PLASTID PREVIEW 2023

7th and 8th of September 2023

New College, University of Oxford

Oxford, OX1 3BN

Organisers:

Jani Bolla, University of Oxford (jani.bolla@biology.ox.ac.uk)

Paul Jarvis, University of Oxford (paul.jarvis@biology.ox.ac.uk)

Locations

Venue:

The entire conference will be in New College.

Transportation:

New College is within walkable distance of Oxford train station and main bus station. Taxis are available outside the stations if you prefer getting to the college by taxi.

Accommodation:

We have rooms (B&B) booked (for those of you indicated on the form) on the main site of New College. Please see the attached welcome guidance in the email.

Registration:

Registration will be in the North Undercroft.

Presentations:

All talks will be in Lecture room 6.

Poster Session and Tea/Coffee sessions:

Poster session and Tea/coffee sessions will be in the North Undercroft.

Meals:

All meals will be in the main Hall.

Wifi:

Network: NC-Conference network

Password: Penny-Housing-Sheep

Please see attached guidance in the email for more details

New College

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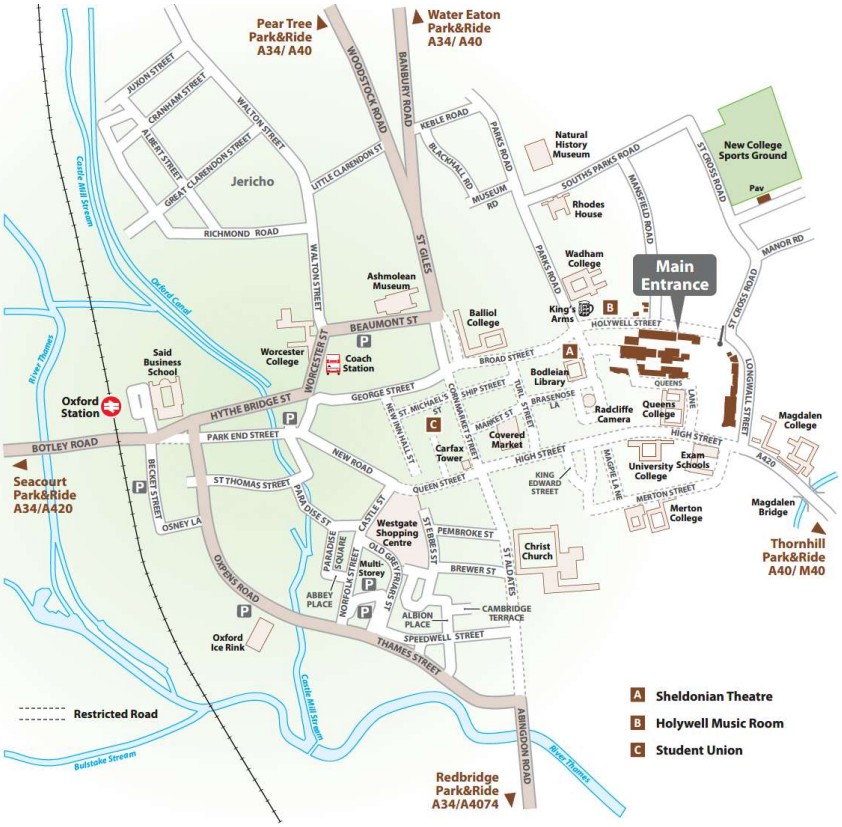

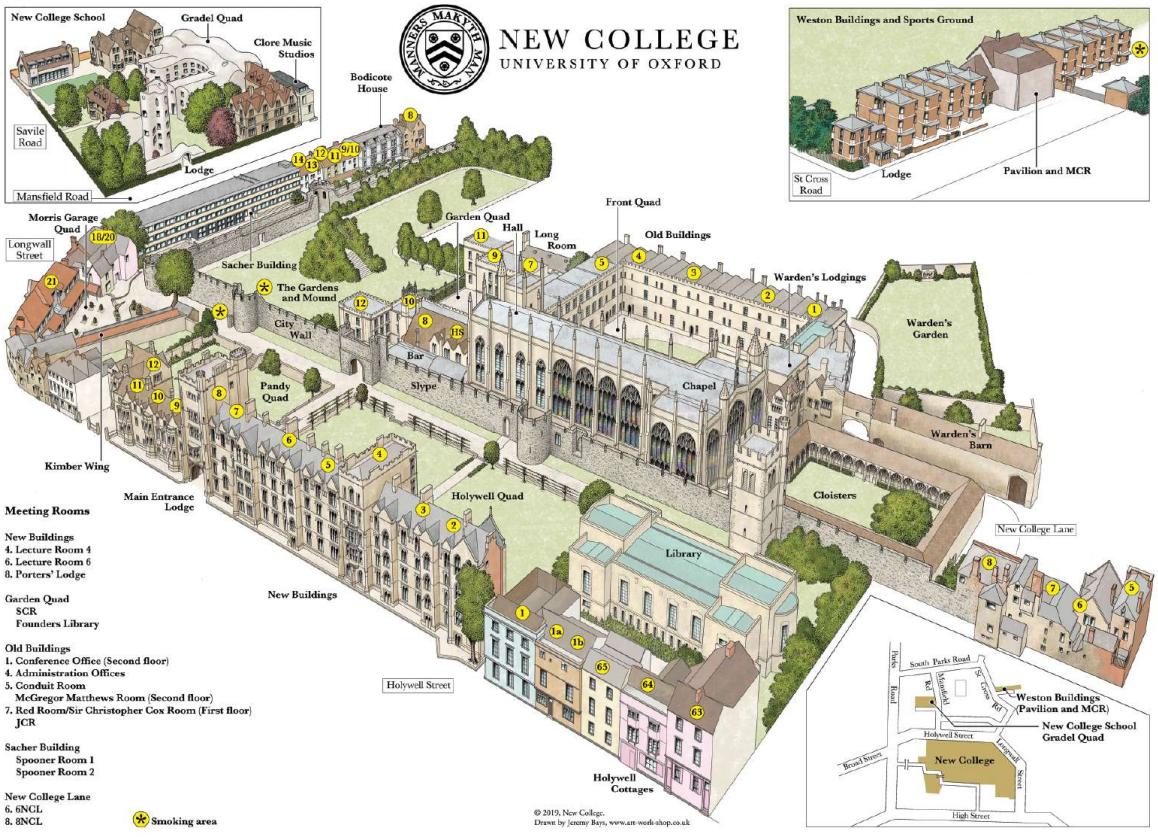

Programme

Thursday 7th September

13:00 – 14:00 Accommodation check-in, welcome refreshments, registration, poster hanging

14:00 – 14:10 Opening remarks (Jani Bolla and Paul Jarvis)

Session I: Photosynthesis and Chloroplast biogenesis

Chair: Yi Sun, University of Oxford

14:10 – 14:30 Jacques Bouvier, University of Oxford
The "rubisco paradox": why is the world's most important enzyme inefficient?

14:30 – 14:50 Audrey Short, University of California, Berkeley
Kinetics of the Xanthophyll Cycle and its Role in the Photoprotective Memory and Response

14:50 – 15:10 Changsong Ri, University of Oxford
Genetic Analysis of Chloroplast Biogenesis in *Arabidopsis thaliana*

15:10 – 15:30 Tamara Hernández-Verdeja, Lancaster University
The role of GLKs in C2 photosynthesis

15:30 – 16:00 Coffee break

16:00 – 16:30 **Workshop**
Gogulan Karunanithy, Refeyn Ltd
Analysing Proteins, their Complexes and Interactions using Mass Photometry

16:30 – 16:50 Poster flash talks, 4 mins each with 1-2 questions

Declan Perry (Royal Holloway University of London)
Ziad Soufi (University of Oxford)
Julanie Stapelberg (University College London)
Ana Lobo (Lancaster University)

16:50 – 18:00 Poster session

18:00 – 19:00 Drinks reception

19:00 – 22:00 Conference dinner

Friday 8th September

08:00 – 09:00 Breakfast

Session II: Chloroplast biology

Chair: Tamara Hernandez-Verdeja, Lancaster University

09:00 – 09:20 Sreedhar Nellaepalli, University of Oxford
Toc75 orchestrates the biogenesis and assembly of two TOC protein import complexes in plastids

