoring can be a time consuming and tedious process. We propose an open-source platform for automatic (flat – e.g. Marchantia) plant and colony scanning, which extends plate-reading functionalilty to morphological and long-term analysis and will also be more flexible, considering growth plate size. This is an extension of the OpenScope initiative from the Cambridge 2015 iGEM team and will combine video and optical microscopy techniques with CNC technology. Outputs will include a more precise and more affordable CNC translation stage, technical interfaces that allow easy integration of open source measurement and preparation tools and new open source tool that allows to identify and pick or mark colonies and their positions. The set up will be tested on seed germination experiments and the hardware will be replicated in Norwich, Cambridge and Slovenia to lay a foundation for easy feedback and collaboration.

Development of an Open Source Autonomous Imaging Station for Distribution in High Schools, Universities, and Emerging DIY Scientific Communities (ongoing)
Fernán Federici (University of Cambridge/Universidad Catolica, Chile), Neil Pearson (Earlham Institute), Tim Rudge (Department of Engineering, Universidad Catolica, Chile), Tim Marzullo (Backyard Brains, Inc), Juan Keymer, (Universidad Catolica, Chile)

We propose to develop a standalone tool for imaging and analysing fluorescence in biological samples at a range of scales from individual bacteria, through colonies, plant cells and even whole organisms such as C. elegans. The system will be self contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. timelapse), and quantitative analysis of samples. We also propose the development of a simple genetic toolkit for the production of fluorescent and pigmented bacteria complementing the device. The entire system, optics, frame, electronics, genetic resources and software will be open source. This robust and affordable package will enable independent, inexpensive experiments and observation for scientists in emerging scientific cultures in Latin America as well as in schools, colleges and universities.

This project also wishes to highlight the benefits of employing an open framework for academic collaborations that seek to deliver Open Access resources and information. We have formed an industry partnership with the Open Source company Backyard Brains (TM), which has significant experience in creating and distributing open educational and research technology for neuroscience in Latin America and worldwide (backyardbrains.com, backyardbrains.cl).

Biology

Development of novel selection markers for plant transformation to advance live-imaging techniques
Dr Fernán Federici (University of Cambridge/Universidad Catolica, Chile), Dr Katharina Schiesl (John Innes Centre), Leonie Lugjinbuehl (John Innes Centre), Guru Rhdakrishnan (John Innes Centre)

A total of 25 DNA parts were synthesised, including tissue specific promoters and coding sequences of fluorophores and chromophores. Level 1 and level 2 GOLDEN GATE plasmids were generated and transformed into Medicago Hairy roots. Subsequently, selection markers were tested to see if they were detectable under the stereomicroscope and images were taken using confocal microscopy. It was found that the nuclear-envelope localised fluorophore dtomato, expressed under the Lotus UBIQUITIN promoter, was detectable under the stereomicroscope and could therefore provide a novel selection marker for live imaging. Furthermore, it was found that the BEARKSXXXXXXX marker was not detectable in the lateral root cap but expressed at the root surface.
base of the induced hairy root callus. No significant colour change was observed in the roots transformed with the chromoproteins.

**Development of new codon optimisation tools and development of a synthetic gene expression system in the green alga**

*Chlamydomonas reinhardtii*

Francisco Navarro (Department of Plant Sciences, University of Cambridge), Marielle Vigouroux (John Innes Centre)

Most organisms share the same genetic code, based on three nucleotide codons that encode for one amino acid. However, synonymous codons (which specify a single amino acid) are not used at equal frequency by different species. We were interested in assessing the impact of codon usage in protein production in the green alga *Chlamydomonas reinhardtii*. We have performed sequence analysis, and developed a platform for measuring the production of a reporter protein, which can be used for testing gene variants. Our analysis, protocols, and materials will be useful for transgene design and expression in the alga.

**The use of synthetic biology tools to define the roles of LysM receptor-like kinases in legumes and cereals**

Feng Feng (John Innes Centre), Ronelle Roth (Department of Plant Sciences, University of Cambridge)

We have synthesised a number of golden gate modules including gene promoters, coding sequences and terminators and got the final constructs required for this project using Golden Gate cloning technology. Secondly, we have already expressed these constructs in *Nicotiana benthamiana* to check the protein expression, now we are focusing on transforming these constructs in *Medicago* and rice to detect defence and symbiosis phenotype.

**Quick analytical system for plastid genome modifications**

Mario Juhas (Department of Pathology, University of Cambridge)

We set out to provide the synthetic biology community with a quick Pulsed-Field Gel Electrophoresis (PFGE)-based analytical system for plastid genome modifications. The project led to a number of educational resources, including protocols for the sample plugs preparation for PFGE of plastid and BAC DNA and for PFGE analysis of plastid and BAC DNA using CHEF-DRII PFGE system. All protocols will be open and publicly available and the protocol has been published in: Juhas, M. and Ajikoka, J.W., 2016. Integrative bacterial artificial chromosomes for DNA integration into the *Bacillus subtilis* chromosome. Journal of microbiological methods, 125, pp.1-7.

