OpenPlant Handbook 2017
Multi-spectral fluorescence image of a Marchantia polymorpha gemma (clonal propagule) expressing MpEF1a:mTurquoise2-N7 and MpAGL:Venus-N7 fluorescent reporter genes with propidium iodide-stained cell walls. Image captured by Bernardo Pollak using confocal laser scanning microscopy in the Haseloff laboratory at the University of Cambridge. (Synthetic Botany, Boehm et al. CSH Perspectives in Biology, 2017, doi: 10.1101/cshperspect.a023887)
What is OpenPlant?

Institutional strengths in plant sciences
We believe that there is a crucial need to accelerate the development and open sharing of new tools and methods for plant synthetic biology. OpenPlant is a joint initiative between the University of Cambridge, the John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme. The initiative promotes (i) interdisciplinary exchange, (ii) open technologies and (iii) responsible innovation for improvement of sustainable agriculture and conservation.

Interdisciplinary exchange
The UK provides an ideal hub for interdisciplinary exchange between foundational sciences like botany, agronomy, physics, chemistry, computer sciences and engineering. This exchange drives innovation for the engineering of biological systems. OpenPlant promotes and funds the development of novel foundational technologies, the creation of international standards for plant synthetic biology, and open tools for trait development. We believe that advances in plant synthetic biology will provide a key to securing and sustaining future food and materials production, and that there should be worldwide open access to these benefits.

Open technologies for innovation
Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore novel “two-tier” intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for innovators in business and social enterprises. We are building new frameworks and collaborations for open innovation in plant synthetic biology.

Responsible innovation for sustainable agriculture and conservation
Past experiences with GM technologies have shown that they cannot be developed in isolation from social, ethical and environmental considerations, and OpenPlant supports work on the wider implications of the technology at local and global scales, including discussions on the potential impact of Synthetic Biology on environmental conservation and sustainable human practices. These bring together a wide range of engineers, scientists and policy developers to explore the implications for adopting new technologies and different models for sustainable agriculture, bioproduction and land use.

Marchantia polymorpha plants

OpenPlant supports the standardisation and global sharing of open technologies for plant synthetic biology. We aim to catalyse responsible innovation for sustainable agriculture and conservation. Further, OpenPlant provides new tools and a point of exchange for young scientists and entrepreneurs in the UK.
OpenPlant objectives

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.

OpenPlant Work Programme

The OpenPlant initiative supports two tiers of activities.

First, we are developing open technologies that will underpin systematic approaches to bioengineering of plants. These include:

**Workpackage A**: Development of the lower plant Marchantia as a simple and facile chassis for Synthetic Biology, to enable high throughput screening and analysis at the cellular scale.

**Workpackage B**: A common syntax for plant DNA parts and assembly of genetic circuits. Establishment of a moderated archive for publication of DNA part descriptions.

**Workpackage C**: New DNA parts for the control and quantitative imaging of genetic circuits.

**Workpackage D**: Techniques for routine genome-scale engineering in plants.

**Workpackage E**: 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:

**Workpackage F**: Altered photosynthesis and leaf structure.

**Workpackage G**: Changes in plant carbohydrate content.

**Workpackage H**: Engineered pathways for the metabolic engineering of natural products.

**Workpackage I**: New forms of symbiosis and nitrogen fixation for crop plants.

**Workpackage J**: Methods for high level production of biomolecules by transient expression.

**Workpackage K**: Annual funding round to support small-scale interdisciplinary grants.

**Workpackage L**: Outreach activities, training and tools for open exchange of DNA parts and other reagents in biotechnology.
Year 1 progress

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.

Foundational work
- Refurbishment and equipping of new OpenPlant laboratories in Cambridge and Norwich.
- Establishment of a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al., 2015; RFC106).
- 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.
- Implementation of a “single-click” OSX-installable version of the IBIER/ICE open source DNA registry and DNA manipulation software.
- Development of routine methods for transformation and gene editing in Marchantia polymorpha.
- Characterisation of mR1175 and mR1162 precursors for use as synthetic gene regulators in Chlamydomonas reinhardtii.

Trait Development
- Development of Marchantia pallescens as a new system for engineering actinomycorhizal associations.
- Generation of draft genome and transcriptome maps for M. polymorpha and M. pallescens.
- Generation of system for transient induction of gene expression in tomato fruit, based on the HyperTrans vectors.
- Refactoring and use of the HyperTrans system for rapid testing of DNA circuits for terpene synthesis in Nicotiana benthamiana.
- New vectors created for fine tuning of protein levels using the HyperTrans system (Mescheriakova et al., 2015).

Outreach and Responsible Innovation
- 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.
- Support for a joint Cambridge-JIC iGEM2015 team in the Hardware Track.
- Support for a Synbio Beta Activate event in Cambridge, to promote entrepreneurial interactions.
- Organisation of the OpenPlant Forum and international exchange.
- Delivery of two summer schools on Plant Synthetic Biology and CRISPR Technology in Plants, co-sponsored by ERA-SynBio and Plant Methods/GarNet, respectively.
- Delivery of three Science, Art and Writing educational workshops, and two school outreach events.
- Delivery of international workshops on IP, MTAs and open sharing of DNA parts as a new standard (Phytobricks) in the iGEM 2016 competition.

Year 2 progress

Foundational work
- Commissioning of advanced imaging and robotics equipment at the Cambridge OpenPlant laboratory.
- 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.
- High resolution map of the time course of gene expression during spermatogenesis and germination differentiation.
- 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.
- Development of Phytobrick and UNS standards for efficient hierarchical assembly of DNA circuits.
- Expansion of a Chlamydomonas DNA toolkit for target gene expression and assay of mRNAM-dependent gene silencing.
- Development of a suite of Cas9 variants and toolkit for targeted mutagenesis and gene deletion in multiple plant species.

Trait Development
- Design and synthesis of an artificial protein scaffold library, built to the Phytophobic standard and verified by BiFC.
- Production of cell-specific epitope tags for identifying DNA motifs that drive gene expression in photosynthetic tissues in Arabidopsis.
- Publication of a novel reporter for chloroplast transformation, and identification of transit peptides for chloroplast localisation of nuclear encoded proteins in Marchantia.
- Identification of a large repertoire of carbohydrate active enzymes in Euglena gracilis.
- Transformation of gene editing constructs into potato, to control diterpene and genistin production.
- Generation of a提高 specific protein database for enzyme discovery.
- Production of a choline-specific protein for expression in Chlamydomonas, obtained co-sponsorship from Cambridge Consultants and Wellcome Trust/BSR/CSEB fund.
- Responsible innovation workshop with Kathy Liddell, Law Faculty, Cambridge.
- OpenPlant continues to collaborate with Linda Koh, Bio-bricks 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.biobet.net).
- Organised OpenPlant All Hands meeting for scientific exchange, Newmarket.
- Participated in Open Technology for Biology workshop, Chile.
- OpenTechnology Week events in Cambridge, including Technology for the Bottom Billion workshop and Makethon, coordinated with the Centre for Global Equality (http://centreforglobalequality.org).
- Workshops on ethics and openness run at OpenPlant (Mar 2015), outreach with the SAW trust (Mar 2016), and BBSRC Media Training for OpenPlant (Mar 2016).
- Nineteen postgraduate students are participating in projects funded by the OpenPlant Fund. In addition, three PhD students were recruited directly to OpenPlant (Cambridge) this year.
- Undergraduates have formed a student society for Synthetic Biology at the University of Cambridge (http://cusb soc.socnet).
- 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.
- Testing of the HyperTrans system in Marchantia and BY2 cells.
- Consultation on the design of the LeafSystems high throughput production facility, due for completion in Q2 2017.

Outreach and Responsible Innovation
- Funding of 14 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich. Applicants were given a over SynBio training in Africa, increase SynBio capacity in Africa, and produce resources for schools and universities in South America.
- 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 RaspberryPi and customised software.
- Responsible innovation workshop with Kathy Liddell, Law Faculty, Cambridge.
- OpenPlant continues to collaborate with Linda Koh, Bio-bricks 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.biobet.net).
- Organised OpenPlant All Hands meeting for scientific exchange, Newmarket.
- Participated in Open Technology for Biology workshop, Chile.
- OpenTechnology Week events in Cambridge, including Technology for the Bottom Billion workshop and Makethon, coordinated with the Centre for Global Equality (http://centreforglobalequality.org).
- Workshops on ethics and openness run at OpenPlant (Mar 2016), outreach with the SAW trust (Mar 2016), and BBSRC Media Training for OpenPlant (Mar 2016).
- Nineteen postgraduate students are participating in projects funded by the OpenPlant Fund. In addition, three PhD students were recruited directly to OpenPlant (Cambridge) this year.
- Undergraduates have formed a student society for Synthetic Biology at the University of Cambridge (http://cusb soc.socnet).
- 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.
Year 3 progress

Foundational work
- Consolidation of the Phytobrick standard for Type III based DNA parts for Marchantia under the accepted standard for eukaryotic DNA parts, and introduction of the Plant in iGEM 2016.
- Design, construction and ingestion of the first 500 DNA parts for Marchantia, headed by Susana Sauret-Gueto.
- Establishment of the Loop assembly technique by Bernardo Pollak and Mihails Delmans. Built in the PDRA of Lukas Mueller (PDRA) building.
- Development of a new vector system (gFET) which combines the high translational benefits of the CPW-HT system with the regulation abilities of the plant virus X, in collaboration with the Centre for Bioengineering at the Russian Academy of Sciences. The system will be extremely useful where virus spread throughout a host is desirable.