09:20 – 09:40 Ángel Vergara-Cruces, John Innes Centre

Structure and function of the plastid-encoded RNA polymerase (PEP)

09:40 – 10:00 Na Li, University of Oxford
Recruitment of Cdc48 to chloroplasts by a UBX-domain protein in chloroplast-associated protein degradation

10:00 – 10:15 Poster flash talks

Simon Thomson (University of Oxford)
Adrian Barbrook (University of Cambridge)
Sabina Musial (University of York)

10:15 – 11:40 Coffee break and poster session

Session III: Crop improvement

Chair: Jun Fang, University of Oxford

11:40 – 12:00 Rose McNelly, John Innes Centre
Starch Trek: Exploiting Natural Variation in *Aegilops tauschii* to Discover Genetic Factors Influencing Cereal Endosperm Starch

12:00 – 12:20 Thomas Navarro, John Innes Centre
Profiling the mRNA and microRNA transcriptome of diploid potato to understand the regulation of starch metabolism

12:20 – 14:00 Lunch

Session IV: Synthetic biology

Chair: Adrian Barbrook, University of Cambridge

14:00 – 14:20 Abigail Perrin, University of York
CyanoTag: Novel insights from high-throughput protein tagging in photosynthetic bacteria

14:20 – 14:40 Ravendran Vasudevan, University of Cambridge
RNA engineering for gene expression in Marchantia chloroplasts

14:40 – 15:00 Angelo J. Victoria, University of Edinburgh
Engineering the highly productive cyanobacterium Synechococcus sp. PCC 11901

15:00 – 15:20 Onyou Nam, University of York
Identifying the proteins underpinning the diatom pyrenoid using high-throughput fluorescent protein tagging in the diatom *Thalassiosira pseudonana*

15:20 – 15:50 Coffee break

Session V: Synthetic biology continued

Chair: Gaurav Kumar, University of York

15:50 – 16:10 Luyao Yang, University College London
Could transgenic microalga *Chlamydomonas reinhardtii* boost shrimp growth and protect shrimp from viral diseases?

16:10 – 16:30 Darius Kosmützky, University of Cambridge
Stayin' Alive in the DISCO Light: Unravelling the Mystery of Cytochrome c6A in *Chlamydomonas reinhardtii*

16:30 – 17:00 Concluding remarks/Poster and presentation prize giving

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Talk Abstracts

Photosynthesis/Chloroplast Biogenesis

The "rubisco paradox": why is the world's most important enzyme inefficient?

Jacques William Bouvier [1], *Steven Kelly*[1]

[1] *Department of Biology, University of Oxford, Oxford, UK*

Rubisco assimilates CO2 to form the sugars that fuel life on earth. However, despite representing the primary entry point for carbon into the biosphere, rubisco is a surprisingly inefficient catalyst which exhibits a modest CO2 turnover rate and a costly secondary reaction with O2. Combined, these shortcomings of rubisco present an evolutionary paradox: why does this enzyme of paramount biological importance appear poorly adapted for its primary role in CO2 fixation? To help answer this question, we have performed a phylogenetically resolved analysis of the molecular and kinetic evolution of Form I rubisco. We demonstrate that the rubisco kinetic trait trade-offs previously proposed to explain the above rubisco paradox are weaker than previously thought when accounting for phylogenetic biases in the data. In actual fact, we discover that a previously unidentified phylogenetic constraint has played a more significant role in limiting rubisco adaptation compared to the combined action of all kinetic trait trade-offs. When we investigated the source of this phylogenetic constraint, we reveal that it is primarily driven by a slow pace of molecular evolution in the rubisco large subunit. Thus, although canonical kinetic trait trade-offs exist, these are less severe than previously thought and co-limit rubisco adaptation in combination with its slow pace of molecular sequence evolution.

Kinetics of the Xanthophyll Cycle and its Role in the Photoprotective Memory and Response

Audrey Short, [1, 2, 3], *Thomas P. Fay,*[4] *Thien Crisanto,* [2, 5, 6] *Ratul Mangal,*[4] *Krishna K. Niyogi,* [2, 5, 6] *David T. Limmer,* [3, 4, 7, 8] *and Graham R. Fleming* [1, 2, 3, 4]

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[7)] *Chemical Science Division Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA*

[8)] *Material Science Division Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA*

Light levels fluctuate in nature frequently, and the survival of photosynthetic organisms depends on their ability to adjust to sudden changes in light intensity. Plants and algae need to balance photochemistry and photoprotection to maximize efficient energy use in all light conditions. Photosynthetic organisms have evolved several mechanisms to dissipate excess energy through non-photochemical quenching means, minimizing the formation of reactive oxygen species to prevent damage. These processes occur on or across the thylakoid membrane within chloroplasts. In the alga *Nannochloropsis oceanica* we observed an ability to respond rapidly to sudden increases in light level

which occur soon after a previous high-light (HL) exposure. This ability implies a kind of memory of recent (1 min- 1 hour) HL exposure. In this work, we explore the xanthophyll cycle in *N. oceanica* as a photoprotective memory system. By combining snapshot fluorescence lifetime measurements with a biochemistry-based quantitative model, we show that both short-term and medium-term "memory" arises from the xanthophyll cycle. In addition, the model enables us to characterize the relative quenching abilities of the three xanthophyll cycle components. Given the ubiquity of the xanthophyll cycle in photosynthetic organisms the model described here will be of utility in improving our understanding of vascular plant photoprotection with important implications for crop productivity.

Genetic Analysis of Chloroplast Biogenesis in *Arabidopsis thaliana*

Changsong Ri[1], *R. Paul Jarvis*[1]

[1]*Department of Biology, University of Oxford, UK*

Chloroplasts are organelles ubiquitously found in plants and algae, which are photoautotrophic. They are primarily responsible for photosynthesis, which plays a vital role in the survival and development of photoautotrophic eukaryotic systems. Since chloroplasts are separate but dependent entities, their biogenesis and development are synchronised with the global biogenesis and development of the entire cell and the whole plant. Chloroplast biogenesis and development are thus exceedingly intricate and complicated processes. Their development is regulated by a variety of intricate signalling and regulatory networks. As of now, only a limited amount of information about chloroplast biogenesis and development has been elucidated. The present study aims to explore the regulatory factors which govern the chloroplast biogenesis and development in *Arabidopsis thaliana*. In *Arabidopsis thaliana*, EMS mutants, indicative of novel mechanisms related to chloroplast biogenesis, were found and forward genetic screens were carried out to find out the causative gene loci. The ongoing research will investigate the mechanism by which causative genes influence chloroplast biogenesis and development.

The role of GLKs in C2 photosynthesis

Tamara Hernández-Verdeja[1], *George Rumble & Marjorie Lundgren*[1]

[1]*Lancaster Environment Centre, Lancaster University, LA1 4YQ*

C2 photosynthesis is a carbon concentrating mechanism (CCM) with biotechnological potential to improve photosynthetic efficiency in C3 crops. Engineering a C2 phenotype into C3 crops will require the development of photosynthetically functional chloroplasts in bundle sheath cells. Currently, the best candidates to turn small BS plastids into photosynthetically active chloroplast are Golden2-like (GLK) transcription factors, which are master regulators of chloroplast development. Moreover, we know that constitutive overexpression of GLKs in rice results in BS cells with larger mitochondria with active GDC, enhanced photosynthetic enzymes, and increased plasmodesmatal connections to the mesophyll, which are all intrinsic characteristics of C2 leaves. Therefore, to clarify the role of GLKs in the evolution of photosynthetic diversity, we are presenting an updated GLK phylogenetic tree to include new lineages and C2 species for the first time. This will identify whether specific motifs or gene number are associated with specific photosynthetic types. We will also present our study of C2 phenotypic traits in GLK over-expressor and knock-down lines of Arabidopsis to discuss the potential for GLK overexpression to recreate C2 leaf characteristics in a eudicot species.

Workshop

Analysing Proteins, their Complexes and Interactions using Mass Photometry

***Gogulan Karunanithy*[1]**

[1]*Refeyn Ltd, Unit 9, Trade City, Sandy Ln W,Oxford OX4 6FF, United Kingdom*

Mass photometry is a novel, label-free analytical tool based on detecting light scattered by single molecules, enabling the monitoring of the molecular mass of biomolecules in solution. This technique can analyze small (µL) sample volumes and concentrations (100 pM up to 100 nM) under native conditions within a short analysis time of a few minutes. Mass photometry is very versatile and can be used in protein sample characterisation, the analysis of protein complexes as well as interaction studies. Compared with alternative techniques, mass photometry is rapid, requires minimal sample and few optimization steps.