**Channeling targeted DNA double strand breaks into alternative repair pathways**

Dr Ian Henderson, Dr Natasha Yelina, Patrick Diaz (Department of Plant Sciences, University of Cambridge), Dr Sebastian Schornack (The Sainsbury Laboratory, University of Cambridge), MeioGenix (Paris)

We have expressed TAL DNA binding domains fused to the FokI nuclease under meiotic promoters (e.g. DMC1, SPO11) in Arabidopsis. The aim of this work is to target DNA double strand breaks to specific sites in the genome, in order to bias initiation of meiotic recombination. Our preliminary data show that while these nucleases are expressed in meiotic-stage floral buds they do not support wild type levels of crossover recombination when the endogenous nuclease (SPO11-1) is mutated. Additionally these transgenic lines show occurrence of developmental phenotypes, leading us to the hypothesis that the resulting DSBs enter a mutagenic pathway. To investigate this in this project we are performing whole genome DNA sequencing and mutation discovery. This has been performed using support from the OpenPlant project and bioinformatic mutation discovery is ongoing. In parallel we have crossed these nuclease lines to mutants in canonical and alternative end joining pathways to test the hypothesis that we can shunt DSBs into crossover recombination via removing competing repair pathways. These lines will be grown and DNA sequencing repeated, in addition to phenotypic analysis in the next part of this project.

**Engineering Marchantia polymorpha chloroplasts for the production of high-value specialised terpenes**

Aymeric Leveau (John Innes Centre), Tessa Moses (John Innes Centre), Christian R. Boehm (Department of Plant Sciences, University of Cambridge)

Originally, three independent operon-like synthetic constructs should be built to achieve de novo synthesis of mono-, sesqui- and triterpenes in *M. polymorpha* chloroplasts. GoldenGate modules of coding sequences to be expressed in *M. polymorpha* were synthesized. However, two major issues were encountered during the project, including problems with transforming *M. polymorpha* chloroplasts with large constructs, and an assembly defect of the 2A peptide system used for generating the clusters. To circumvent these obstacles, constructs allowing nuclear transformation of *M.*
polymorpha and subsequent chloroplast targeting of the proteins were designed and a new 2A peptide system has been created and is currently being evaluated.

**Hot Tomato: Complementation of the Capsaicin Biosynthetic Pathway to Engineer Spicy Tomatoes**

Greg Reeves (Department of Plant Sciences, University of Cambridge), Chris Boursnell (Department of Plant Sciences, University of Cambridge), Jie Li (John Innes Centre)

This proposal seeks to utilise synthetic biology approaches to overexpress capsaicin pathway enzymes missing from tomatoes but found in chilli peppers, yielding spicy tomatoes. Transient expression in tomato fruit and leaves would be used for fast screening and validation of the key genes mentioned above. The project would utilise the current models for the capsaicin pathway as a blueprint and would provide a clearer picture of capsaicinoid evolution in Solanaceae. This would demonstrate that the path to capsaicin production is relatively straightforward and that other members of Solanaceae may be evolving capsaicin production. This proposed experiment offers a tool to building synthetic pathways in plants through complementation of existing components and furthers understanding the evolution of secondary metabolites in plants.

**Implementation of a synthetic transcriptional AND gate in the chloroplast of Chlamydomonas reinhardtii**

Christian Boehm (Department of Plant Sciences, University of Cambridge), Payam Mehrshahi (Department of Plant Sciences, University of Cambridge), Hannah Laeverenz-Schlogelhofer (Department of Physics, University of Cambridge)

Chloroplasts are among the most attractive substrates for biological engineering and one of the major limitations to realisation of its potential has been a lack of suitable systems for controlling the expression of transgenes from the chloroplast genome. Over the past decade, several conditional expression systems have been developed responding to a single input only. In order to enable more sophisticated control over chloroplast gene expression based on multiple conditions, we propose to develop a synthetic transcriptional AND gate in the chloroplast of *Chlamydomonas reinhardtii*. The nuclear component of the proposed circuit is composed of two chloroplast-targeted halves of split T7 RNA polymerase, which are conditionally expressed under control of two different input promoters. Co-induction of the two polymerase halves will lead to expression of a fluorescent transgene.

**Advancing the ability to image single RNA molecules at the cellular level**

Susan Duncan (John Innes Centre), Susana Sauret-Guet (Department of Plant Sciences, University of Cambridge), Christian Boehm (Department of Plant Sciences, University of Cambridge)

Plant biology currently lags behind other fields in the study of cell-to-cell variation and subcellular localisation of mRNA. Susan Duncan (John Innes Centre) helped to establish the first Single molecule Fluorescent In situ hybridisation (smFISH) method for plants where each RNA molecule can be visualised as a single fluorescent dot in *Arabidopsis thaliana* root meristem tissue (Duncan et al., Plant methods, 2016 in press). This technique revealed subcellular localisation of coding and non-coding RNA and provided data to enable the estimation of the frequency of transcriptional firing events. The high level of back ground autofluorescence emitted by many green plant tissues currently limits smFISH analysis to a single tissue type. With the support of OpenPlant we propose to promote and optimise this existing technique. In addition, we aim to adapt the methodology for use in other *Arabidopsis* tissues and to enable RNA imaging in the liverwort *Marchantia polymorpha*.