Trait Development
- Hibbard urd has characterised promoter elements that drive specific expression in leaves, and a negative regulator that represses expression in mesophyll and venous cells.
- Compilation of transformation lines that are preferentially expressed in bundle sheath cells of Arabidopsis, including the cognate TF for identified promoter element.
- Development of the circular clock in Marchantia (a new species) to establish an Arabidopsis line.
- Establishment of the Loop assembly technique by Bernardo Pollak and Mihails Delmans.
- CRISPR-Cas9 mediated gene KO to produce “giant chloroplasts”. Missing ORFs have been identified, partial annotation and published frequency doubling of the clock in Molecular Systems Biology.
- Synthesis of a panel of codon optimised fluorescent reporters for Chlamydomonas are now constructed according to the approved standards.
- Validated the use of fluorescent protein based reporter for evaluating miRNA mediated gene silencing in Chlamydomonas.
- Optimised miRNA abundance, extent of sequence complementarity and target sites for gene silencing in Chlamydomonas.
- Constructing synthetic genetic circuits with miRNA mediated co-repressor feed forward loop to confer robust levels of gene expression.
- Gene parts for Chlamydomonas are now constructed according to the approved standards.
- Synthesis of a panel of codon optimised fluorescent reporters spanning the visual spectrum, including five variants of the fluorophore AvecA (Underwood, M), MCherU, mNeptune, mRaspberry, mTurquoise, mWabi-sabi, eBFP, Sirius and TagCFP, all modified for chloroplast expression in the Ajaloba.
- The Marchantia chloroplast genome has been re-annotated. Missing ORFs have been identified, partial annotations completed and likely promoter sites identified throughout the chloroplast genome using BPRom.
- CRISPR-Cas9 mediated gene KO to produce “giant chloroplast” phenotypes in Marchantia, (Male, Pollak, Sau- ret-Gueto, Silvestri in Hasse1ob lab).
- Established reproducible colonisation of several liverwort species (Marchantia spp., Lunularia cruciata) with Glomeromyces fungii (Funneliformis mossae, Rhizophagus irregularis) in custom vermiculite system and detection using staining and high resolution confocal fluorescence microscopy, by Philip Carella (PDRA) in the Schonack lab.
- Established constructs for secretion system pathway and tonoplast labelling and have confirmed functionality in Marchantia cell polymorphs.
- Enhance trap screen underway in Hasse1ob lab, led by Linda Silvestri, Susan Parrett, Marta Tomaselli and Dave Preston, Bernat Pollak.
- In planta cytomter techniques developed in Marchantia air chamber by Ben Froning and Michael Olenick.
- Marta Tomaselli (OpenPlant PhD student) develops clea- ring techniques for image reconstruction of Marchantia air chambers.
- Lukas Mueller (PDRA) building ubiquitin-tagged rapid turn over fluorescent markers for imaging dynamic genetic responses in Wbs-Hasse1ob labs.
- New synthetic version of the 5’LTR used in Hyper-Trans system has been shown to be twice as effective as the origi- nal HT sequence, Lomonosov.
- Developed a new vector system (gFET) which combines the high translational benefits of the CPW-HT system with the regulation abilities of the plant virus X, in collaboration with the Centre for Bioengineering at the Russian Academy of Sciences. The system will be extremely useful where virus spread throughout a host is desirable.

Outreach and Responsible Innovation
- Revision and launch of new OpenPlant branding and communication strategy.
- OpenPlant organised and participated in a wide range of workshops over the last year:
  - Built the Workshop to enable dissemination and share best practice with other research centres.
  - Delivered two of these workshops for SynSys and the School of Biological Sciences, University of Edinburg.
  - And plan similar collaboration with Warwick Integrative Systems Biology Centre.
- Training workshop on the BP2 cell pack system (“cuki- kies”), developed by Fraunhofer Institute, Aachen, Ger- many, run by the Lomonossoff group (July 2016).
- Norwich Science Festival - OpenPlant Exhibit (22nd Oct 2016), and talk ‘The green vaccine machine’.
- Youth STEMM Award Mid-Year Conference OpenPlant Exhibit (Jan 2017).
- CSER Biorisk workshop (Mar 2017).
- Standards & responsible governance, (PGMT) project meeting, Mar 2017.
- Talk on “Finding drugs in the jungle, Just Eat Your Greens - A New Way of Vaccinating?” and ‘20,000 Lea- gues Below: A tale of Nanomachines and Synthetic Biology’ at the second Patent Science Festival in Norwich.
- Talk and discussion panel at Kew Garden’s “State of Greens - A New Way of Vaccinating?” and ‘20,000 Lea - gues Below: A tale of Nanomachines and Synthetic Biology’ at the second Patent Science Festival in Norwich.
- The SAW Trust organised workshops in primary schools inspired by work in the Lomonossoff lab with particular note of using paper “protein pieces” decorated by visitors.
- Colette Matthewman and Jenni Rant (SAW) secured a BBSRC funded workshop in South Africa for SAW in 2017.

Presentations
- 17th International Conference on the Cell and Molecu- lar Biology of Chlamydomonas Kyoto, Japan, June 2016.
- 8th Annual S2.0 and Synthetic Genomes Conference, Edinburgh, Apr 2016.
- Carbon Capture discussion meeting, Woods Hole, MA, USA, August 2016.
- Garfiet meeting, Cardiff, September 2016.
- OpenPlant presentations at SLUK & BBSRC Workshop, Nov 2016.
- Plant Omics and Biotechnology for Human Health, Ghent, Belgium, Nov 2016.
- Genetic Resources and Sustainable Develop- ment Goals, Rockefeller Foundation Bellagio Center, Italy, Nov 2016.
- Synthetic Biology for Natural Products, Cancun, Mexi- co, Mar 2017.
- SEED, Vancouver, Canada, Jun 2017.
- Plant Transformation and Biotechnology IV, Vienna, Austria, Jun 2017.
- OpenPlant presentations at SLUK & BBSRC Workshop, Nov 2016.
- Plant Omics and Biotechnology for Human Health, Ghent, Belgium, Nov 2016.
- Genetic Resources and Sustainable Develop- ment Goals, Rockefeller Foundation Bellagio Center, Italy, Nov 2016.
- Synthetic Biology for Natural Products, Cancun, Mexi- co, Mar 2017.
- SEED, Vancouver, Canada, Jun 2017.
- Plant Transformation and Biotechnology IV, Vienna, Austria, Jun 2017.
DNA PARTS

Synthesis of DNA parts

COMMUNITY STANDARD

With wide support from the international plant science community, we have established a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al. 2015). This common syntax for plant DNA parts is at the core of RFC 106, posted at OpenWetWare, and accepted as an official standard for DNA parts in the iGEM synthetic biology competition.

The Phytobrick standard is a consolidated and consistent standard for Type IIS restriction endonuclease based assembly of DNA parts to make synthetic genes. It is based on the widely used “Golden-Gate” type standard, and allows highly efficient assembly of multiple standard parts into genes without the need to isolate DNA fragments. A range of existing techniques such as Gibson assembly, MoClo and Golden Braid can be used for higher order multi-gene assemblies, however we have developed a simple and flexible protocol for assembly of plant vectors, the Loop Assembly technique.

The Phytobrick standard is general, and applicable to all plants, and other eukaryotes

Principal contacts: Nicola Patron & Jim Haseloff

OPEN SCIENCE

OpenMTA to promote free exchange of DNA parts

OPENPLANT & BIOBRICKS FOUNDATION

Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new “two-tier” intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for small companies in commercial applications of Synthetic Biology.

We are collaborating with the BioBricks Foundation on an Open Materials Transfer Agreement (OpenMTA). This is a simple, standardised legal tool that enables individuals and organizations to share their materials and associated data on an open basis.

The primary purpose of the OMTA is to eliminate or reduce transaction costs associated with access, use, modification, and redistribution of materials and associated data. This in turn will help minimize waste and redundancy in the scientific research process and promote access to materials and associated data for researchers in less privileged institutions and world regions. (http://www.openmta.org)

Principal contact: Linda Kahl

DNA ASSEMBLY

Loop Assembly for efficient & simple construction of DNA

CAMBRIDGE, PUC CHILE

As part of a collaboration between the University of Cambridge and the Universidad Católica de Chile, Pollak and Federici have devised a new method for gene assembly based on two Type IIS restriction endonucleases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and can be easily automated.

Loop Assembly requires the alternating use of two Type IIS enzymes, BsaI (6-base-pair recognition sequence, 4 base overhang) and SapI (7 base-pair recognition sequence, 3 base overhang), and two sets of complementary plasmid vectors that allow efficient and ordered construction of 1, 4, 16, 64 gene fragments.

Principal contacts: Bernardo Pollak & Fernan Federici

AUTOMATION

Automated Loop assembly and validation

CAMBRIDGE, PUC CHILE, EARLHAM INSTITUTE

Like other “Golden-gate” based protocols, Loop Assembly does not require purification of individual DNA fragments, side products are recut during the ligation reaction to drive efficient formation of end-products. Loop Assembly is well suited to automation. OpenPlant researchers at the Earlham Institute and Cambridge are developing methods using acoustic-focusing non-contact liquid handling robots, which increases speed and scale of assembly, while reducing consumable costs and allowing reactions to be performed in nanolitre volumes.

Principal contacts: Bernardo Pollak & Nicola Patron
MarpoDB: a gene-centric database

CAMBRIDGE

For work with the model plant Marchantia polymorpha, we have produced MarpoDB, which is an open source database for MarpoDB describes that presents the Marchantia genome from an engineer’s perspective, rather than a geneticist’s. The database handles the Marchantia genome as a collection of parts. This is highly useful for automatically mining new parts, and managing part description, and part characterisation. We think that this break from standard genome database architecture is essential for tackling the refactoring of synthetic plant genomes. MarpoDB also provides a useful container for gene expression data, and integration of cellular features via Plant Ontology terms. (http://marpodb.io)

Principal contact: Mihails Delmans

DNA PARTS

Mining the Marchantia genome for DNA parts

CAMBRIDGE

MarpoDB has been designed to facilitate the definition and extraction of synthetic DNA elements to be synthesised as standardised DNA parts. For example, we have identified core promoter candidates, and extracted these from the Marchantia genome. The extracted sequences have been domesticated, removing Bsa1 and Sap1 recognition sequences if necessary, and sent for DNA synthesis. The refactored parts will be cloned into pUAP1, a specially prepared vector designed for public distribution.

Principal contacts: Mihails Delmans & Susana Sauret-Gueto

The liverworts (or Marchantiophyta) are descendants of the earliest terrestrial plants. The group is characterised by morphological simplicity. Liverworts have been a largely neglected area of plant biology, but show great promise as model plant systems after recent developments in transformation methods, genome characterisation and biotechnology.

Marchantia polymorpha is the best characterised liverwort. It is a thalloid liverwort, forming a body of sheet-like tissues that possess distinct upper and lower surfaces. The upper surface has a modular structure, with repeated cellular units that form simple cell complexes adapted for photosynthesis and gas exchange. Like other Bryophytes, the gametophyte or haploid generation is dominant phase of the life cycle. Marchantia has a global distribution, and is often found as a weed in horticulture. The plants grow vigorously on soil or artificial media. Marchantia plants spontaneously produce clonal vegetative propagules, or gametogenesis can be induced by exposure to far red light. Male and female plants can be sexually crossed to produce spores. The plants are extraordinarily prolific. A single cross can produce millions of propagules in the form of single-cell spores. Spores can be harvested in huge numbers and stored indefinitely in a cold, desiccated state. Each spore can germinate to produce a new plant, and, unlike higher plants, can undergo the entire developmental sequence to produce an adult plant under direct microscopic observation.

Sequencing efforts have provided a draft of the ~280Mbp genome. Most of the major gene families present in more advanced plants are represented by a single or few orthologues in Marchantia, meaning that there is low genetic redundancy. The apparent simplicity of genetic networks in liverworts, combined with the growing set of techniques for genetic manipulation, culture and microscopy, are set to make this primitive plant a major new system for analysis and engineering.