In this talk we will introduce the principles behind mass photometry, demonstrate a range of applications and showcase some of the latest advances in the technique.

Chloroplast Biology

Toc75 orchestrates the biogenesis and assembly of two TOC protein import complexes in plastids

Sreedhar Nellaepalli*[1], Ziad Soufi[1], Sireesha Kodru[1], Shuyang Cheng[1], Jun Fang[1], Yi Sun[1], Pablo Pulido[2], Paul Jarvis[1]*

[1] *Section of Molecular Plant Biology, Department of Biology, University of Oxford, Oxford OX1 3RB, UK*

[2] *Department of Plant Molecular Genetics, CNB-CSIC, Madrid, Spain*

The plastid proteome is dynamic in plants reflecting the transitions of plastids between forms (green and non-green) regulated by developmental and environmental cues. The plastid protein import apparatus plays a key role in determining the proteome of plastids. A multiprotein complex, TOC, consisting of a channel protein, Toc75, and two intimately associated preprotein receptors (isoforms of Toc33 and Toc159), drives protein import across the outer envelope membrane of plastids. Although components of the TOC complex are well-studied, little is known about the existence of different TOC complex configurations in different plastid types and their biogenesis processes. Here we report that Toc75 is the master player in the biogenesis of the TOC complex, controlling the assembly and stability of all the receptor proteins. It forms a photosynthetic-type TOC configuration with Toc33 and Toc159 (TOC-P) in green plastids, chloroplasts, and a non-photosynthetic-type TOC configuration with Toc34 and Toc132/120 (TOC-NP) in non-green plastids, leucoplasts. Functionally, the preference for different preprotein substrates is the key difference between TOC-P and TOC-NP. We propose a step-wise assembly process of the TOC complex in chloroplasts, in which Toc75 is the initial component. Then, Toc75 is stabilized by the Toc33 component to form a Toc75-Toc33 module, which final integrates the Toc159 component to reach a mature TOC configuration.

Structure and function of the plastid-encoded RNA polymerase (PEP)

Ángel Vergara-Cruces*[1], Ishika Pramanick[1], David Pearce[1], Matt Byrne[2] and Michael Webster[1]*

[1] *Department of Biochemistry and Metabolism, John Innes Centre, Norwich, UK*

[2] *Electron Bio-Imaging Centre, Didcot, UK*

The plastid-encoded RNA polymerase (PEP) is a key element of chloroplast gene expression: it transcribes plastid genes, some of which encode components of the photosynthetic complexes. PEP is a 1 MDa multi-subunit complex with an enzymatic core similar to bacterial RNA polymerase and at least 12 associated proteins (PAPs). PAPs are not found in bacterial RNA polymerases, but they are

necessary for PEP to be functional. Mutations in PAP proteins result in disruption of chloroplast biogenesis; plants carrying these mutations are albino or pale green and are unable to complete their life cycle. However, we do not yet know the exact role of PAPs in PEP activity. In order to address this knowledge gap, we need a molecular understanding of how the transcriptional activity of PEP is regulated.

We purified native PEP from the plastids of *Sinapis alba* (white mustard). Using single-particle cryo-EM, we obtained the first model of the full PEP complex at high resolution. This revealed extensive interactions between the core subunits and PAPs. Based on the density map, we identified new subunits as constitutive components of the PEP complex. Future work will further characterise the architecture of transcriptional complexes by a number of biophysical, biochemical and structural techniques. This will yield a more sophisticated understanding of plastid gene expression, which ultimately will further our comprehension of plastid biology, and potentially find applications in the engineering of plastids.

Recruitment of Cdc48 to chloroplasts by a UBX-domain protein in chloroplast-associated protein degradation

***Na Li*[1]** *& R. Paul Jarvis[1]*

[1]Section of Molecular Plant Biology, Department of Biology, University of Oxford, Oxford, United Kingdom

The translocon at the outer chloroplast membrane (TOC) functions as the gateway for chloroplast protein import, and so it is vital for photosynthetic establishment and plant growth. Chloroplast-associated protein degradation (CHLORAD) is a ubiquitin-dependent proteolytic system that regulates the TOC apparatus. In CHLORAD, Cdc48 provides motive power for the retrotranslocation of ubiquitinated TOC proteins to the cytosol. Here, we identify the plant UBX-domain protein PUX10 as a component of the CHLORAD machinery. We show that PUX10 is an integral chloroplast outer membrane protein that projects UBX and ubiquitin-associated (UBA) domains into the cytosol. It interacts with cytosolic Cdc48 via its UBX domain, recruiting it to the chloroplast surface; and with ubiquitinated TOC proteins via its UBA domain. Genetic analyses in Arabidopsis revealed a requirement for PUX10 in the CHLORAD-mediated regulation of TOC function and plant development. Thus, PUX10 coordinates the ubiquitination and retrotranslocation activities of CHLORAD to enable efficient TOC protein turnover.

Crop improvement

Starch Trek: Exploiting Natural Variation in *Aegilops tauschii* to Discover Genetic Factors Influencing Cereal Endosperm Starch

Rose McNelly*[1], Jesús Quiroz Chávez[2], Ruth Franklin[1], David Gilbert[1], Amy Briffa[3], Sanu Arora[1], Alexander Watson-Lazowski[1], Cristobal Uauy[2] and David Seung[1]***

[1] Department of Biochemistry and Metabolism, John Innes Centre

[2] Department of Crop Genetics, John Innes Centre

[3] Department of Computational and Systems Biology, John Innes Centre

Cereal endosperm starch is of huge commercial importance, providing 25% of calories in our diets, whilst also being extensively used in the production of paper, biodegradable polymers and biofuels. In the grains of the Triticeae (e.g., wheat, barley and rye), there are two types of granules: large, lenticular

A-type granules and smaller spherical B-type granules. These are both synthesized in non-photosynthetic plastids called amyloplasts. The size distribution and composition of starch granules influences grain quality, but the mechanisms determining these traits remain poorly understood. Here we have characterised endosperm starch in a diversity panel of *Aegilops tauschii*, the wheat D-genome progenitor. We have discovered huge variation in starch granule size distributions within the panel, with examples of novel variation not found in modern wheat cultivars. This large intraspecies variation has been exploited in a genome wide association study, allowing us to identify novel quantitative trait loci on chromosomes 3, 5 and 7 influencing B-type granule diameter and additional loci on chromosome 3 influencing B-type granule number. These results reveal that starch granule size and number are under independent genetic control and can both be exploited in wheat breeding. Candidate genes within these regions have been identified and are being pursued to provide new mechanistic insights into starch granule formation in crops.

Profiling the mRNA and microRNA transcriptome of diploid potato to understand the regulation of starch metabolism

Thomas Navarro[1] *and David Seung*[1]

[1]*Department of Biochemistry and Metabolism, John Innes Centre*

Potatoes are among the most important food crops globally and genetic approaches to improve nutritional and post-harvest quality are of great interest. However, in the common tetraploid potato, the highly heterozygous genome, inbreeding depression, and long generation cycles pose challenges for genetic improvement. Inbred diploid lines are emerging as a promising breeding platform for generating high-performing hybrid potato and are particularly suitable for gene-editing technologies as they provide a means to cross-out editing transgenes as well as a route to introduce target traits into commercial germplasm. However, due to their novelty, metabolic pathways relevant to key quality traits in the tuber have not yet been studied in these lines.

Starch is the major carbohydrate in potatoes. It is produced during tuber development and is partially degraded post-harvest during cold storage. To understand the regulation of these processes in diploid potato, we generated an mRNA and microRNA (miRNA) transcriptome dataset using RNA sequencing. We grew a diploid potato research line in the glasshouse until tuber maturation and harvesting, and then kept the tubers at low temperatures to simulate cold storage. We sampled leaves and developing tubers at three different stages of development, as well as two stages of cold storage. By analysing and comparing gene expression patterns, we are developing a broad understanding of the genes that mediate key processes of starch biosynthesis and degradation in different tissues, as well as the role of miRNAs in gene regulation in potato tubers.

