ISPR 2022 CONGRESS

ŌTEPOTI DUNEDIN, AOTEAROA NEW ZEALAND.

JULY 31 - AUGUST 5, 2022

CONTENTS

TOPIC	PAGE

CONFERENCE COORDINATION

2022 Organising Committee

Julian Eaton-Rye - Convenor
Tina Summerfield
Grant Pearce

In association with
akB Conference Management Limited
www.akb.nz | +64 3 4 745165
PO Box 994 Dunedin 9054

Audio Visual and Online delivery by

A WORD FROM THE CONVENOR

Tēnā koutou, tēnā koutou, tēnā koutou katoa
Greetings, Greetings, Greetings to you all

On behalf of our local organising committee, it is my pleasure to welcome you to the 18th International Congress on Photosynthesis Research. This is the first time that the Congress has been presented in a hybrid format and we hope those of you attending on-line, as well as those who are here in Ōtepoti Dunedin, have a fruitful and enjoyable experience. Our Congress encompasses all aspects of photosynthesis research with applications in energy and food security as well climate change mitigation. It is our hope that basic and applied science presented here will act a stimulus to us all, and particularly those just entering the field, to embrace the challenges and necessity of applying photosynthesis research to improving our world.

Thank you to our three sponsors who have supported us in a variety of ways; Heinz Walz, Li-Cor and Agrisera your support in these challenging time is greatly appreciated.

For those of you who have joined us in person, I do hope you take the opportunity to get out and enjoy all that Ōtepoti Dunedin has to offer.

Ngā manaakitanga
Best regards,

Julian Eaton-Rye

ISPR Congress 2022 Convenor
University of Otago

THANK YOU TO OUR SPONSORS & EXHIBITORS

GOLD SPONSOR / VIRTUAL EXHIBITOR

From a vision about gas exchange measurements, Heinz Walz GmbH developed in the last 50 years into one of the world's top producers of highly sophisticated photosynthesis measuring systems. In 1985, pulse-amplitude-modulated fluorescence (PAM) measurements were added to the portfolio. A method that formed the basis for expansion into both physiological (high time resolution, P700, P515) and eco-physiological (field measurements, monitoring) directions. Close connections to leading scientists in basic and applied research initiated and stimulated many of these innovative developments. In addition, excellence in product quality and service has been a tradition in this company since its founding in 1972.

IN-PERSON EXHIBITOR

LI-COR Environmental is a leading environmental technology innovator for plant physiology, ecosystem, soil, light, water, and greenhouse gas research. LI-COR provides complete solutions for plant and aquatic photosynthesis with the LI-6800 Portable Photosynthesis System, 6800-18 Aquatic Chamber, and LI-600 Porometer/Fluorometer. In addition to eddy covariance systems, trace gas analyzers, and soil gas flux systems, LI-COR also offers atmospheric and underwater light sensors, leaf area meters, and software. Our global team of scientists provides industry-leading professional support and training in an effort to help scientists improve the human condition and solve challenges that are facing humanity. For more information, visit www.licor.com/env.

POSTER SPONSOR

Agrisera is a Swedish company offering the most well-published antibodies for a wide range of model species including Arabidopsis thaliana, crops, trees. algae and diatoms. For the last 20 years Agrisera Antibodies helped to speed up research in various areas of plant biology, including developmental biology, environmental stress, nitrogen metabolism and photosynthesis, to name a few, Our well characterized primary antibodies and antibodies to epitope tags, are accompanied by matching secondary antibodies and outstanding detection reagents. Our experienced scientific staff are on hand to answer all your antibody queries promptly, and we are looking forward to meeting you during the conference and help you to accelerate your work.

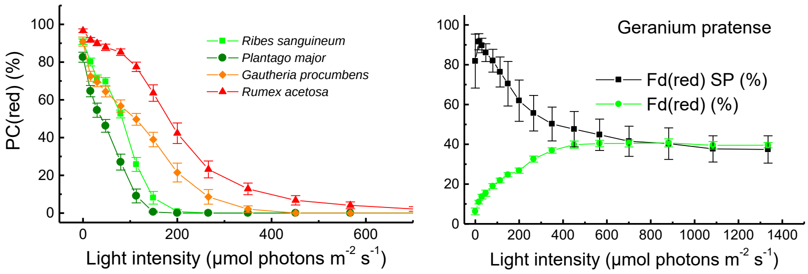
Walz, solutions for your photosynthesis needs

Innovation and tradition: these are the driving forces which have made of Heinz Walz GmbH one of the world's top producers of highly sophisticated photosynthesis measuring systems.

Close connections to leading scientists in basic and applied research have initiated and stimulated many innovative developments. Excellence in product quality and service have been a tradition in this company since its founding in 1972.