OpenPlant has adopted Marchantia as a simple testbed for plant synthetic biology.
Marchantia polymorpha

- Simple to culture and propagate
- Easy to transform and regenerate
- 280MB genome with reduced gene redundancy
- Efficient Cas9 mediated gene editing
- Haploid genetics
- Male and female gamete bearing structures induced by far-red light
- Single sexual cross results in millions of progeny spores
- Spores germinate and subsequent development is entirely exposed to observation
- Plants spontaneously produce clonal propagules (gemmae)
- Growing sporelings and gemmae can be observed directly using quantitative microscopy
- Marchantia contains the genetic machinery found in higher plants
- Best plant system for fundamental bioengineering work

Left: Spontaneous production of clonal propagules (gemmae) during vegetative growth of Marchantia polymorpha

Right: Spore germination and exposed form of development in Marchantia polymorpha

Images: Bernardo Pollak

Images: Jim Haseloff
GENE EXPRESSION

Gene expression in germinating sporelings

CAMBRIDGE

Marchantia spores can be harvested and germinated in synchronised fashion. Germination is accompanied by rapid expansion, differentiation of chloroplasts, the first cell divisions, formation of a pronounced apical-basal axis and continued growth and specialisation. Samples can be collected across this period, RNAs extracted, and analysed by high-throughput RNA sequencing to obtain a map of shifting patterns of transcription during these initial phases of plant development. The transcriptome data has been used to investigate genes involved in early chloroplast differentiation and division.

Principal contact: Bernardo Pollak

CHLOROPLASTS

Giant chloroplasts in Marchantia

CAMBRIDGE

Cas9 can be codon-modified for efficient use in Marchantia and other plants. Marchantia plants tolerate presence of the gene, and transgenic lines can be maintained, where simple delivery of a suitable guide sequence will trigger genome modification events, after Cas9-mediated cleavage of the genome. This system has been established by Bernardo Pollak. Owen Male used the system to target the homologues of genes known to be important for chloroplast division in higher plants. Gene knockouts produced aberrant, oversized chloroplasts in the targeted lines - see the images above taken with Susana Sauret-Gueto. Eftychis Fragedakis is now targeting a wider set of genes to determine if the chloroplast numbers per cell can be further reduced, as found in Arabidopsis mutants. These manipulations may be useful for improved chloroplast transformation and homoplasty techniques.

Principal contact: Eftychis Fragedakis

TISSUE ARCHITECTURE

Reconstruction of Marchantia air chambers

CAMBRIDGE

As Marchantia grows, cell division at growing points, or meristems, produces tissues that undergo self-organisation via additional cell divisions and differentiation events, to form air chambers. These chambers are comprised of cellular “floors,” “walls,” “roof” and air pore. The air chambers are packed with specialised cell filaments, that consist of highly photosynthetically active cells. The air chambers form uninterrupted arrays on the top surface of the plant, and are likely a relic of an early attempt to adapt to gas exchange and photosynthesis in a terrestrial environment. Marta Tomaselli has been applying optical clearing and image reconstruction techniques to analyse these cell complexes, including 3D printing of cellular features.

Principal contact: Marta Tomaselli
Plants as Bio-factories
JOHN INNES CENTRE

The Hypertrans® system, developed by Professor George Lomonossoff and Dr Frank Sainsbury at the John Innes Centre, has established a unique position for the UK for rapid transient expression of proteins in plants. The technology is extremely powerful and was used under licence by the Canadian company Medicago to produce 10m effective doses of H1N1 (swine flu) VLP Vaccine in just 30 days, meeting the US Defense Advanced Research Projects Agency test requirements for control of emerging diseases.

Traditional methods for vaccine production using chicken embryos take 6-9 months, and limitations in egg supplies can create bottlenecks. In contrast, plant-based production is much faster and can be rapidly up-scaled. Hypertrans® technology has also been used for expression of biosynthetic pathways, production of antibodies and antigens, and human gastric lipase enzyme for use in a model gut system, and to solve the structure of particles to near atomic resolution when no parent virus particle is available. The system has huge potential for nanotechnology and nanomedicine applications with virus-like particles delivering cargo to cells (Brillault et al., 2016). When coupled with construct design tools and modular “build” techniques, the Hypertrans® system stands out as a rapid testing platform and a valuable teaching and “build” techniques, the Hypertrans® system stands out as a rapid testing platform and a valuable teaching and training tool, as was demonstrated during the OpenPlant ERASynBio plant synthetic biology summer school for early career researchers in 2014. Participants were able to move through the entire design-build-test cycle, learning a multitude of technical skills, in just one week.

Principal contact: George Lomonossoff
Synthetic Biology meets the real world

OpenPlant funds interdisciplinary team-based projects that explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology - and policy workshops and outreach events. These projects aim to build open technologies and promote development of research skills and collaborations. They tap into existing open standards and a rich ecosystem of resources for microcontrollers, first established to simplify programming and physical computing for designers, artists and scientists. These resources provide a simple environment for biologists to learn programming and hardware skills, and develop real-world laboratory tools. Further, the OpenPlant projects provide a direct route for physical scientists and engineers to get hands-on experience with biological systems, and we are developing low-cost open reagents and protocols for easier access to cell-free DNA programmable systems.

Enabling the innovators

History

Since 2014, we have funded small interdisciplinary projects and catalysed new collaborations between several hundred students, researchers and academics across Cambridge, Norwich and beyond. A listing of recent OpenPlant projects is provided here. A more comprehensive collection of information can be found at www.biomaker.org. The projects have generated a large number of electronic prototypes, software, 3D printed devices and biological elements. We hope that these resources prove useful and can be built upon by others, especially to initiate new low-cost approaches to quantitative biology and engineering for teaching and research.

SynBio Strategic Research Initiative Fund

The University of Cambridge SynBio Fund supported eighteen innovative, open and interdisciplinary projects relevant to Synthetic Biology over 2015-16. The aim of the fund was to promote the development of Synthetic Biology as an interdisciplinary field at the University of Cambridge. (www.synbio.cam.ac.uk)

OpenPlant Fund mini-projects

OpenPlant Fund aims to promote the development of plant Synthetic Biology as an interdisciplinary field and to facilitate exchange between The University of Cambridge, the John Innes Centre and the Earlham Institute for the development of innovative and open projects relevant to plant Synthetic Biology, and responsible innovation and outreach in this context. The projects receive £4000 over six months, with an additional £1000 for outreach or follow-on work after reporting on their progress. Funds are managed through a cost centre managed by a faculty sponsor, to help manage integration of the project with existing research loads. All outputs of the projects are open and shared. (www.openplant.org/fund)

Biomaker Challenge micro-projects

Starting in Summer 2017, the Biomaker Challenge is a four-month programme challenging interdisciplinary teams to build low-cost sensors and instruments for biology. From colorimeters to microfluidics and beyond, we’re looking for frugal, open source and DIY approaches to biological experiments. Participants receive a £250 Biomaker Starter Kit and a discretionary budget for additional sensors, components, consumables and 3D printing worth up to an additional £750. Up to 50 grants will be awarded and all teams will exhibit their device at a public Open Technology event and Biomaker Fayre in October.

The Biomaker Challenge leverages additional support from the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative and SynBridgeSens Strategic Research Network. We are actively promoting wide participation both within Cambridge and Norwich, and with external partners - including international collaborations with individuals, companies and institutions. In particular, the new Biomaker Challenge has been designed to be easily portable between institutions and open to industrial collaboration. (www.biomaker.org)

Cell-free gene expression

We are encouraging applications related to use of cell-free extracts to transcribe and translate engineered DNA. Cell-free synthetic biology is gaining popularity for prototyping genetic circuits and metabolic pathways and has many applications from production of biologics to paper-based diagnostic tests and biosensors.

Innovative projects

OpenPlant funding has proved to be a highly effective way of providing key support for independent small projects and promoting valuable new collaborations among young researchers, along with the development and documentation of open source biology, hardware and bioinstrumentation. In a short period of time, we have seen some notable outcomes. For example, our funds have provided seed money for the evolutionary development of 3D printed microscopes across several projects: "Open source 3D-printed microscope", Richard Bowman, Stefanie Reichelt, Hugh Matthews & Jeremy Baumberg, £5K; “High Performance Mechanisms for Low Cost Science”, Richard Bowman, Stefanie Reichelt, Hugh Matthews, £5K; "OpenScope", Cambridge-JIC iGEM2015 team, £10K; and "OpenScope", SynBio Student Society, £5K. These early projects promoted experimentation with a novel, clever and open design. This has subsequently mutated into a family of 3D printed microscopes, optical devices and accessories - and found global use in community labs, schools, social enterprises and research labs.

An example: evolution and spread of open technology

OpenFlexure 3D printed microscope

1. Richard Bowman and colleagues in Cambridge develop a design for a monolithic, 3D printed microscope stage, based on a novel plastic flexure translation stage. This design and its implementation is later published as: A one-piece 3D printed flexure translation stage for open-source microcopy James P. Sharkey, Darryl C. W. Foo, Alexandre Kabla, Jeremy J. Baumberg, and Richard W. Bowman. Review of Scientific Instruments 87, 025104 (2016); http://doi.org/10.1063/1.4941068


3. The Cambridge-JIC iGEM2015 team builds an upright version of the microscope in consultation with Richard. They add motorised focus and translation, and develop software package for remote image collection and data processing (http://2015.igem.org/TeamCambridge-JIC)

4. Richard Bowman, Alex Patto and colleagues develop an award-winning social enterprise, WaterScope, around use of the low-cost microscope for rapid automated screening of micro-colonies from water-borne bacterial contamination. The microscope technology is made freely available to all. Microscopes are now being printed in Africa (http://www.waterscope.org).

5. Richard Bowman continues to develop the microscope optics, producing versions that incorporate low-cost, high-performance objectives and cheap tube lenses.

Versions of the OpenFlexure microscope are being built and modified worldwide. The stage design is modular and has been used for fibre optic alignment, and integrated into other microscope design.

The current version can be found at https://github.com/rwb27/openflexure_microscope pe.
Wireless, portable, low cost, open source hardware for monitoring plant electrophysiology

Dr Pakpoom Subsoontorn (Plant Science, University of Cambridge), Sakonwan Kuhaudomlarp (JIC), Dr Kyle Lopolin (Naresuan University, Thailand), Dr Settha Tangkwakanit (Naresuan University, Thailand)

Plant electrical signaling in grasses and legumes is a wide range of physiological functions including stress responses to drought and wounding. Existing tools for monitoring such functions often require the use of cumbersome and expensive equipment in well-controlled laboratories. We aim to create a low-cost measurement tools that can function robustly in the field, collecting electrical activity profiles from multiple plants. We have developed a new 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.

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

Jan Lyczanski (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 analyze 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. WhiskerScope was also applied to study Arabidopsis thaliana stems with altered composition of cell walls.