Synthetic Biology

CyanoTag: Novel insights from high-throughput protein tagging in photosynthetic bacteria

Abigail Perrin[1]*, Guoyan Zhao*[2]*, Matthew Dowson*[1]*, Adam Dowle*[1]*, Grant Calder*[1]*, and Luke Mackinder*[1]*.*

[1] *Department of Biology, University of York, UK*

[2] *Shandong Normal University, China*

Photosynthetic bacteria are fundamentally important to aquatic ecosystems and global carbon cycling and have exciting potential for use in sustainable bioindustries. They can also be used as a genetically tractable model system to understand aspects of fundamental plant and algal biology. However, much

of their basic biology is not yet understood and their genomes still contain large numbers of uncharacterised protein-coding genes. To accelerate our understanding of these essential microorganisms we have developed a high-throughput pipeline for the scarless integration of tags that facilitate protein localisation, expression and interactome profiling in the model cyanobacterium *Synechococcus elongatus* PCC7942. We have now used this CyanoTag platform to fluorescently label around 20% (>500 proteins) of the S. elongatus proteome. Localisation of these proteins via super-resolution imaging has provided exciting novel biological insights into a diverse range of processes including the regulation of photosynthesis and cell division. By analysing our CyanoTag lines with an affinity-purification mass-spectrometry based approach we have also mapped a preliminary interactome. We are now expanding the CyanoTag library to target the whole *S. elongatus* proteome, and exploring the ways in which this resource may be best used and shared to facilitate novel discoveries within the wider scientific research community.

RNA engineering for gene expression in Marchantia chloroplasts

Ravendran Vasudevan[1] *and Jim Haseloff*[1]

[1]*Department of Plant Sciences, University of Cambridge, UK.*

Over the last several decades, plants have been developed as a platform to produce useful recombinant proteins, where the plastid offers many advantages for production of foreign proteins. Plastids can promote very high levels of gene expression due to the presence of high numbers of genome copies in the cell and lack of gene silencing. To fully exploit this potential, it is necessary to regulate, optimize and tune the expression of transgenes in the chloroplast. A most promising approach to date employs nucleus-encoded, chloroplast-targeted helical repeat RNA binding PPR proteins, that act post-transcriptionally to stabilize specific chloroplast mRNAs and activate gene expression. This type of approach, however, is complicated by interaction, and possible competition with endogenous regulatory networks. To avoid such complication, we have engineered RNA motifs that stabilize chloroplast mRNAs and enhanced translational efficiency in *Marchantia polymorpha* chloroplast.

Engineering the highly productive cyanobacterium Synechococcus sp. PCC 11901

Angelo J. Victoria[1,2], *Tiago Toscano Selão*[3], *José Ángel Moreno-Cabezuelo*[4], *Lauren A. Mills*[4], *Grant A. R. Gale*[1,2], *David J. Lea-Smith*[4], *Alistair J. McCormick,*[1,2]

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Synechococcus sp. PCC 11901 (PCC 11901) is a fast-growing marine cyanobacterial strain that has a capacity for sustained biomass accumulation to very high cell densities, comparable to that achieved by commercially relevant heterotrophic organisms. However, genetic tools to engineer PCC 11901 for biotechnology applications are limited. Here we describe a suite of tools based on the CyanoGate MoClo system to unlock the engineering potential of PCC 11901. First, we characterised neutral sites suitable for stable genomic integration that do not affect growth even at high cell densities. Second,

we tested a suite of constitutive promoters, terminators, and inducible promoters including a 2,4-diacetylphloroglucinol (DAPG)-inducible PhlF repressor system, which has not previously been demonstrated in cyanobacteria. Lastly, we developed a DAPG-inducible dCas9-based CRISPR interference (CRISPRi) system and a modular method to generate markerless mutants using CRISPR-Cas12a. Based on our findings, PCC 11901 is highly responsive to CRISPRi-based repression and showed high efficiencies for single insertion and multiplex double insertion genome editing with Cas12a. We envision that these tools will lay the foundations for the adoption of PCC 11901 as a robust model strain for engineering biology and green biotechnology.

Identifying the proteins underpinning the diatom pyrenoid using high-throughput fluorescent protein tagging in the diatom *Thalassiosira pseudonana*

***Onyou Nam*[1]** *and Luke Mackinder*[1]

[1] *Department of Biology, University of York, York, UK*

Diatoms are a diverse phytoplankton group responsible for up to 20% of global CO2 fixation. They run a CO2-concentrating mechanism from the aqueous environment to efficiently concentrate CO2 at the active site of Rubisco packaged in a subcellular compartment called the pyrenoid. Despite the importance of diatom CO2 fixation, there are many unknowns about diatom pyrenoid structure and function. Here, we aim to unveil the proteins underpinning the diatom pyrenoid using *Thalassiosira pseudonana* as a model. To understand the spatial distribution of the proteins, we established a high-throughput fluorescent protein (FP) tagging pipeline using modular cloning (MoClo) framework based on the Golden Gate system for episomal delivery via conjugation. In parallel, the potential pyrenoid proteins were identified by co-immunoprecipitation coupled with mass spectrometry (CoIPMS), using Rubisco, the major pyrenoid component, as bait. Subsequently, the identified proteins were fluorescently tagged, and their spatial distribution was analysed by confocal microscopy. Furthermore, we built a pyrenoid protein-protein interactome by utilising the FP-tagged lines as baits for affinity purification coupled with mass spectrometry (APMS). This study reports several uncharacterised proteins localised to distinct sub-regions of the *T. pseudonana* pyrenoid providing novel insights into diatom pyrenoid structure and function.

Could transgenic microalga *Chlamydomonas reinhardtii* boost shrimp growth and protect shrimp from viral diseases?

***Luyao Yang*[1*]**, *Patai Charoonnart*[2,3], *Harry Jackson*[1], *Brenda Parker*[4], *Vanvimon Saksmerprome*[2,3], *Saul Purton*[1]

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Keywords: Chlamydomonas; Chloroplast; Fish growth hormone (fGH); double stranded RNA (dsRNA)

Growth rates and disease control are two major limiting factors in aquaculture, but both could be addressed through oral delivery of affordable therapeutics. The edible microalga *Chlamydomonas reinhardtii* has emerged as a promising platform for producing recombinant therapeutics for the aquaculture industry. Fish growth hormones (fGHs) have been shown to promote the growth of fish and shellfish, and specific double-stranded RNA (dsRNA) molecules designed to key viral genes can serve as RNA-based vaccines. When taken up by animals, the dsRNA can trigger the RNA interference (RNAi) mechanism and produce small interfering RNA (siRNA) that silence viral genes. Traditional methods for delivering therapeutics in aquaculture involve purification, cold chain storage, and injection by hand, often making them technically challenging, prohibitively expensive and hindering widespread use. Our study sets out to produce fGHs and dsRNAs in the chloroplast of *C. reinhardtii* to develop a system for whole-cell bio-encapsulation and oral delivery. Initial studies this summer using shrimp as model will focus on the optimisation of fGHs and dsRNA administration doses, shrimp growth and viral challenge performance, as well as optimisation of a low-cost 'hanging bag' photobioreactor system used for scale-up production of the algae to produce sufficient dried biomass for further shrimp feeding trials.

Stayin' Alive in the DISCO Light: Unravelling the Mystery of Cytochrome c6A in *Chlamydomonas reinhardtii*

***Darius Kosmützky*[1]**, *Laura T Wey[2], Lauri Nikkanen[2], Yagut Allahverdiyeva[2] and Christopher J Howe[1]*

[1] Department of Biochemistry, Tennis Court Rd, University of Cambridge, United Kingdom

[2] Molecular Plant Biology, Department of Life Technologies, University of Turku, Finland

The electron transfer protein cytochrome c6 (c6) was believed to have been lost in plants until a ubiquitous and highly conserved homologue, named cytochrome c6A (c6A), was identified throughout the plant and green algal lineages. Despite its homology to c6, c6A cannot replace c6 (or plastocyanin) functionally in the photosynthetic electron transport chain (PETC). The function of c6A in green photosynthetic eukaryotes therefore remains unknown. To uncover the function of enigmatic c6A, a mutant strain of *C. reinhardtii* lacking c6A was tested for growth and photosynthetic performance under a variety of different high intensity and fluctuating light regimes, including a novel light regime we call DISCO Light - Darkness Interrupted by Short COntinuous Light phases. We found that under DISCO Light in photomixotrophy, the c6A knock-out strain showed a difference in growth due to altered photosynthetic ability. This phenotype was investigated using fluorescence and absorbance spectroscopy probing the functionality of both photosystems as well as the cytochrome b6f complex. Our results indicate that c6A plays an important role in modulating the photosystem and/or antennae composition under stressful fluctuating light conditions in *C. reinhardtii* and probably in all green photosynthetic eukaryotes. Ongoing research aims to elucidate the precise underlying mechanisms by which c6A operates.