Visit us in our virtual booth at the Photosynthesis Congress in Dunedin and find out more.

Unveil the whole electron transport chain with the DUAL-KLAS-NIR

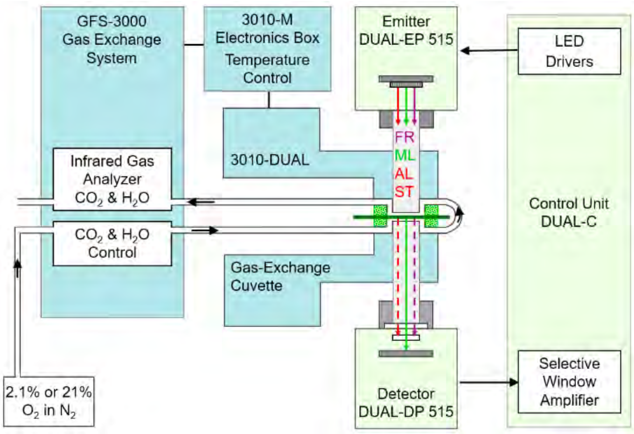


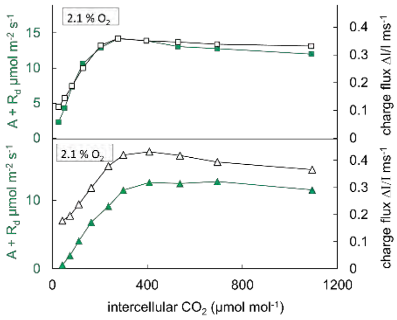
Four wavelength pairs and two fluorescence channels to get, in a single measurement, a complete picture of the state of the photosynthetic electron transport chain.

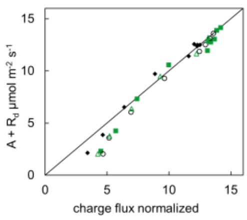
Understand P700 measurements better, by knowing the redox state of plastocyanin (before) and ferredoxin (after). See how light intensity changes affect the PS I acceptor side redox state, probe Photosynthetic Control and much, much more.

3010-DUAL, bridge between electron transport chain and gas exchange measurements

The 3010-DUAL gives the user full control over the gas conditions around a leaf and allows the combination of gas exchange measurements with fluorescence and NIR measurements or in this case electro-chromic shift measurements.







Schematic representation of the DUAL-3010 with the gas exchange part in aquamarine and the part for the optical measurements in light green. To the right an example measurement.

Soon available, the new HEXAGON-IMAGING-PAM

Up to 20 x 24 cm can be measured with the HEXAGON-IMAGING-PAM in high resolution and it provides the best homogeneous illumination over the whole imaged area, far-red light for F_0', a range of measuring setups to accommodate a variety of sample types. The HEXAGON-IMAGING-PAM can be fully remote controlled.

BARRY BRUCE

Dr Barry D. Bruce is the Charles P. Postell Distinguished Professor of Biochemistry. He is a professor in multiple departments, including Biochemistry and Cellular and Molecular Biology, Microbiology, Chemical, Biomolecular Engineering, Genome Science and Technology, and an inaugural member of the Bredesen Center Interdisciplinary Research. Dr Bruce has BAs in chemistry and biology from the University of California at Santa Cruz and holds an MS in Biochemistry/Biophysics from the University of Massachusetts at Amherst. In 1990, he received his PhD in Molecular Plant Biology from the University of California at Berkeley. Dr Bruce joined the UT Knoxville faculty in 1994 after completing a National Science Foundation post-doctoral Fellowship in Plant Biology at the University of Wisconsin at Madison. He is a Fellow of the American Association for Advancement of Science.

ABSTRACT

Emerging Approaches for PSI-Based Bio-hybrid Devices

Barry D. Bruce[1,2,3,4], Nathan Brady[1], Jyotirmoy Mondal[1], Alex Teodor[4]
[1] Departments of Biochemistry, Cellular and Molecular Biology, [2] Chemical and Biomolecular Engineering, [3] Microbiology, Program in Genome Science &Technology, [4] The University of Tennessee at Knoxville, USA 37996