OpenLabware 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 Vighnesh Radakrishnan, (John Innes Centre), Dr Marielle Vigouroux (Independent, previously 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 ‘I/Os’ 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 skills such as manipulators and signal transducers are published on a dedicated 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

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 mostly are optimised for plant growth, 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

Sakonwan Kuhaudomlarp (John Innes Centre), Dr Pakpoom Subsoontorn (Department of Plant Sciences, University of Cambridge), Dr Kyle Lopolin (Naresuan University, Thailand), Dr Settha Tangkwakanit (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 use 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.

Plant electro-mechanics: improving low-cost plant electrophysiology for research and education

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

We prototyped a very low-cost plant electrophysiology 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

Steven Burgess (Department of Plant Sciences, University of Cambridge), Tom Moany (Department of Plant Sciences, University of Cambridge), Richard Bohan (Department of Physics, University of Cambridge), Oleg Ratskin (Earlham Institute), Neil Pearson (Earlham Institute)

With synthesis of DNA becoming cheaper and plasmid construction automated, the testing of biological parts is becoming bottleneck in the design-build-test-analysis cycle. Single cells offers a procedure 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

Tobias Wenzel (Department of Physics, University of Cambridge), Luka Mustafa (Institute RNNAS Race), Ji Zhou (Earlham Institute), Nick Pearson (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 flat (i.e. – Marchantia) plant and colony scanning, which extends plate-reading functionality to morphological and long-term analysis and will also be more flexible, considering growing plate size. This is an extension of the OpenScope initiative from the Cambridge 2015 GEM 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 setup will be tested on seed germination experiments and the hardware will be replicable 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

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 as small as C. elegans. The system will be self contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. time lapse), 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).

Light sheet microscopy of cell sheet folding in Volvox

Stephanie Hoehn (DAMPT, University of Cambridge), Pierre Haas (DAMPT, University of Cambridge), Karen Lee (JIC)

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 the quality of the generated LSFM data. Optical sectioning is achieved by moving the sample through a light sheet and thereby creating a 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 aquaculture carnivorous plant Bdellovibrio.


Tyler McCleery (JIC), Ziyi Yu (Chemistry, UCam), Zhijun Meng (Chemistry, UCam), Veronica Greeneisen (JIC)

Single cell growth typically involves heterogeneous nutrient conditions, but such experimental conditions are rarely homogeneous. Interestingly patterns in root architecture arise from heterogeneous conditions, or even dynamic conditions through time. Such patterning calls into question the underlying, likely non-linear processes among root cells that can generate diverse, plastic architecture. Indeed, understanding such phenomena is crucial for development of true synthetic plant systems. I propose development of a low-cost microfluidic device that can finely control rapid changes in the micro-
environment surrounding the root structure. A prototype of such a device could easily be tested with cut vinyl ridges for P/MAX rather than soft lithography. The device would produce heterogeneous nutrient conditions along the root structure, either by laminar flow or gradient generator. The goal is to build a proof-of-concept device, and use it in conjunction with fluorescence imaging for a preliminary test of a well-documented response to heterogeneous nutrient conditions.

The Green Mother Machine Reloaded
Christian Schwall (Biochemistry, UCam), Philipp Braeuninger-Weimer (Engineering, UCam), Bruno Martins (Sainsbury Laboratory, UCam), Anjilt Das (Sainsbury Laboratory, UCam), Chao Ye (Sainsbury Laboratory, UCam), Toby Livesey (Biochemistry, UCam), Antony Hall (UEA)
In this project we wanted to build a microfluidic device which allows the observation of Synechococcus elongatus PCC 7942, a well-studied cyanobacterium, at the single cell level. We based our design on a well-established device called the mother machine and tailored it to the specific needs of Synechococcus elongatus. One of the biggest challenges in adapting the mother machine to Synechococcus elongatus is to keep the cells alive and to load the cells into the growth channels. Here we optimized the loading and survival of Synechococcus elongatus in the green mother machine by improving the loading protocol and the experimental setup. In addition, we tested various prototypes for the robust media switching between different media.

OpenPlant Fund: Biology projects

Developing novel selection markers for plant transformation to advance live-imaging techniques
Dr Katharina Schlassl (John Innes Centre), Dr Femán Féderici (University of Cambridge/Universidad Catolica, Chile), Leonie Luginbuehl (John Innes Centre), Guru Rhadakrishnan (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 localized fluorophore diamino, expressed under the Lotus UBQUITIN promoter, was detectable under the stereomicroscope and could therefore provide a novel selection marker for live imaging. Furthermore, it was found that the BEARKIN promoter was not detectable in the lateral root cap but expressed at the base of the induced hairy root callus. No significant colour change was observed in the roots transformed with the chroomopsin.

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 same 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. Second, 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 Pulled-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: Juhás, M. and Ajiki, 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 John Innes Centre, University of Cambridge)
We have expressed TAL DNA binding domains fused to the FokI nuclease under mitotic promoters (e.g. DMC1, SP011) in Arabidopsis. The aim of this work is to target DNA double strand breaks to specific sites in the genome, in order to induce mitotic recombination. Our preliminary data show that while these nucleases are expressed in mitotic-stage floral buds they do not support wild type levels of crossovers recombination when the endogenous nucleases (SPO11, DMC1) are mutated. Additionally we show onset of developmental phenotypes, leading us to the hypothesis that the resulting DSBs enter a mutagenic pathway. To investigate this in our 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 shut DSBs into crossovers recombination via 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, Jie Li (John Innes Centre))
Originally, one open-inherent synthetic constructs should be built to achieve de novo synthesis of monoo-, sesqui- and terpene in M. polymorpha. We have used the GoldenGate method for constructing the recombine expression cassettes and have already expressed 24 cassettes in M. polymorpha. We have used GoldenGate to introduce the expression cassette for another 5 genes in M. polymorpha plasmid. We have developed a new selection marker for M. polymorpha and have tested this marker in the expression of the same genes in M. polymorpha and new a 20-plasmid system has been created and is currently being evaluated.

Hot Tomato: Complementation of the Capsaicin Biosynthetic Pathway to Engineer Spicy Tomatoes
Greg Reeve (Department of Plant Sciences, University of Cambridge), Chris Boursnell (Department of Plant Sciences, University of Cambridge), Jie Li (John Innes Centre)
This project 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 was used for fast screening and validation of the key genes mentioned above. The project will use the current models for the capsaicin pathway as a blueprint and would provide a clearer picture of capsaicin 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 Lawrence, Schlegelmikhoff (Department of Plant Sciences, 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 different two input promoters. Co-expression of the two polymerase halves will lead to expression of a fluorescent reporter.

Advancing the ability to image single RNA molecules at the cellular level
Susan Duncan (John Innes Centre), Susana Sauret-Gueto (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 Fluorescence in situ Hybridisation (smFISH) method for plants where each RNA molecule can be visualised as a single fluorescent spot 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 exciting technique. In addition, we aim to adapt the methodology for use in other Arabidopsis tissues and to enable RNA imaging in the lwernort 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 aim of 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 used 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 toil 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 polymorpha, 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 synthesised homologs of M. polymorpha and M. polymorpha. (2)the CRISPR-Cas9 based editing of the M. polymorpha locus to assess the functional requirement of MdD14L for AM symbiosis. The project utilises gene synthesis, Golden Gate cloning, the CRISPR/Cas9 system and established protocols for isolation available in the BDmonkey laboratory.

Plant-ProChip 2.0: High throughput transformation of plant protoplasts

Ivan Reyna-Llorens (Plant Sciences, UCam), Steven Burgess (Plant Sciences, UCam), Ziyi Yu (Chemistry, UCam), Gregory Reeves (Plant Sciences, UCam), Christian R. Boehm (Plant Sciences, UCam)

A current limitation for plant synthetic biology involves high-throughput screening of genetic parts in plants. Current approaches require testing circuits in individual plants, through transient or stable transgensics. Applying these techniques to entire libraries is not feasible at a laboratory scale. In the first stage of the project we aimed to develop a high-throughput screen for the analysis of promoter sequences in plant protoplasts. As a result, we successfully isolated, encapsulated and analysed protoplasts from the model species, Marchantia polymorpha and Arabidopsis thaliana using a PEG microfluidic device. Despite of this, there are considerable limitations in terms of protoplast transformation for making these assays high-throughput. The aim of this project is to use microfluidics to develop both transient and stable protoplast transformation protocols at a high-throughput scale. Encapsulated protoplasts will be transformed by PEG transformation and screened for reporter activity. The transformed cells will be sorted and plated onto regeneration media for whole plants regeneration. We envisage this system to be applicable to a range of plant species not just for testing DNA parts but to other applications such as the generation of random mutagenesis lines, enhancer trap lines or inserting novel pathways in plants using minimal amount of resources.

Translating Nitrogen Use Efficiency from models to crops

Marina Fazenda (Plant Sciences, UCam), Matthew Milner (NIAB), Mario Caccamo (NIAB), Dan Swan (Earlham Institute)

Optimising biological nitrogen (N) use is pivotal to maximising crop yields and ameliorating the adverse environmental impacts of excessive agricultural N application. Most cereal crops only take up about 50% of the applied N (Robertson, 1997). The other 50% of applied nitrogen is lost either to soil microbes, leached from the soil during rains or chemically lost to the environment. New opportunities exist to provide gains in efficiency via the translation of basic research into application in crop species. The proposal sought to help better indentify orthologous genes as well as indentify how much variation exists in nitrogen use efficiency targets in wheat. We proposed to collect RNA Seq data on the eight parents of a widely distributed and publically available mapping population developed at NIAB (MAGIC elite) to add experimental evidence to help build connections between current knowledge in model species and wheat. The RNA samples are currently awaiting sequencing and the collaboration have visited and given talks at their respective sites.

DNA-mediated fusion of spheroplasts with synthetic liposomes

Lorenzo Di Michele, (Physics, UCam), Martin Howard (ARC), Pietro Cicuta (Physics, UCam)

We have very good control over phospholipid (liposome) formation, transport, and fusion; we also know how to isolate the external cell membrane of gram negative bacteria, yeast and single cell algae, all of which then form a ‘spheroplast’ state, from which the whole cell can be recovered under appropriate culture. Removing the external cell membrane/wall is indeed a standard step in various protocols for uptake of material into the cells. The key idea of our proposal is to demonstrate a hybrid system, engineering controlled adhesion and fusion of artificial liposomes to spheroplast cells. This could represent a new high throughput and selective tool for delivering cargo into cells, not limited to genetic material and very flexible in terms of size and chemical nature of the cargo.
New funding model for interdisciplinary exchange, project-based learning and DIY bioinstrumentation

The Biomaker Challenge is an interdisciplinary team-based opportunity to explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology. The Biomaker Challenge aims to promote collaboration between disciplines, tapping into commodity electronics and open technologies for instrumentation to build research skills and collaborations.