Posters and Flash Talks

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1. [Flash] Green to the fullest: Genes controlling the total chloroplast compartment of photosynthetic cells

***Declan Perry*[1]**, *Suresh Leonel*[1], *Enrique Lopez-Juez*[1]

[1]*Royal Holloway University of London*

The population of chloroplasts within a plant mesophyll cell is present within the cytoplasm as a distinctive monolayer, which can be referred to as the chloroplast compartment. The opportunity to engineer the "chloroplast index", the proportion of the cell taken up by the chloroplast compartment, has significant applications towards food security. However, our understanding of the governing genetic factors responsible for its regulation was completely lacking until the identification of the REC genes, whose loss results in cells with a REduced Coverage or chloroplast index (Larkin et al. 2016, PNAS 113, E1116-E1125). To uncover novel genetic regulators responsible for the control of the chloroplast compartment, two genetic screens have been initiated. Within *Arabidopsis thaliana*, 35S activation-tagging and chemical mutagenesis by ethyl methanesulfonate (EMS) are being used to discover suppressor mutations of the rec phenotype. 35S activation-tagging mutagenesis is currently in progress, while EMS putative suppressor mutant demonstrating an enhanced greening phenotype have been identified, and their mesophyll cellular phenotype has been quantified and confirmed in one to present more abundant and less heterogeneous chloroplasts than the rec mutant cells do. Bulked segregant analysis through next generation sequencing is underway, to identify the position of a rec EMS suppressor mutation. The end goal is to utilize this knowledge to enhance the plastid compartment within specific cell types in organs of crop plants, with purposes ranging from improving the photosynthetic performance within leaf cells to improving the production of pigments within fruit pericarp cells.

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2. [Flash] Safeguarding wheat production by editing the CHLORAD pathway

Joseph Oddy[1], ***Ziad Soufi***[1], *Najiah Mohd Sadali*[2], *Qihua Ling*[3], *Paul Jarvis*[1]

[1]*Section of Molecular Plant Biology, Department of Biology, University of Oxford, Oxford, UK*

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[3]*National Key Laboratory of Plant Molecular Genetics, CAS Centre for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China*

Wheat is the second most widely consumed cereal worldwide, with wheat-based food products making up approximately 18% of all calories consumed every day. Climate change will likely decrease wheat yields due to increased extreme weather events, making yield forecasting more difficult and negatively impacting food security in vulnerable regions. Consequently, the global food system needs to implement strategies to safeguard wheat production. One potential way to achieve this, in part, is through the introduction of stress tolerant wheat varieties with consistent yields. Stay-green wheat varieties (varieties that stay photosynthetically active for longer than average) possess some of these high-yielding and stress tolerant qualities. Mutations in certain components of the chloroplast-associated protein degradation (CHLORAD) pathway have the potential to create the stay-green phenotype in wheat varieties. We are investigating how editing of the SP1 and SP2 genes (components of the CHLORAD system) impacts stay-green qualities in wheat. Future work will look at the

performance of edited SP1 and SP2 plants in the field and whether they are promising candidates to address upcoming challenges in commercial wheat cultivation.

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3. [Flash] **Exploring the production of super-sweet proteins in the chloroplast of Chlamydomonas**

Julanie Stapelberg[1] *and Saul Purton*[1]

[1]*Algal Research Group, Institute of Structural and Molecular Biology Department, University College London, Gower Street, London WC1E 6BT, UK*

The chloroplast of green microalgae, *Chlamydomonas reinhardtii* is being developed as a cellular factory for producing high value proteins. Super-sweet proteins could offer a range of health, economic and social benefits to society. The most characterised and FDA-approved is the natural sweet protein thaumatin from the West African plant, *Thaumatococcus daniellii*. Thaumatin is 100,000 times sweeter than sucrose and therefore represents an attractive alternative sweetener that would help address dietary issues such as obesity, diabetes and dental caries. Natural extractions from the plant is not feasible and producing thaumatin in expression systems, such as bacterial, yeast, plant and mammalian systems is challenging because thaumatin requires correct folding and the formation of its eight disulphide bonds for functionality. Furthermore, with its Generally Recognized as Safe (GRAS) status and its rich nutritional profile, the whole cell of *C. reinhardtii* may be utilised, mitigating downstream costs.

Utilising newly developed synthetic biology tools, ways to enhance thaumatin production within the chloroplast of *C. reinhardtii* have been explored. An iterative design-build-test-learn approach has been adopted to investigate innovative strategies such as targeting the transgene into different plastome loci, assessing the effect of various cis elements (promoters, etc.) on expression levels, and examining protein accumulation within the stroma and the lumen. Improved organoleptic properties have been achieved by producing a 'sweet Chlamy' in a 'yellow-in-the-dark' mutant. Further genetic attributes have been incorporated for strain improvement regarding safety, scalability, and taste enhancement. With the use of refined molecular tools and advanced strain optimisation, the latest advancements in cultivating the ""sweet Chlamy"" will be showcased as an innovative functional food ingredient.

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4. [Flash] **Rubisco regulation by chloroplast metabolites in rice**

Ana KM Lobo[1]*, Doug Orr*[1]*, Elizabete Carmo-Silva*[1]

[1] *Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK*

Rubisco activity often limits photosynthesis and crop yields. Sugar phosphate derivatives and other metabolites in the chloroplast can inhibit or affect the enzyme performance by binding to Rubisco catalytic or allosteric sites. Ancillary enzymes such as Rubisco activase and sugar phosphatases are required to remove and degrade the inhibitors and reactivate the enzyme. We assessed rice Rubisco activity in vitro in the presence of chloroplast metabolites individually to provide a holistic understanding of the active enzyme regulation and potential interaction with these compounds. Rubisco activity strongly decreased (80%) in the presence of inhibitory sugar phosphates (CABP – 2-carboxy-D-ribitol-1,5-bisphosphate and CA1P – 2-carboxy-D-arabinitol 1-phosphate). 3PGA (3-phosphoglyceric acid), the product of Rubisco carboxylation, and Glu (glutamate) decreased activity by ~25%. Most metabolites tested, which included photorespiratory intermediates, carbohydrates, and amino acids, did not directly affect Rubisco in vitro activity. By quantifying the apparent Vmax and Km for RuBP (at ambient O2), and docking analysis interactions, we concluded that sugar phosphates

and 3PGA inhibit Rubisco activity by binding tightly and loosely, respectively, to the catalytic sites (i.e., competing with the substrate RuBP). The interaction with glutamate is less clear but might negatively affect Rubisco activity by interacting with allosteric sites. These findings will aid the design and modelling of new strategies to improve the regulation of photosynthesis by Rubisco and enhance the efficiency and sustainability of rice production.

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5. [Flash] Characterising the CDC48 Adapter Proteins NPL4 and UFD1: are they involved in Chloroplast-Associated Protein Degradation?

***Simon Thomson*[1]** *and Paul Jarvis[1].*

[1] Department of Biology, University of Oxford, Oxford, UK

The vast bulk of the chloroplast proteome is imported post-translationally through protein translocation complexes called TOCs (translocon at the outer envelope of chloroplasts). In response to environmental and developmental cues, the TOC proteins are targeted by the UPS (ubiquitin-proteasome system) for degradation. This pathway – termed CHLORAD (chloroplast-associated protein degradation) – therefore acts as an important mechanism to modulate the chloroplast proteome. Membrane-embedded TOC proteins must first be extracted from the outer membrane before degradation by the cytosolic UPS. This is accomplished by retrotranslocation through a protein channel, with the motive force provided by an ATPase called CDC48. CDC48 functions in a variety of cellular processes at different subcellular locations. The localisation and activity of CDC48 is determined by a wide range of adapter proteins. In UPS processes, CDC48 is typically recruited by a heterodimer of NPL4 and UFD1. Putatively, there are two NPL4, and four UFD1 isoforms in the model higher plant *Arabidopsis thaliana*. To examine their potential role in CHLORAD, we present bioinformatic analysis on protein sequences and predicted structures; interaction and localisation experiments; and finally, a mutant analysis of knockout and overexpressing lines, examining developmental and stress responses. NPL4 and UFD1 proteins show a seemingly high degree of functional conservation with model eukaryotic orthologs, though some key differences are apparent. While NPL4 and UFD1 demonstrate interactions within the CHLORAD pathway, their functional significance requires much additional consideration in the future.