**Establishing a Procedure for Rapid Identification of Genetic Parts for Use in Algal Biotechnology**

Kher Xing Chan (Cindy) (Department of Plant Sciences, University of Cambridge), Steven Burgess (Department of Plant Sciences, University of Cambridge), Marielle Vigouroux (John Innes Centre)

We propose to run a pilot experiment to investigate the feasibility of using DNase-SEQ to identify of regulatory elements in *Chlamydomonas reinhardtii*; with the view to producing a genetic toolkit for this alga. DNase-SEQ is a powerful approach to identify transcription factor (TF) binding sites (He et al. 2014) which can then be utilised as genetic parts. To date there have been no reports of DNase-SEQ being applied to *C. reinhardtii* so the first stage of the project will be to establish the procedure. As a test case we will focus on identifying regulatory elements that control the induction of the algal carbon concentrating mechanism (CCM). We propose to develop an open access, online tool to facilitate the bioinformatics pipeline for DNase-SEQ.
A synthetic biology approach to investigating arbuscular mycorrhizal symbiosis in *Marchantia paleacea*

William Summers (Department of Plant Sciences, University of Cambridge), Uta Paszkowski (Department of Plant Sciences, University of Cambridge), Giles Oldroyd (John Innes Centre), Andrew Breakspear (John Innes Centre), Guru Radhakrishnan (John Innes Centre)

D14-LIKE (D14L) encodes an alpha/beta hydrolase receptor that has been well characterised for its role in the perception of the smoke constituent karrikin; whilst in recent years it has been heavily studied for functions in development and light responses. Recently however it has also been identified as being vital for the establishment of arbuscular mycorrhizal (AM) symbiosis in rice (*Oryza sativa*). Mutation of this gene results in a complete breakdown in communication between the plant and fungus (Gutjahr et al 2015). The evolutionary origin of the AM symbiosis coincides with the occurrence of the early land plants with affinity to liverworts approximately 450 million years ago. The liverwort lineage includes members of the Marchantiaceae of which some species, such as *Marchantia paleacea*, engage in AM symbioses; whilst others, including *Marchantia polymorpha*, do not.

Here, we propose to determine the relevance of the ancient D14L for AM-symbiosis. The approach is two-fold and involves (1) genetic complementation of the rice d14l mutant with synthesized homologs of *M. paleacea* and *M. polymorpha*. (2) the CRISPR-Cas9-based editing of the *M. paleacea* locus to assess the functional requirement of MpD14L for AM symbiosis. The project utilises gene synthesis, Golden Gate cloning, the CRISPR/Cas9 system and established protocols for liverworts available in the Oldroyd laboratory.

Open Pi-Image: A low cost-open source plant growth imaging and analysis platform

Professor Alex Webb (Department of Plant Sciences, University of Cambridge), Dr Dan MacLean (The Sainsbury Laboratory)

We have designed and constructed a near infrared image capture system based on a Raspberry Pi computer, PiNoir camera and custom 3D printed parts. This runs an extensible and modular open source software suite we developed called Open Pi Image that controls automated image capture and spawns image analysis. The Pi software can be accessed on any external system (e.g. a laptop) via a web server running on the Pi and the system can be embedded in inaccessible places. Open Pi Image is designed to incorporate new user provided scripts for analysis and can be easily extended and customised.

Facilitating synthetic biology literature mining and searching for the plant community

Dr Robert Davey (Earlham Institute), Dr Ksenia Krasileva (Earlham Institute/ TSL), Dr Nicola Patron (Earlham Institute), Richard Smith-Unna (CU), Dr Peter Murray-Rust (CU)

A two-day workshop in March 2016 centred on novel methods for discovering information about plants from the existing literature (“Content Mining”). Most people were running within an hour and a typical example was “find all you can about diseases of oats” using EuropePubMedCentral (with over 1 million Open Access papers). This retrieves about 500 papers, which were further filtered for chemicals, diseases, species, etc. and displayed within a minute or two, significantly increasing the speed of knowledge-driven scientific discovery. Participants contributed code to the project and helped construct scrapers and dictionaries to extract more information from papers related to plant synthetic biology. The group will run a second workshop and are seeking external grant funding for further collaboration.

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Software

Documentation Tool for Open Plant Technologies

Tobias Wenzel (Department of Physics, University of Cambridge), Johan Henrikkson (EMBL-EBI), Carlos Lugo (EMBL-EBI), Luka Mustafa (Shuttleworth Foundation Fellow, IRNAS)

We have successfully built an open source hardware documentation software and an online repository called DocuBricks (DocuBricks.com). We arrived at a software tool that is (according to feedback of users) easy to use and helpful in a wide range of hardware projects and saves documentations in a modular and accessible XML format. The database is citable and the first biology related documentations have been uploaded – many more are to follow from Open Plant Fund projects and the Open Science Hardware Movement. We will continue to develop DocuBricks to serve as a high quality repository for Open Science Hardware.
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