We have chosen Arduino-based hardware (www.arduino.cc) as our starting point. The Arduino community has established open standards and rich ecosystem of resources for simple microcontrollers, first established to simplify programming and physical computing for designers and artists. Arduino circuit boards can be plugged into the USB port of any laptop, and a simple cross-platform programming environment used to program the board. A program is simply loaded to non-volatile memory on the Arduino board, which will execute this program whenever the board is powered on - behaving as a dedicated appliance or instrument. Arduino boards include many input/output ports, and are intended to interface with sensors and actuators.

The Arduino system provides a simple environment for learning programming and hardware skills, and developing real-world laboratory tools for biologists. Further, the Biomaker Challenge provides a direct route for other scientists and engineers to get hands-on experience with biological systems.

The effort is sponsored by BBSRC/EPSRC through OpenPlant Synthetic Biology Research Centre (www.openplant.org) and the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative (www.symbio.cam.ac.uk) and CamBridgeSens, the Sensors Strategic Research Initiative (www.sensors.cam.ac.uk).

The Biomaker Challenge Starter Kits contain teaching materials to allow anyone with no previous experience to learn programming and interfacing to the Arduino microcontroller board.

Biomaker Starter Kit

Each team in the Biomaker Challenge receives a Starter Kit that will allow even inexperienced individuals to develop skills, and provide a platform for exploring more challenging applications. The kit includes:

- ARDX Prototyping Kit. The ARDX Starter Kit for Arduino is a great learning resource with components to build 13 different projects. The kit provides a manual with instructions arranged as a series of lessons. These provide a simple way of learning how to wire electronics circuits and programming the Arduino microcontroller. For example, the kit comes complete with a set of paper circuit templates that you lay over the breadboard and push the components through - to remove the worry of wiring the project incorrectly. No experience necessary.
- Grove Modular Sensor/Actuator Kit. Grove is a modular electronics platform for Arduino-based quick prototyping that does not involve soldering. Simply plug the Grove modules into the Grove shield and leverage the example code provided for each Grove module. Grove is a modular, ready-to-use tool set. Much like Lego, it takes a building block approach to assembling electronics. The Grove Starter Kit contains 10 of the most popular Grove modules and an Arduino shield with Grove connectors.
- Sidekick Basic Component Starter Kit. This contains basic components to build 7 different projects, and include an additional small circuit board and more hook-up wires. The kit is provided by Seeed Studios (http://wiki.seeed.cc/Sidekick_Basic_Kit_for_Arduino, V2).
- Giant Prototyping Board for Arduino. The Gronics Proto Shield Plus allows you to plug in Arduino boards, and to integrate these with custom shields and components on a large plug board - minimising tangled hook-up wires. On-board push buttons and an LCD are provided to facilitate debugging of program flow and to interrogate hardware during testing.
- Programmable Touchscreen. The Biomaker Starter Kit will contain a 4D Systems 3.2” 410 g pure touch-responsive programmable display from 4D Systems (with memory card, Arduino interface and programmer), with information about programming environments. An Arduino library for direct serial communication with the display is available - along with more sophisticated Workshop4 development tools, including ViSi-Genie, a graphical programming tool that allows simple access to a wide range of display widgets like gauges, switches, sliders, readouts, etc., for creating customised interfaces for Arduino-based instruments. The programmable displays can be easily adapted for Raspberry Pi board computers. These programmable touchscreens allow the simple prototyping of sophisticated user interfaces, to match the flexible and programmable control of hardware by microcontroller-based instruments.

The teams are also provided with additional support of up to £750 over the summer, for additional components and materials, including access to a 3D printing service with both fused deposition modelling (FDM) and stereolithography (SLA) printing services, and teams will be expected to share their projects on Github. The Biomaker Challenge culminates in a public Open Technology exhibition. All teams will be expected to demonstrate their creations at this public event. Prizes will be awarded for especially creative and/or enabling projects.

For more information, go to https://www.biomaker.org
Practices for responsible innovation

The global cultivation of crops and pastures are driven by global population pressure, and are responsible for unsustainable impacts on natural environments. An overarching aim of the OpenPlant project is to provide a map of feasible technical approaches for improving bioproduction and agriculture – considering possible economic models, opportunities and social implications. This includes consideration of the adoption of different forms of IP ownership, open source technologies, new business models in biotechnology, scientific codes of practice, responsibility for design and implementation, bioengineering accreditation, third world exchange, design for sustainability, decentralisation, UK policy development, evaluation of environmental impact (at the point of conception and design, rather than implementation), guidelines for best practice in new biological systems and real-world agronomy.

Responsible Research and Innovation (RRI) activities are integrated into the OpenPlant SBRC through a number of cross-cutting activities. Central to this are efforts to create mechanisms for the exchange of resources and information by developing enabling tools for sharing such as standards and IP solutions, DNA part collections, shared protocols, and an open community for plant synthetic biology; along with OpenPlant Fund workshops to strengthen synthetic biology capacity in Latin America and Africa.

The OpenPlant Forum is an important vehicle for bringing together a multidisciplinary community to discuss important questions in Responsible Research and Innovation. Smaller meetings such as the OpenPlant All-Hands meeting, ROC meetings, and interdisciplinary workshops provide opportunities to explore issues related to responsible innovation. To support these activities and enable our PDRAs to contribute more extensively, we deliver workshops on RRI, ethics and argumentation, and openness attended by OpenPlant-funded PDRAs and many associates. OpenPlant participates in quarterly meetings of the Virtual Institute of Research and Innovation (VIRI) in Cambridge. These meetings bring together members of the science departments with members of the Centre for the Study of Existential Risk (CSER) and the Centre for Science and Policy (CSaP) to discuss matters related to RRI and to discuss opportunities for collaboration. Resulting from these collaborations, OpenPlant researchers from all three institutes have become involved in a Bioengineering Horizon Scanning Exercise organised by CSER.

Global Garden Workshop

NORWICH

A Global Garden workshop was run as a collaboration between OpenPlant researchers at the John Innes Centre, the Science Art Writing (SAW) Trust, Social Scientist Dr Nick Lee (Social Scientist, Warwick Integrative Synthetic Biology Centre) and the Writers Centre Norwich. The workshop was advertised to the public, and explored biodiversity, traditional and modern uses of plants, access and benefit sharing and feelings on natural vs synthetic products. Participants were immersed in the theme through practical science, art, poetry and a set of case studies that raised a variety of questions leading to discussions of issues around the use of plants as sources of drugs and other high value products. This co-learning experience highlighted to researchers the values, concerns and optimisms of publics in relation to the use of plants as a source of natural products.

Principal contact: Jenni Rant

Norwich Science Makers Network

NORWICH

OpenPlant has supported the establishment of a Norwich Science Makers meetup group to bring together a cross-disciplinary network of people to learn from each other, share ideas and skills and shape interdisciplinary and collaborative project plans. The network will provide an umbrella under which a variety of activities can be captured, and can feed into programmes such as the OpenPlant Fund and Biomaker Challenge. The first meetup will be in September.

https://www.meetup.com/Norwich-Science-Makers/

Principal contact: Colette Matthewman
Cell-free biology workshops
CAMBRIDGE-NORWICH

Recent technical advances in the preparation of microbial cell-free extracts have given rise to a new class of highly efficient systems for gene expression that are cheap to deploy and have huge potential benefit for the provision of a wide variety of diagnostics, sensors, vaccines and research materials. Further, the extracts can be stored desiccated, stable for over a year, and reactivated at the point of use by hydration. The cell-free extracts can be programmed by the addition of DNA to allow rapid and simple prototyping of gene circuits for diagnostics or bioproduction.

In vitro biology provides a number of key advantages for the design, assembly and testing of DNA encoded circuits for diagnostics and environmental sensing. Cell-free extracts avoid the complications, delays and regulatory uncertainty associated with uncontained of GMOs, while providing opportunities for high level, low cost training and capacity building.

The emerging technology enables engineering of DNA circuits without the need for genetic modification and in a low-cost manner that makes it accessible for researchers in low resource settings. OpenPlant is sponsoring efforts to develop new educational and training materials for use in the UK and developing countries.

Principal contact: Jim Haseloff

Building the Biomakespace in Cambridge
CAMBRIDGE

A Biomakespace in Cambridge is being built by a group of researchers, scientists, engineers, technologists and curious minds - to create an innovation space for biology and biological engineering. This effort is being driven by OpenPlant personnel and supported by OpenPlant initiatives. The Biomakespace is situated within the historic old MRC Laboratory of Molecular Biology building.

It intends to make bioengineering and manufacturing technologies accessible to a wide spectrum of innovators and enthusiasts to develop projects and ideas in an informal setting, with space for experimental biology and fabrication tools focused on scientific applications. The space aims to build a cross-disciplinary and cross-sector community for synthetic biology in the city, with a focus on open technology and innovation. It will also provide activities such as training and skills sharing sessions, networking events and foster links with innovation and seed funding schemes and local incubator spaces and accelerator programmes. (https://biomake.space)

Principal contact: Jenny Molloy

Bakubung Report
AFRICA

Synthetic biology and open-source applied biology tools that are pragmatic, safe and cost-effective have the potential to stimulate bioeconomic growth and address African challenges in healthcare, agriculture, education and the environment. OpenPlant is recruiting leading international experts to explore the latest developments in synthetic biology, bioengineering and DIY biology, their potential as training tools for students and future innovators, and practical opportunities for deployment in Africa. This effort has been started with a symposium and workshop that was held in Pretoria and Bakubung, South Africa in February 2017, supported by the BBSRC and Global Challenges Research Fund (GCRF), a £1.5bn UK fund dedicated to the support of challenge-driven initiatives.

This effort has been started with a symposium and workshop that was held in Pretoria and Bakubung, South Africa in February 2017, supported by the BBSRC and Global Challenges Research Fund (GCRF), a £1.5bn UK fund dedicated to the support of challenge-driven initiatives. The workshop resulted in the publication of a report that canvassed the difficulties and opportunities for promoting innovation in the emerging bioeconomy in Africa. In response, OpenPlant is coordinating an international programme for development of open materials for biological education in low resource environments. (https://www.openplant.org/global-challenges/)

Principal contact: Jenny Molloy
OpenPlant Fund: Responsible Innovation projects

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. But we need to understand more about their biology. With this in mind, we have set up the Big Algal Open 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 biosensor 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 thanks to a smartphone app: The Algae-app. The Algae-app will enable everyone having access to a smartphone to get involved.

During the OpenPlant Fund project, biosensors, the website and app were constructed [http://bigalgap.e](http://bigalgap.e)/about). The project has since been on the road at Latitude Festival 2016 and exhibited at London Zoo, further experiments with schools and universities are planned for the future and the concept is being developed into an "Algae-getch" pet with the last Advanced Architecture Group in Barcelona.