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6. [Flash] Evolution of the Dinoflagellate Chloroplast Genome

***Adrian Barbrook*[1]** *and Christopher Howe[1]*

[1]Department of Biochemistry, University of Cambridge

The chloroplast genomes of dinoflagellates have a large number of unusual features which set them apart from other algal lineages. Dinoflagellates which possess chloroplasts that contain the carotenoid peridinin have an extremely reduced and fragmented genome. Only genes for a small number of key photosynthetic proteins, as well as ribosomal rRNA genes, have been retained within the organelle. Furthermore, rather than residing on a large circularly mapping DNA circle, genes are found on much smaller plasmid-like DNA molecules termed minicircles. Typically, one gene is found per minicircle though some incidences of minicircles with a few genes have been discovered. We have extensively characterized this form of chloroplast genome by analysis two species; *Amphidinium carterae* and *Symbiodinium microadriaticum*. As well as revealing the extent of the genome and its organisation, we have shown the presence of highly conserved non-coding sequences that most likely function in replication and transcription of the genes, mapped the extent and processing of transcripts derived from the minicircles and shown the presence of extensive editing within certain species. More recently we have developed a transformation system for the chloroplast genome of *Amphidinium carterae* with

which we are now further defining the key elements of the genome. Within *Symbiodinium microadriaticum*, we are investigating how minicircle loss may shape the evolution of this species and can lead to a transition to heterotrophy.

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7. [Flash] **Biochemical characterisation of peripheral pyrenoid proteins of *Thalassiosira pseudonana***

Sabina Musiał[1], *Onyou Nam*[1], *Luke Mackinder*[1]

[1] *Centre for Novel Agricultural Products, Biology Department, University of York, UK*

Diatoms, like many other algae, operate CO2 concentrating mechanisms (CCM) – a cellular process allowing the supercharging of Rubisco with its substrate CO2. At the heart of the CCM is the pyrenoid – a lenticularly shaped Rubisco aggregation in the chloroplast. Our knowledge of the components and structure of the pyrenoid is limited. Recently, we identified a class of six structurally similar proteins localising to the periphery of the pyrenoid in *Thalassiosira pseudonana*. These proteins form a proteinaceous layer around the pyrenoid. We hypothesise that the presence of homologs modulates the different curvatures associated with the pyrenoid surface. This study focuses on characterising these pyrenoid surface proteins to understand their oligomeric state and interactions between the six proteins using biochemical approaches. Overall, this work will contribute to a more complete understanding of the diatom pyrenoid structure.

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8. Identification of a retro-translocation apparatus acting in CHLORAD

Anne Sophie Lau[1,2], *Yi Sun*[2], *and Paul Jarvis*[2]

[1] *Department of Plant Physiology, RPTU, Kaiserslautern, GER*

[2] *Department of Biology, Oxford University, Oxford, UK*

Chloroplasts evolved in the plant lineage as a result of an endosymbiotic event more than one billion years ago. During evolution, chloroplasts lost independence and most genes were transferred to the nucleus which necessitated a system of protein import. This import process occurs post-translationally and is mediated by molecular machines called translocon of the outer/inner chloroplast envelope membrane (TOC/TIC). The regulation of the import machineries is therefore of high importance to ensure a balanced and functional chloroplast proteome. TOC proteins are known to be degraded by the ubiquitin proteasome system (UPS), where ubiquitin is attached to lysine residues of target proteins, marking them for proteasomal degradation. The ubiquitination cascade involves three enzymes: an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ubiquitin ligase. The multi-component system is called chloroplast associated protein degradation (CHLORAD); identified components are an E3 ligase Sp1 (suppressor of ppi1 locus 1), a channel protein Sp2 (suppressor of ppi1 locus 2), and the cytosolic motor protein CDC48 (cell division cycle 48). Recent findings showed that besides TOC proteins, the chloroplast interior is affected by CHLORAD as well. However, the process by which chloroplast localised proteins are exported for proteasomal degradation has not been discovered. Here, by using biochemical methods a suitable candidate that might mediate the export has not been identified. Sec2, an inner envelope membrane localised translocation apparatus shows an interaction with known CHLORAD targets and seems to affect their stability. However, the proof of its involvement in CHLORAD as well as some questions remain unanswered and still need to be explored.

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9. Unravelling the untapped potential of *Aphanizomenon flos-aquae* – a high-value cyanobacterium

Gabriel Scoglio[1] *and Saul Purton*[1]

[1]*Department of Structural and Molecular Biology*

My project focuses on *Aphanizomenon flos-aquae* (AFA): a filamentous nitrogen-fixing edible cyanobacterium known for its high nutritional value. Specifically, I am working with a wild AFA strain that blooms naturally in Klamath Lake, Oregon, since this strain is already distributed worldwide as a nutritional supplement. I am further investigating the commercial and biotechnological potential of two AFA strains from culture collections: CCAP AFA and NIES AFA. Overall, my project is divided into three main aims: 1) whole genome sequencing (WGS) and characterisation; 2) cultivation optimisation with the goal of commercial exploitation; and 3) AFA genetic engineering for the generation of bespoke strains with improved traits for biotechnology.

WGS and annotation have been obtained for all strains. From the obtained genetic data, proof-of-concept experiments to transform CCAP AFA have been attempted. Specifically, a method to deliver antibiotic resistance genes into CCAP AFA's genome via either natural transformation or electroporation is currently being developed and honed. In terms of cultivation, focus has been placed on CCAP AFA: its growth has been optimised to achieve the fastest doubling time to date (<30h) and has been demonstrated to grow well in 10L photobioreactors, specifically a low-cost bubble column PBR made from cheap polythene tubing, reaching final biomass values of 2–3 g/L. Next steps include optimisation of growth of the Klamath and NIES AFA strains in the laboratory, CCAP AFA growth evaluation in other photobioreactor systems, from open ponds to flat panels (particularly in relation to its production of C-phycocyanin, a high-value pigment), and the generation of a CCAP AFA transformant line.

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10. Engineering cereal crop productivity through enhancement of organelle biogenesis

***Masab Umair Khan*[1]**, *Priyanka Mishra[1], Lei Hua[2], Julian Hibberd[2], Enrique Lopez-Juez[1]*

[1]*Department of Biological Sciences, Royal Holloway University of London, Egham, UK*

[2]*Department of Plant Sciences, University of Cambridge, Cambridge, UK*

Chloroplasts are the cells' organelles responsible for photosynthesis, which directly affects crop yield. The main crops (maize, wheat, and rice) appear to have reached their maximum yield potential since the ''Green Revolution'' and can barely be enhanced with conventional crop breeding. We are seeking new approaches to increase the total chloroplast content of cells, the chloroplast index, which may result in higher photosynthetic yield. Regulatory networks control the development of proplastids in the early stage of plant leaves and their conversion into fully green chloroplasts. The expression of genes for many photosynthetic proteins in chloroplasts is regulated by nuclear transcription factors GOLDEN2-LIKE 1 and 2 (GLK1 and GLK2)., loss of their function reduces chloroplast activity. A mutation identified in our laboratory in *Arabidopsis thaliana* increases the extent of greening of a glk1glk2 double mutant. We named this mutation as suppressor of GLKs 1 (sgl1) and mapped the SGL1 gene. This mutation, as a single mutant, enhances the chloroplast index of mesophyll cells (MCs) and bundle sheath cells (BSCs) in Arabidopsis leaves. One of the steps necessary for the conversion of C3 plants into C4 plants is to increase the chloroplast index in BSCs. The aim of this study is to silence SGL1 in specific cells in rice. A relevant construct was transformed into Nipponbare rice by the National Institute of Agricultural Botany (NIAB). Resulting plants are being analyzed for their chloroplast index using Nomarski microscope, and for photosynthesis (CO2 assimilation) analysis using a Li-6800 instrument. Ongoing results will be presented.

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11. Safeguarding wheat production by editing the CHLORAD pathway

***Joseph Oddy*[1]**, *Ziad Soufi[1], Najiah Mohd Sadali[2], Qihua Ling[3], Paul Jarvis[1]*

[1] *Section of Molecular Plant Biology, Department of Biology, University of Oxford, Oxford, UK*

[2] *Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603 Kuala Lumpur, Malaysia*

[3] *National Key Laboratory of Plant Molecular Genetics, CAS Centre for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China*

Wheat is the second most widely consumed cereal worldwide, with wheat-based food products making up approximately 18% of all calories consumed every day. Climate change will likely decrease wheat yields due to increased extreme weather events, making yield forecasting more difficult and negatively impacting food security in vulnerable regions. Consequently, the global food system needs to implement strategies to safeguard wheat production. One potential way to achieve this, in part, is through the introduction of stress tolerant wheat varieties with consistent yields. Stay-green wheat varieties (varieties that stay photosynthetically active for longer than average) possess some of these high-yielding and stress tolerant qualities. Mutations in certain components of the chloroplast-associated protein degradation (CHLORAD) pathway have the potential to create the stay-green phenotype in wheat varieties. We are investigating how editing of the SP1 and SP2 genes (components of the CHLORAD system) impacts stay-green qualities in wheat. Future work will look at the performance of edited SP1 and SP2 plants in the field and whether they are promising candidates to address upcoming challenges in commercial wheat cultivation.

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12. The production of recombinant SBV-N protein for use in an edible algal vaccine

Jonathan Scarfe[1] *and Ellen Nisbet*[1]

[1]*University of Nottingham*

This project aims to produce an algal vaccine for use in agriculture. Schmallenberg virus (SBV) causes congenital malformations and abortions in ruminants, characterised by arthrogryposis-hydranencephaly-syndrome. Edible algal vaccines offer a novel, pragmatic and cost-effective solution for the prevention of such viral diseases. Codon optimised and his-tagged DNA encoding the SBV nucleoprotein (SBV-N) was precisely inserted into the *Chlamydomonas reinhardtii* (a green alga) plastome via glass bead transformation. Selection was based on phototrophic rescue of the cell wall deficient ΔpsbH TN72 strain (Wannathong et al., 2016). Transformant colonies were screened by PCR, confirming SBV-N DNA integration in all transformant colonies. Reverse transcription PCR (RT-PCR) was used to confirm RNA transcription. Finally, western blot analysis confirmed the expression of SBV-N protein. These results show that SBV-N is being successfully expressed in the *Chlamydomonas reinhardtii* plastid. Future research is now being focused on expressing viral proteins of other viruses, such as tilapia lake virus and rotavirus, following the same method. The immunogenicity of such proteins will be analysed to investigate their suitability as vaccine candidates.

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13. Characterising a novel pyrenoid: ft *Chlorella Sorokiniana*

Mihris Naduthodi[1]*, James Barret*[1] *and Luke Mackinder*[1]

[1]*CNAP, Department of Biology, University of York, United Kingdom*

Pyrenoid is a liquid-liquid phase separated organelle found in the chloroplast of some microalgae and hornworts that acts as the heart of carbon concentrating mechanism (CCM). Developing a pyrenoid in plants that has evolutionarily lost this organelle can theoretically improve plant productivity by 60%

and address the global challenge of food production to a degree. Pyrenoid concentrates CO2 in the vicinity of closely packed Rubisco to improve the carboxylation rate of this protein which can also otherwise fix oxygen in a wasteful-energy consuming process. Current knowledge on the pyrenoid formation and functioning comes from the characterization of this organelle in model microalga *Chlamydomonas reinhardtii*. The phase separation of this organelle in Chlamydomonas was triggered in the presence of a multivalent Rubisco binding protein called EPYC1. Additionally, multiple transmembrane proteins (RBMP1 and RBMP2) and starch binding proteins (SAGA1 and SAGA2) are determined to be essential for an active pyrenoid in Chlamydomonas. A morphologically simpler pyrenoid was observed in the microalgal genus of Chlorella. Investigation into the proteome of *Chlorella sorokiniana* revealed a potentially simpler protein composition in the pyrenoid with following observations: Identification of a linker protein analogous to EPYC1, that is capable to phase separate Rubisco from Chlorella and other microalgal and plant species. Identification of a single analogous protein named PMTT in Chlorella (Pyrenoid Matrix Tubule Tether) to RBMP1 and RBMP2 from Chlamydomonas. Identification of a single analogous protein named PMST in Chlorella (Pyrenoid Matrix Starch Tether) to SAGA1 and SAGA2 from Chlamydomonas.

Having fewer pyrenoid components in Chlorella compared to Chlamydomonas could result in comparatively easy adaptation into plants and pyrenoidless microalgae to improve their productivity. In this study, we perform an in-silico, ex-vivo and in-vivo characterization of the potential Chlorella pyrenoid proteins to understand their role in the formation and functioning of this organelle.

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14. Synthesising ncAA-containing therapeutic proteins in the chloroplast of Chlamydomonas

***Rinad Alhedaithy*[1]**, *Harry Jackson[1] and Saul Purton[1]*

[1] Department of Structural and Molecular Biology, University College London, London, UK

Genetic code expansion aims to reassign codons to incorporate non-canonical amino acids (ncAA) at specific sites in a polypeptide chain to expand the functionality of the target protein. In nature, the full potential of protein functionality has not been unlocked due to limited set of 20 proteogenic amino acids, although some archaeal species naturally contain an extended number of amino acids, which can be exploited. The incorporation of a ncAA at a specific site in a target protein relies on a translation system that has an unused codon that can be reassigned to the ncAA, an orthogonal pair of aminoacyl tRNA synthetase (aaRS) and its cognate tRNA for the ncAA, and the presence of the ncAA itself within the system.

Chlamydomonas reinhardtii is a 'generally recognised as safe' (GRAS) certified microalga, which allows for its use as a platform for the manufacturing of therapeutic proteins. The chloroplast is ideal for genetic engineering due to its small chloroplast genome that benefits from a lack of gene-silencing mechanisms, no overlapping genes, and DNA integration via homologous recombination. Importantly, the chloroplast genome does not use the UGA stop codon, allowing for its reassignment. Using synthetic biology techniques, elements of the ncAA-incorporating machineries can be engineered into the chloroplast to synthesise novel therapeutic proteins in *C. reinhardtii*.

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15. Heterologous small subunits complement growth and modify Rubisco catalysis in Arabidopsis

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[2] Department of Cell and Molecular Biology, Uppsala University, S-751 24 Uppsala, Sweden

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[4] *Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA*

Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) is the primary enzyme for CO2 fixation and continues to be a key target for improving photosynthetic efficiency. In plants, Rubisco is composed of eight large subunits (LSUs) encoded in the chloroplast genome and eight small subunits (SSUs) encoded in the nuclear genome. Previous work has indicated that structure of the SSUs can modify the catalytic properties of the active sites on the LSUs. Here we have used an Arabidopsis SSUs deficient mutant 1a2b3b which contains only ~3% of wild type Rubisco levels to explore the capacity of five different non-native SSUs to assemble into hybrid Rubisco complexes, complement growth and photosynthesis, and impact Rubisco catalysis. We transformed SSUs from tobacco, rice, sorghum, Limonium or Chlamydomonas into 1a2b3b. All five non-native SSUs complemented growth and photosynthetic CO2 assimilation rates in the mutant. Cryogenic electron microscopy (cryo-EM) analysis revealed that all five SSUs were assembled into hybrid-Rubisco complexes, indicating that the LSU octamer can tolerate a broad range of sequence and structural variability. The catalytic properties of the hybrid Rubiscos are currently being analysed. Based on our results to date, the tolerance of the LSUs suggests that there may be significant scope for modifying Rubisco performance in plants by engineering the nuclear encoded SSU family.