Responsible Innovation and Open innovation with Large BioResources: Goals, Challenges and Proposals

Dr John Liddicoat (Centre for Responsible Innovation and Open Innovation with Large BioResources: Goals, Challenges and Proposals)

In addition, the project has taken further steps in exploring the practical realisation of their electronic counterparts, allowing students to gain experience in circuit design, and implementing openness effectively in bio-resources intellectual property policies? Discussions were started on this question: How do they implement these genetically and molecularly? How are bistable switches, hysteretic systems, all-or-none responses, proportional control mechanisms etc. implemented in living cells? Can we reconstruct simple molecular biology capacity in Kenya through bioinformatic training

Richard Smith-Umna (CRI, Dr Vicky Schneider (Earlham Institute), Dr Jenela Alekic (TRInGo), Richard Pilling (Intel)

From 8th November to 5th December 2015, 17 students from nine African countries attended our course, held at CEPS 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 project group, coordinated online, will help the students keep the momentum from the course going and the course also repeated with a new cohort in 2016. The course materials are available at [https://github.com/jenela121/NGS_analysis_icipe](https://github.com/jenela121/NGS_analysis_icipe).

Setting up an open synthetic biology lab in Abuja, Nigeria

Richard Smith-Umna (CRI, Dr Chinyere Okoro (Sanger Institute), Dr Ibuken Akinnadie (University of Bingham, Nigeria), Dr Jenela Alekic (TRInGo), Dr Vicky Schneider (Earlham Institute)

The team were able to develop a synthetic biology lab in Bingham University, Abuja, Nigeria by collecting over 150 kg of equipment donations from institutes in Switzerland and the UK, and shipping to Nigeria in May 2016. This included molecular biology equipment such as a PCR machine, centrifuges and consumables. Preparations for the workshop is now in top gear as logistics are been arranged and course materials are being prepared. A course was run in January 2017 providing a robust introduction to molecular biology and gene editing techniques (e.g. cloning, CRISPR, RNAi and protein methods). The course also included a Science Policy Lecture supported by the European Molecular Biology Organization.

Development of Cell-Free Genetic Circuits and their Electronic Counterparts as Educational Tools for SynBio Students

Cambridge University Synthetic Biology Society

There is a notable absence of Synthetic Biology (SynBio) concepts and methods within the STEM courses at Cambridge, particularly in the first two years of study. In addition, the course structures preclude any interdisciplinary student research. The need for a society bridging departmental divides and giving students a platform on which to develop experience in Synthetic Biology outside the conventional course structure was clear. In 2015, CUSBS was established with the aim of increasing understanding and involvement in SynBio within the student body, and to allow students from different backgrounds to share ideas and skills. This project will allow us to work on understanding gene regulation by engineering oscillating genetic networks in cell-free TK-1 systems. Generally, the project aims to develop and implement two parallel "biological" and "physical" branches designed to be biomolecularly and complementary. Theoretical design of genetic networks will run alongside the practical realisation of their electronic counterparts, allowing students to gain experience in circuit design and implementing their ideas on biological systems. Do cells compute information in the same way as biological circuits? How do they implement these genetically and molecularly? How are bistable switches, hysteretic systems, all-or-none responses, proportional control mechanisms etc. implemented in living cells? Can we reconstruct simple and complex network motifs outside of a cell?

Accessible 3D Models of Molecules

Fernando Federici, Bernardo Pollak, Nicola Patron

This project aims to create series of 3D models of molecules far schools and curriculum activities. The models will be used to facilitate the understanding of in vivo structures, polymers and synthetic biology projects. The kits will include complete structures and also pieces to be assembled as 3D puzzles and will be a tool for teachers and researchers to teach about their subject in an interactive manner.

Developing teaching resources for rapid, open and combinational genetic circuit fabrication in cell-free systems

Co-lab OpenPlant - interdisciplinary workshops of science art and design

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

Co-lab OpenPlant ran a series of three workshops and a hackathon event during 2016, with the objectives of creating new ideas around plant synthetic biology applications and fostering further collaboration by establishing links between designers, artists and scientists. Some projects have continued and one, VRiCS, achieved its own OpenPlant Fund to develop accessible 3D models of molecules for schools.

Synthetic Biology for Schools: A multidisciplinary approach

Dr Colette Matthewman (John Innes Centre); Dr Benni Rant (The Saw Trust), Dr Tim Rudge (Universidad Catolica, Chile), Tim Marzullo (Backyard Brains, Inc), Juan Keymer (Universidad Catolica, Chile), Nadia Radzman (John Innes Centre), Samantha Fox (John Innes Centre), Lawrence Pearce (John Innes Centre), Dr Nicola Patron (Earlham Institute), Dr Fermin 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

Prof 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 biosafety management. This workshop in November 2016 acted 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. A report is in preparation.

Devising Cell-Free Genetic Circuits and their Electronic Counterparts as Educational Tools for SynBio Students

Cambridge University Synthetic Biology Society

there is a notable absence of Synthetic Biology (SynBio) concepts and methods within the STEM courses at Cambridge, particularly in the first two years of study. In addition, the course structures preclude any interdisciplinary student research. The need for a society bridging departmental divides and giving students a platform on which to develop experience in Synthetic Biology outside the conventional course structure was clear. In 2015, CUSBS was established with the aim of increasing understanding and involvement in SynBio within the student body, and to allow students from different backgrounds to share ideas and skills. This project will allow us to work on understanding gene regulation by engineering oscillating genetic networks in cell-free TK-1 systems. Generally, the project aims to develop and implement two parallel "biological" and "physical" branches designed to be biomolecularly and complementary. Theoretical design of genetic networks will run alongside the practical realisation of their electronic counterparts, allowing students to gain experience in circuit design and implementing their ideas on biological systems. Do cells compute information in the same way as biological circuits? How do they implement these genetically and molecularly? How are bistable switches, hysteretic systems, all-or-none responses, proportional control mechanisms etc. implemented in living cells? Can we reconstruct simple and complex network motifs outside of a cell?
OpenPlant Global Challenges

Synthetic Biology in Africa

The OpenPlant obtained funding for a ‘Practical Synthetic Biology’ workshop in Africa - to exploit recent technical advances in biology that have given rise to cell-free and transient expression systems that are cheap to deploy and have large potential benefit for diagnostics, sensors, vaccines and research materials. The workshop was help in collaboration with the University of Pretoria, which has initiated the construction of the Future Africa campus, intended to provide a hub for Africa’s leading scientists and scholars.

The workshop found:

1. The field of Synthetic Biology is introducing low-cost, breakthrough technologies for a wide range of practical challenges including diagnostics, environmental conservation, microbial bioproduction, crop improvement and human health. These are of critical importance to the future well-being and economic development of sustainable societies across Africa.

2. Synthetic biology offers new tools and approaches:
   - Standardised, modular DNA parts and rapid assembly of genetic circuits for reprogramming biological systems.
   - Cell free expression systems that do not require containment, and can be freeze-dried and stored at ambient temperatures to eliminate the need for refrigeration.
   - Transient gene expression in contained hosts, and transgene-free genome editing to avoid the costs, resources and regulatory hurdles associated with the deployment of genetically modified organisms.
   - Legal frameworks, repositories and open technologies for the open exchange of genetic materials.

3. These new technologies are relatively low-cost, but their adoption in Africa is limited by deficits in technical training, poor access to new research materials, inadequate laboratory facilities, and lack of strategic partnerships with other African and international research institutions.

4. The UK and Africa share a common goal with the need to develop improved synthetic biology training in schools, universities, community labs and industry.

5. International efforts to develop open standards and protocols for DNA parts and tools will provide a major impetus for technology transfer to Africa.

6. We recommend that (i) biotechnology is fertile area for UK-Africa exchange, and that (ii) capacity-building based on open technologies and exchange be a major component of any funding initiative.

7. Synthetic biology can provide better solutions for: (i) rapid-response production of vaccines and biologics, (ii) point-of-use diagnostics and field biosensors, (iii) agricultural crop improvement using non-transgenic (genome editing) tools, and (iv) harnessing local biodiversity to build a sustainable bioeconomy.

8. In each of these applications, the development of practical solutions and social impact requires:
   - Standardised curricula for training and biotechnology education in resource-poor communities and institutes.
   - Building local expertises through exchange and shared knowledge.
   - Establishing in-country facilities for generation and exchange of open-source tools and materials.

We have organised follow-up workshops and meetings, focusing on knowledge transfer for cell-free biology.
**Leadership group**

Prof. Anne Osbourn, Norwich Director
Anne 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.

Prof. Jim Haseloff, Cambridge Director
Jim 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.

Prof. Sir David Baulcombe, Principal Investigator
David’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.

Prof. Dale Sanders, Principal Investigator
Dale’s 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.

Prof. Nicola Patron, Earlham Institute
Nicola 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.

**Coordination group**

Dr. Colette Matthewman, Manager
Colette 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.

Dr. Jim Ajioka, University of Cambridge
Jim’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.

Dr. Jenny Molloy, Coordinator
Jenny 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.

Dr. Susana Sauret-Gueta, Cambridge Research Manager
Susana is an experienced molecular biologist and microscopist. She has established new facilities for robotic liquid-handling and advanced microscopy in the Cambridge OpenPlant laboratory, and is coordinating the sharing of standardised Marchantia resources. These include libraries of DNA parts and transformed plant lines. With a scientific backround in plant growth and development, she supports researchers and strengthens the integration of research projects. Susana is the main organiser of the ROC Group (Researchers with OpenPlant Cambridge).

Dr. Fernan Federici, OpenPlant International Fellow
Fernan is an assistant professor at PUC (Santiago, Chile) and manages a research group that explores spatial patterning, open science and educational efforts across Latin America. Fernan has a long association with Cambridge as a Gates Scholar and research fellow. He is continues to play a pioneering role, contributing open technologies for bioengineering, science and education across Latin America, and exploring international collaborations with OpenPlant colleagues.

Dr. Nicola Patron, Earlham Institute
Nicola 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.

**Research leaders**

Dr. Jim Ajioka, University of Cambridge
Jim’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.
Prof. Sarah O’Connor, John Innes Centre
Sarah 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. 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 understanding of the complex metabolic pathways that produce these high-value chemicals.

Prof. Rob Field, John Innes Centre
Rob 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 to 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 and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Prof. Paul Dupree, University of Cambridge
Paul 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 synthesize 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.

Prof. Giles Oldroyd, John Innes Centre
Giles 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 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.

Prof. Christopher Howe, University of Cambridge
Chris 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.

Prof. Cathie Martin, John Innes Centre
Cathie 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 healthpromoting properties in preclinical studies. Her expertise on transcriptional regulation 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.

Prof. Sarah O’Connor, John Innes Centre
Sarah 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. 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 understanding of the complex metabolic pathways that produce these high-value chemicals.

Prof. Rob Field, John Innes Centre
Rob 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 to 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 and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Prof. Paul Dupree, University of Cambridge
Paul 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 synthesize 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.

Prof. Giles Oldroyd, John Innes Centre
Giles 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 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.

Prof. Christopher Howe, University of Cambridge
Chris 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.

Prof. Cathie Martin, John Innes Centre
Cathie 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 healthpromoting properties in preclinical studies. Her expertise on transcriptional regulation 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.

Prof. Sarah O’Connor, John Innes Centre
Sarah 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. 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 understanding of the complex metabolic pathways that produce these high-value chemicals.

Prof. Rob Field, John Innes Centre
Rob 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 to 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 and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Prof. Paul Dupree, University of Cambridge
Paul 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 synthesize 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.

Prof. Giles Oldroyd, John Innes Centre
Giles 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.

Prof. Christopher Howe, University of Cambridge
Chris 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.

Prof. Cathie Martin, John Innes Centre
Cathie 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 healthpromoting properties in preclinical studies. Her expertise on transcriptional regulation 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.
Scientific Advisory Board

Tom is an experienced technology venture entrepreneur with an R&D background in both the physical and life sciences. Before Synthace, he was Chief Operating Officer of CellCentric, a leading epigenetics drug discovery company, Chief Technology Officer of Arrow Therapeutics and co-founder and General Manager of DNA microarray tools company, Oxford Gene Technology (Operations). Prior to this, Tim spent 13 years performing highly interdisciplinary research at the University of Oxford holding post-doctoral positions in three different departments (Biochemistry, Engineering and Materials). He has a DPhil in Semiconductor Materials and an MBA from London Business School. Tim is the Chairman of the UK Biotechnology Association’s Synthetic Biology Advisory Committee and also a member of the Synthetic Biology Leadership Council.

Pietro's group uses optical tweezers, microrheology, advanced confocal microscopy and image analysis methods to address dynamics both in colloidal and cellular systems. The lab's research includes self-assembly of phospholipids, including physical properties of lipid bilayers, hydrodynamic synchronisation of motile cilia, including model colloidal systems and living ciliated cells, particularly human airways; and physical mechanisms of regulating gene expression in bacteria.

The main theme of research in Lisa's Analytical Biotechnology Group is in 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.

Dr. James Locke, Sainsbury Laboratory Cambridge University

James is an expert in mathematical modelling and single cell analysis of genetic networks. He developed the first model of the plant circadian clock, and experimental data and modelling to correctly predict a new feedback loop. He co-developed a high-throughput time-lapse single cell analysis and tracking system for bacteria, and used the system to discover a new mode of prokaryotic gene regulation; stochastic frequency modulated pulsing. He is studying stochasticity and signal integration at the single cell level in B. subtilis, plants and Cyanobacteria.

Prof. François Képes, Genopole, CNRS, France

François is a noted leader in the Synthetic Biology field in Europe. He studies and engineers genome architecture. For this purpose he uses various approaches including bioinformatics and molecular, systems and synthetic biology. François Képes is a Research Director at CNRS. He is the Founding Director of the Epigenomics Project (Genopole), an Institute of Complex Studies that is dedicated to the emerging discipline of Synthetic Biology. He is a permanent Invited Professor at Imperial College London. He is a member of the National Academy of Technologies of France.

Christopher is one of the few “dyed-in-the-wool” synthetic biologists exploring plant systems, outside OpenPlant. She has a very high profile in the field, with a fast-track career at Berkeley-Caltech-Stanford - working on the engineering of RNA-based control mechanisms and natural product biosynthesis. She's been president of the Society for Biological Engineering, and has string of awards to her name: NIH Director's Pioneer Award, National Institutes of Health (2012), World Technology Award in Biotechnology (Individual), World Technology Network (2009), Alfred P. Sloan Foundation Fellow, Alfred P. Sloan Foundation (2008), National Science Foundation CAREER Award, National Science Foundation (2006),

Dr. Drew Endy, Stanford University

Drew is a well-known evangelist for synthetic biology. As well as his scientific work at MIT and Stanford, Drew has provided early leadership and support for many open biotechnology programs. He was the co-founder of the iGEM competition, OpenWetWare.org, the Biofab and Bionet, efforts to share high-quality standard biological parts. Drew is founder and President of the BioBricks Foundation, which supports open technical and legal standards. He has worked with Congress, the White House, DOE, OGC, the National Academy, etc. on policy matters. Drew is a “big ideas” person, with an exceptional track record in promoting successful international open science initiatives in synthetic biology.

Dr. David Rejeski, Woodrow Wilson Institute

David directs the Science and Technology Innovation Program (STIP) at the Woodrow Wilson Center in Washington DC, including synthetic biology (www.synbioproject.org). STIP focuses on emerging technologies and the critical choices innovation presents to public policy. He has graduate degrees in public administration and environmental design from Harvard University and Yale University and a degree in industrial design from the Rhode Island School of Design. He founded and co-directed a non-profit involved in renewable energy technologies, was head of the Future Studies Unit at the US Environmental Protection Agency, and worked at the White House Council on Environmental Quality (CEQ) and the Office of Science and Technology (OSTP) on a variety of technology, R&D, and policy initiatives.

Drew has provided early leadership and support for many open biotechnology programs. He was the co-founder of the iGEM competition, the BioBricks Foundation, the National Academy, etc. on policy matters. Drew is a “big ideas” person, with an exceptional track record in promoting successful international open science initiatives in synthetic biology.

Dr. Scott Steedman, British Standards Institute

Director at the British Standards Institution (BSI), where he is responsible for the work of the UK National Standards Body, representing the UK internationally and for advising industry and government on the role of standardization in the economy. Prior to joining BSI in January 2012, Scott spent around twenty years working on major infrastructure and building projects in the UK and around the world for consulting and contracting companies, including GIBB, WhittbyHendal and Foster Wheeler Energy. Formerly a lecturer at Cambridge University, he has specialised in natural disasters, forensic engineering, risk and innovation strategy. Scott chaired the European Council for Construction, Research and Innovation for over a decade, and is former Vice President of the Royal Academy of Engineering.
Dr. Dr. Philip Carella
Postdoctoral Fellow, Schornack Lab, Sainsbury Laboratory, University of Cambridge
Dr. Philip Carella
Postdoctoral Fellow, Schornack Lab, Sainsbury Laboratory, University of Cambridge
I recently completed my PhD in Dr. Robin Cameron’s lab (McMaster University, Canada), where I studied phloem-mediated long-distance immune signalling induced by a bacterial pathogen in Arabidopsis thaliana. Feeling a need to branch out a little, I joined Dr. Sebastian Schornack’s group (Sainsbury Laboratory, University of Cambridge, UK) to study interactions between filamentous microbes and non-vascular early land plants. Our goal is to identify core developmental processes required for the colonization of early land plant tissues by filamentous microbes and to understand how these processes evolved into the defense and symbiotic programs employed by higher plants. Our work will generate transcriptomics data, fluorescent marker lines and microbe inducible promoters for cell biology, and other molecular-genetic tools that will enable the OpenPlant community to explore early land plant biology.

Dr. Dr. Alex Broomslore
EPSRC
Dr. Alex Broomslore
EPSRC
I did my bachelor and master in Biotechnology in Pisa, where I discovered how fascinating plants can be. In the past, I have worked with CRISPR/Cas9 systems in two different plant models: Arabidopsis thaliana and Marchantia polymorpha. These were my first experiences related to synthetic biology and they really got me involved. I started as an OpenPlant PhD student at the University of Cambridge in 2016. During a rotation in the Haseloff Lab, I developed optical clearing techniques for microscopy of Marchantia gemmae. These tools allow 3D reconstruction of the plant tissue. In my second rotation in the Schornack lab, I focused on plant-pathogen interactions: looking for pathogen-responsive promoters in Marchantia. These sequences can be exploited to generate new reporter lines.

Dr. Dr. Eftychis Frangedakis
Postdoctoral Fellow, Haseloff Lab, University of Cambridge
Eftychis did his PhD at Oxford University focusing on the evolution of developmental mechanisms in land plants. During his doctoral research he developed a strong interest and fascination for bryophytes. He then moved to the University of Tokyo to work with the least studied group of bryophytes, hornworts. After a short detour in Hong Kong he is now back to the UK working on the development of new synthetic biology tools in Marchantia. In particular, he is developing tools for engineering the chloroplast genome, where work with the liverwort allows the benefits of single-cell handling through spores, facile transformation and regeneration, and access to a full set of genetic and optical tools for manipulation and quantitative screening of the organism.

Dr. Dr. Henry Temple
Postdoctoral Fellow, Dupree Lab, University of Cambridge
Dr. Henry Temple
Postdoctoral Fellow, Dupree Lab, University of Cambridge
Plant cell walls represent the most abundant renewable source on the planet, but only a small fraction of this biomass is used by humans. With ongoing interest in use of cell wall polysaccharides, we are just starting to understand their biosynthesis in plant cells. Synthesis of polysaccharides occur mainly through the activity of glycosyltransferase (GTs) enzymes which transfer an activated sugar in the form of a nucleotide-sugar onto a specific growing polysaccharide acceptor. I have great interest in the different processes that govern cell wall biosynthesis. In my Master’s and PhD thesis I worked on characterisation of Golgi localised nucleotide sugar transporters (NSTs) responsible for the incorporation of substrates used by GTs enzymes. Now I'm working in Professor Dupree’s laboratory as a Postdoctoral Research Associate on a very exciting project, where our goal is to manipulate polysaccharides synthesis by developing genetic tools expressing different GT activities (and other required activities) under tissue specific promoters to evaluate whether it’s possible to engineer polysaccharide synthesis, proportions/structures and assess the consequences of these changes in the extracellular matrix.
**Dr Bruno Martins**
Postdoctoral Fellow, Locke Lab, University of Cambridge

I am a post-doctoral researcher in James Locke's group at the Sainsbury laboratory. I am interested in how cells discriminate between different environmental states, integrate dynamic outputs from different gene circuits, and make decisions. In my current research, I use a combination of theory and time-lapse microscopy experiments to understand the dynamical coupling of the cyanobacterial circadian clock to other networks, in both endogenous and synthetic systems.

Before coming to Cambridge, I did a PhD in Peter Swain's lab at the University of Edinburgh. In my PhD I used mathematical modelling to gain insight into two simple, yet ubiquitous, sensing and transductions mechanisms: allosteric sensing and phosphorylation-dephosphorylation cycles. I studied the input-output dynamics of these mechanisms in terms of the fundamental constraints inherent in their design.

---

**Dr Francisco Navarro**
Postdoctoral Fellow, Baulcombe Lab, University of Cambridge

Frant's work focuses on the function of small RNA (sRNA) molecules and their use as regulatory elements in synthetic gene circuits. sRNA molecules most likely evolved as a defense mechanism against viruses and retro-transposons, and were co-opted for fine-tuning of gene expression. Their small size and predictable targeting rules make them perfect tools for regulating gene expression in synthetic gene circuits. This project is carried out in the green alga Chlamydomonas reinhardtii, which is amenable to genetic manipulation and possesses a sRNA pathway that resembles that of higher plants. Chlamydomonas provides a testbed for plant RNA-based genetic devices.