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16. Co-culturing biofilms of phototrophic and heterotrophic bacteria and their application in bio-photoelectrochemical systems

Bartosz Witek [1,2], *Joshua M Lawrence*[1,2], *Alberto Scarampi*[1], *Karan Bali*[3], *Robert W. Bradley4, Darius G. Kosmützky*[1], *Linying Shang*[2], *Jenny Z. Zhang*[2], *Christopher J. Howe*[1]

[1.] *Department of Biochemistry, University of Cambridge, UK.*

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[4.] *Department of Life Sciences, Imperial College London, UK.*

Co-culturing biofilms of phototrophic and heterotrophic bacteria and their application in bio-photoelectrochemical systems. In nature microorganisms co-exist in complex communities, interacting with each other and the environment. Mirroring nature, synthetic consortia are a powerful tool for biotechnology, offering greater robustness, expanded metabolic capabilities, and decreased metabolic burden when compared to axenic cultures. There is particular interest in generating phototrophheterotroph co-cultures for renewable bioenergy generation. However, the design of stable consortia remains challenging, requiring optimisation of culturing conditions, starting inocula ratios, and microbial interactions. Furthermore, well mixed co-cultures limit the achievable biodiversity, and favour the growth of fast-growing species. With this in mind, we have designed an accessible workflow for growing, engineering, and analysing spatially heterogenous biofilm co-cultures consisting of phototrophs and heterotrophs. Using our system, we were able to generate stable co-cultures of the cyanobacterium Synechocystis sp. PCC 6803 with the model heterotroph *Escherichia coli*. We have investigated the syntrophic interactions within these biofilms and explored their application with genetically engineered strains for bio-electricity generation in electrochemical devices. We expect this platform to have wide applications in microbial ecology research and the design of green biotechnologies.

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17. Structural attributes of disordered linker protein and their role in pyrenoid assembly

***Gaurav Kumar*[1]**, *Luke Mackinder*[1]

[1] *Department of Biology, University of York, York, UK*

Pyrenoids are algal subcellular organelle that encase CO2 fixing enzyme Rubisco and this organelle is responsible for one-third of global CO2 fixation. The three levels of organization that make up a pyrenoid's structure are the core matrix, starch sheath encircling the core matrix and the tubules produced by a network of thylakoid membranes. The matrix is loaded with the signature enzyme Rubisco and its formation is driven by phase separation of Rubisco molecules in the presence of a disordered linker protein. The linker protein has multiple Rubisco binding motifs that interconnects Rubisco molecules, and these motifs are separated by disordered regions that make the linker-Rubisco network very dynamic. The objective of this work is to understand why linker proteins have a specific number of Rubisco binding motifs and if changing the number of these motifs will affect the Rubisco phase separation and assembly. We are using a combination of biochemical assays and imaging techniques to address these questions. Our findings suggest that the Rubisco phase separation is significantly influenced by the presence of a specific number of Rubisco binding motifs. We further plan to extend our study into in vivo Chlamydomonas sp. model and study the effect of linker variants on their phenotypes. We believe that our present study will help us better grasp intracellular compartmentalization in algae and open opportunities in future for introducing pyrenoid-based systems in agricultural plants to boost crop yields.

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18. Production in the algal chloroplast of a major capsid protein (MCP) subunit vaccine against the infectious spleen and kidney necrosis virus (ISKNV) of Sea bass (*Lates calcarifer*).

***Pokchut Kusolkumbot*[1]**, *Patai Charoonnart,*[2,3], *Sarocha Jitrakorn*[2,3], *Vanvimon Saksmerprome*[2,3],

Harry O. Jackson[1], *Saul Purton*[1]

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The chloroplast of the green microalga *Chlamydomonas reinhardtii* offers various advantages for the sustainable production of recombinant proteins. The alga is Generally Recognized as Safe (GRAS), and the chloroplast compartment represents an enclosed system that allows transgene expression to a high level and correct protein folding. The Infectious Spleen and Kidney Necrosis Virus (ISKNV) causes a severe pandemic in fish. ISKNV infection results in epidermal lesions with significant petechial haemorrhages and abdominal edema. The algal strain selected for this experiment is *C. reinhardtii* CC-4033 which is engineered to contain a bacterial gene that allows selective growth of the strain in media containing phosphite. The strain also loses chlorophyll when cultures are transferred from the light to the dark. The two features will reduce scale-up costs and palatability. A novel method to resolve downstream processing costs is to use the whole of the edible algae, allowing vaccine encapsulation in the dried algae and formulation into the aquaculture feed. Moreover, a codon reassignment strategy results in the biocontainment of the transgenes in the chloroplast and prevents horizontal gene transfer. This approach is improving the genetic features of the *C. reinhardtii* host, which will be beneficial for oral vaccine production in the future.

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19. Insights on photosynthesis and Rubisco activity in cowpea plants treated with silica nanoparticles and exposed to heat stress

Jaqueline da Silva Santos [1,2], *Ricardo Antunes de Azevedo*[2], *Elizabete Carmo-Silva*[1].

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[2] *Genetics Department, Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo (USP), Piracicaba, SP, Brazil.*

Heat stress damage to plants limits overall crop yield, as it affects chloroplast metabolism, decreases Rubisco efficiency and photosynthetic activity. Silicon and silicon nanoparticles (SiO2-NPs) have been described as mitigators of various types of stress in plants, including high-temperature stress. However, little is known about the mechanisms involved in the mitigating action of this compound on stressed plants. This study aims to understand the mitigating role of silicon nanoparticles in the photosynthetic performance, Rubisco activity and growth of cowpea (*Vigna unguiculata* (L.) Walp.) plants exposed to a heat wave. Silicon nanoparticles were added to the substrate before planting, and the plants are in cultivation, and 2 weeks after emergence they will be subjected to a 5-day heat wave, then the analyses will begin. The findings of this study will facilitate efforts to improve the yield and efficiency of crops, and their resilience to climate change.

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20. Understanding the control of starch granule size in wheat and its impact on starch digestibility

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Starch is a ubiquitous polysaccharide in plants and an important source of carbohydrates in human diet. In most plants including modern staple crops like wheat (*Triticum aestivum*), it is synthesized and stored in semicrystalline granules. In wheat endosperm, there are two types of starch granules – larger lenticular A-type granules and smaller spherical B-type granules. The properties of these granules affect the nutritional quality of starch, however, the mechanisms that control those properties are not well understood. B-GRANULE CONTENT 1 (BGC1) protein has recently been implicated in B-type granule initiation and morphology in wheat. In this project, we aim to further our understanding the role of BGC1 in the control of wheat B-type starch granule formation and morphology and its implications on human health. Our previous work has shown that BGC1 interacts with PHS1 (α-Glucan Phosphorylase 1) to regulate B-type granule initiation. Here, we have discovered that BGC1 strongly interacts with itself and forms dimers. Structural solution investigations by SAXS indicated the possibility of even higher oligomerization states, such as the dimer of dimers. We will investigate the functional importance of this oligomerization, along with the structure and function of native BGC1, with in vitro mutagenesis and mutant wheat populations, crystallography, and substrate binding assays. Mutant wheat populations with varying starch granule phenotypes will be used to make pasta and analyse its nutritional properties, such as digestibility and effects on gut microbiome. These investigations will help understand how starch granule morphology is controlled in wheat and its impact on human health.

List of delegates

- Jani Bolla
- Paul Jarvis
- Simon Thomson
- Sabina Musial
- Jun Fang
- Rose McNelly
- Chris Howe
- Abi Perrin
- Jacques Bouvier
- Ravi Vasudevan
- Anne Lau
- Adrian Barbrook
- Gabriel Scoglio
- Masab Khan
- Declan Perry
- Sreedhar Nellaepalli
- Joseph Oddy
- Georgia Yiasoumi
- Ziad Soufi
- Darius Kosmutzky
- Yi Sun
- Gabriela Toledo-Ortiz
- Jonathan Scarfe
- Audrey Short
- Angelo Joshua Victoria
- Mihris Ibnu Saleem Naduthodi
- Rinad Alhedaithy
- Yuwei Mao
- Na Li
- Bartosz Witek
- Shuyang Cheng
- Julanie Stapelberg
- Gaurav Kumar
- Changsong Ri
- Pokchut Kusolkumbot
- Onyou Nam
- Luyao Yang
- Saba Riaz
- Farrukh Makhamadjonov
- Tamara Hernandez-Verdeja
- Ana Lobo
- Gogs Karunanithy
- Jacqueline da Silva Santos
- Tom Reynolds

- Pushan Bag
- Bruno Peixoto
- JA siqueria
- Nattaphorn Buayam
- Enrique Lopez-Juez
- Thomas Navarro
- Sujata Kumari
- Yi Wang
- Angel Vergara-Cruces
- Tina Schreier
- Elizabeth Robbins