Fran completed his PhD in the laboratory of Prof. Jose Manuel Siverio (University of La Laguna, Spain), studying nitrate assimilation in Hansenula polymorpha, a methylotrophic yeast with important biotechnological applications. This was followed by a postdoc in the laboratory of Sir Paul Nurse, at The Rockefeller University, USA, and the London Research Institute, on cell size control and regulation of gene expression by RNA-binding proteins in Schizosaccharomyces pombe.

---

**Linda Silvestri**
Research Technician, Haselhoff Lab, University of Cambridge

As the Research Technician for the Haselhoff group, I work closely with Susana Sauret-Gueto, Research Lab Manager, to ensure the smooth running of the lab. I am responsible for Marchantia polymorpha tissue culture and 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 joined us for 8 weeks to work on this project and I helped with her supervision and provided laboratory training.

---

**Dr Lukas Müller**
Postdoctoral Fellow, Webb and Haseloff Labs, University of Cambridge

I'm interested in the circadian clock and its effect on physiological and agricultural performance in plants. In the OpenPlant project I am investigating the circadian clock in Marchantia polymorpha and analyze the regulation of clock behavior and outputs in this relative of early land plants. In particular, I am focusing on the primary metabolism as an excellent proxy for systemic processes and vegetative growth.

I apply fluorescent imaging tools with computational time-lapse analysis to obtain cell-specific read-outs for the whole plant in real-time. This data is intended to set the stage for both physiological engineering and systems biology approaches. Part of my project is to engineer fluorescent proteins that are standardised and improved reporters for dynamic changes in gene expression.

---

**Dr Noam Chayut**
Postdoctoral Fellow, Martin Lab, John Innes Centre

I am interested in the interface between applied plant breeding and plant metabolism. In my master's thesis we used classical breeding of passionfruit with the goal of releasing new varieties, now used by farmers. In my PhD thesis we studied carotenoid metabolism in melons and established a molecular marker now used routinely by melon breeders around the world. Recently, we suggested a novel non-transgenic path toward pro-vitamin A carotenoid biofortification of food crops. The objective of the current OpenPlant project is to develop pre-breeding lines of beetroot for the production of L-DOPA.

L-DOPA is used to treat Parkinson's symptoms, however, the current costs of chemical synthesis make it unavailable for deprived populations worldwide. L-DOPA, a product of tyrosine hydroxylation, is an intermediate metabolite in biosynthesis of violet and yellow betalain pigments, in Beta vulgaris (table beet). L-DOPA natural steady state levels are very low, usually undetectable. We intend to block the turnover of L-DOPA in beetroot to allow its accumulation to levels that could enable low-tech accessible production in a plant system.

---

**Louis Wilson**
PhD student, Dupree lab, University of Cambridge

I started as an OpenPlant PhD student at the University of Cambridge in September 2016. I am interested in all parts of plant biochemistry, but my projects tend to focus on the characterization and manipulation of enzymes and catalytic pathways.

In my first rotation project, I worked with Prof. Alison G Smith in Cambridge on metabolic gene clusters, developing methods for the expression of higher plant clusters in algae and yeast, and the detection of potential clusters endogenous to algae themselves. I am working with Paul Dupree to study and engineer cell wall-modifying enzymes for improved crops, food and materials. I have been using OpenPlant heterologous expression systems and a transient expression construct from the Lomonossoff lab to assess the stability of glycosyltransferases in vitro, with the aim of finding better enzymes for further study and exploitation. Increasing our understanding of these enzymes may ultimately permit the creation of designer fibres and saccharides, as well as being able to manipulate the properties of plant cell walls.
Dr. Susana Sauret-Gueto  
Postdoctoral Fellow, Smith Lab, University of Cambridge  
Dr. Susana Sauret-Gueto is an experienced molecular biologist and microscopist, with a scientific background in plant growth and development. In the OpenPlant Cambridge laboratory, she coordinates the establishment of semiautomated workflows to accelerate the generation and characterisation of genetically engineered Marchantia lines. Susana is establishing a new facility for robotic liquid handling around the Echo acoustic liquid handler, and an advanced microscopy facility. The microscopy hub includes a Keyence digital microscope for real-time 3D reconstruction of Marchantia plants, as well as a series of fluorescent microscopes with different resolution capabilities, including a Leica SP8 confocal microscope. She is specially interested in the sector analysis project in order to dissect gene function and autonomy at the cell and tissue level. Susana is also the main organiser of the ROC Group (Researchers with OpenPlant Cambridge), which brings together synthetic biology researchers from across Cambridge.

Dr. Orr Yarkoni  
Postdoctoral Fellow, Ajioka Lab, University of Cambridge  
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. 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 Marchantia 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 Marchantia plastid, consisting of a 13 fluorescent reporters across a wide spectrum ranging from near UV to near infrared.

Dr. Aytung Tuncel  
Postdoctoral Fellow, Haseloff Lab, University of Cambridge  
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 microbiod 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 crisper-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.

Dr. Michael Stephenson  
Postdoctoral Fellow, Osbourn Lab, 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 expertise in small organic molecule extraction, purification, and structural characterisation. This strengthens the group’s ability to functionally characterise biosynthetic enzymes; something which is important for many areas of research within the Osbourn lab. As a medicinal chemist I am interested in applying these techniques to engineer chemical diversity and to explore the structure activity relationships of bioactive triterpenes. I have been involved in isolating and characterising several novel triterpenes structures arising from co-expression of ‘un-natural’ combinations of biosynthetic enzymes. In addition, I have solved the structure of a number of novel and usual triterpene scaffolds, produced by oxidosqualene cyclases under investigation within the group.

Dr. Ivan Reyna-Llórenes  
Postdoctoral Fellow, Hibberd Lab, University of Cambridge  
My research involves using synthetic biology and evolution for improving agricultural traits, more specifically to improve photosynthesis. C3 photosynthesis can be very inefficient as Rubisco interacts with oxygen 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. 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.

Dr. Oleg Raitskin  
Post-doc, Patron Group, Earlham Institute  
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 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 have 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 an editing essay in plant protoplasts.
**Dr Benjamin Lichman**  
Postdoctoral Fellow, O’Connor Lab, John Innes Centre  

Plants are incredible chemical factories, capable of producing a host of complex molecules that synthetic chemists struggle to produce. These compounds are produced by plants to interact with their environment, but they also have great significance for humans, as we use them for fragrances, agrochemicals and medicines. This knowledge can be used to produce natural products and novel chemicals in microbial or plant-based platforms. I am currently working on the design of innovative approaches to plant-based production of proteins and metabolites. 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.

---

**Dr Hanz-Wilhelm Nützmann**  
Postdoctoral Fellow, Osbourn Lab, John Innes Centre  

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.

---

**Bernardo Pollak**  
PhD student, Haseloff Lab, University of Cambridge  

I am a final year PhD student at Plant Sciences in the Haseloff lab, with a BA in Biochemistry from Pontificia Universidad Catolica de Chile. I am studying the molecular genetics involved in meristem establishment and maintenance in the simple plant model system Marchantia polymorpha. I have experience with next-generation sequencing technologies, bioinformatics and parts expression in Marchantia gemmae. In collaboration with Bernardo Pollak, he has developed an open source gene-centric database platform for managing genome data and synthetic DNA parts for Marchantia. He maintains a strong interest in engineering approaches to biological problems and exploits his considerable expertise with electronics, optics and 3D printing to build and modify instrumentation for observing Marchantia cell dynamics.

His PhD research combines the construction of new marker genes, expression in Marchantia gemma, quantitative imaging and software analysis in order to map the dynamics of growth in gemmae. He has found evidence of long distance control of cell proliferation which can be deregulated by surgical manipulations.

---

**Dr Eva Thuememann**  
Postdoctoral Fellow, Lomonossoff Lab, John Innes centre  

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.

In addition to my research project, I was involved in the planning stages for the new John Innes Centre spin-out, Leaf Systems International Ltd, which opened on the Norwich Research Park in January 2017 and will enable translation of research to industry through scale-up of plant-based production of proteins and metabolites. 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.

---

**Dr Hanz-Wilhelm Nützmann**  
Postdoctoral Fellow, Osbourn Lab, John Innes Centre  

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.

---

**Mihails Delmans**  
PhD Student, Haseloff Lab, University of Cambridge  

Mihails is a 3rd year PhD student, with an Engineering background as an undergraduate. His research topic is the regulation of cell proliferation in Marchantia gemmae. In collaboration with Bernardo Pollak, he has developed an open source gene-centric database platform for managing genome data and synthetic DNA parts for Marchantia. He maintains a strong interest in engineering approaches to biological problems and exploits his considerable expertise with electronics, optics and 3D printing to build and modify instrumentation for observing Marchantia cell dynamics.

His PhD research combines the construction of new marker genes, expression in Marchantia gemma, quantitative imaging and software analysis in order to map the dynamics of growth in gemmae. He has found evidence of long distance control of cell proliferation which can be deregulated by surgical manipulations.

---

**Dr Jenni Rant**  
SAW Trust Coordinator  

Whilst training as a PhD student and working as a plant pathologist at the John Innes Centre, Jenni became interested in science communication and spent time out of the laboratory volunteering for the Science Art and Writing (SAW) Trust (reg charity no.1113386). Twelve years on and she has transitioned to running SAW fulltime as a social enterprise specialising in working with researchers on the design of innovative outreach activities. SAW delivers cross-disciplinary projects, providing accessible and inclusive starting points for people with varied interests and learning styles to explore scientific concepts and cutting edge research themes. SAW works in partnership with OpenPlant to deliver a range of activities, including workshops in schools, with adult groups, exhibits at science festivals and music festivals. We have also worked with SynthSys and the UK Centre for Mammalian Synthetic Biology at the University of Edinburgh to train scientists, teachers, writers and artists in the delivery of SAW workshops. See www.sawtrust.org for more information about work with OpenPlant.

---

**Dr Bernardo Pollak**  
PhD student, Haseloff Lab, University of Cambridge  

I am a final year PhD student at Plant Sciences in the Haseloff lab, with a BA in Biochemistry from Pontificia Universidad Catolica de Chile. I am studying the molecular genetics involved in meristem establishment and maintenance in the simple plant model system Marchantia polymorpha. I have experience with next-generation sequencing technologies, bioinformatics and parts expression in Marchantia gemmae. In collaboration with Bernardo Pollak, he has developed an open source gene-centric database platform for managing genome data and synthetic DNA parts for Marchantia. He maintains a strong interest in engineering approaches to biological problems and exploits his considerable expertise with electronics, optics and 3D printing to build and modify instrumentation for observing Marchantia cell dynamics.

His PhD research combines the construction of new marker genes, expression in Marchantia gemma, quantitative imaging and software analysis in order to map the dynamics of growth in gemmae. He has found evidence of long distance control of cell proliferation which can be deregulated by surgical manipulations.