Nature has developed remarkable means for harvesting solar energy to drive the process of photosynthesis. In part, the remarkably high quantum efficiency associated with photosynthesis has been enabled by the successful “division of labor” associated with this process. In nature, organisms have evolved largely separate biomolecular structures that have become specialized to either 1) capture photons and facilitate energy transfer via high-efficiency exciton coupling of pigments and 2) convert this exciton into a charge separation that has a quantum yield approaching unity (via the reaction center complex). Interestingly, the light harvesting process has been very adaptive to capture a wide range of the visible solar energy via distinct optical “niches”. Our work has focused on Photosystem I (PSI) as a highly robust reaction center. Utilizing the thermophilic cyanobacteria, Thermosynechococcus elongatus, we have developed new, non-detergent methods of isolation/stabilization of PSI that may enable much more robust and cost-effective methods for PSI utilization. In addition, we are exploring novel means of coupling PSI directly to metal-oxide electrodes via simple self-assembly methods using the native electron acceptor, ferredoxin. Through molecular modeling we have begun to bioengineer Fd and the PSI interface to enhance their affinity permitting a diffusion free mode of electron transfer. Similar efforts on the donor side of PSI will work to utilize natural and bioengineered cyt c_6 proteins to facilitate more rapid re-reduction of $P700^+$. We are also synthetizing novel Co complexes that may mimic cyt c_6 yet be potentially more cost effect and facile in device design. Employing synthetic biology may allow us to improve on nature, advancing the design and fabrication of new biohybrid devices. In addition, I will discuss future designs to further enhance their external quantum efficiency toward the goal of a viable, sustainable, and environmentally benign strategy for bioenergy production.

GARY BRUDVIG

Yale Energy Sciences Institute
Gary Brudvig is the Benjamin Silliman Professor of Chemistry, Professor of Molecular Biophysics and Biochemistry, and Director of the Yale Energy Sciences Institute. He received his BS (1976) from the University of Minnesota, his PhD (1981) from Caltech and was a Miller Postdoctoral Fellow at the University of California, Berkeley from 1980 to 1982. Professor Brudvig has been on the faculty at Yale since 1982. His research involves study of the water-oxidation chemistry in photosystem II and work to develop artificial bioinspired systems for solar fuel production.

ABSTRACT

The Tom Wydrzynski Lecture on Water Oxidation

Gary W. Brudvig
Department of Chemistry, Department of Molecular Biophysics & Biochemistry, and Energy Sciences Institute, Yale University, New Haven, CT 06520, USA

Photosystem II (PSII) uses light energy to split water into protons, electrons and oxygen. In this reaction, Nature has solved the difficult chemical problem of efficient four-electron oxidation of water to yield O_2 without significant side reactions. In order to use Nature's solution for the design of materials that split water for solar fuel production, it is important to understand the structure of the catalytic site and the mechanism of the reaction. The X-ray crystal and cryo-electron microscopy structures of cyanobacterial PSII provide information on the structure of the Mn and Ca ions, the redox-active tyrosine called tyrosine-Z, chloride and the surrounding amino acids that comprise the oxygen-evolving complex (OEC). The structure of the OEC in the intermediate oxidation states of the catalytic cycle, the binding of substrate water molecules to the OEC and the water oxidation chemistry of PSII will be discussed in the light of biophysical, spectroscopic and computational studies, inorganic chemistry, and X-ray crystallographic and cryo-electron microscopy information.

ELIZABETE CARMO-SILVA

Lancaster Environment Centre, Lancaster University
Elizabete is interested in photosynthesis and plant responses to fluctuations in the environment, in particular light and temperature. Her PhD studies focused on photosynthesis and photorespiration in C4 grasses under drought stress. During her post-doctoral research she specialized on Rubisco activation by its molecular chaperone, Rubisco activase, while conducting studies on the interplay between drought and heat stress in cotton. In 2012 she started working on improving photosynthetic performance to increase wheat crop yields at Rothamsted Research, before becoming a lecturer in Plant Sciences
global food security and resource use efficiency by optimising crop performance in response to the changing climate.

ABSTRACT

Rubisco regulation in crops

Elizabete Carmo-Silva
Lancaster Environment Centre, Lancaster University, UK

Rubisco catalyses the first step in the conversion of carbon from CO_2 into plant biomass: the carboxylation of ribulose-1,5-bisphosphate (RuBP). This reaction is essential for life on earth. Rubisco is frequently regarded as an inefficient enzyme because it also catalyses the oxygenation of RuBP and is prone to making mistakes during catalysis. However, the more we know about Rubisco, the more we appreciate how good it is for its job! Many chloroplast components, including proteins, sugar-phosphates, ions, etc, interact with Rubisco. These components respond to fluctuations in the prevailing environment of the leaf, and dynamically modulate the activity of Rubisco, adjusting the rate of CO_2 assimilation as conditions change. Incident light, for example, is constantly changing for leaves within crop canopies in field settings. With increasing frequency of heat waves, leaf temperature changes also impact Rubisco activity. While Rubisco has been evolving and adapting to the specific chloroplast environment, there is scope to fine tune the regulation and thereby improve the efficiency of crop Rubiscos.

PLENARY SPEAKER

ROBERTA CROCE

VU Amsterdam

Roberta Croce (www.robertacroce.nl) studied chemistry in Padova and completed her PhD in Plant Biology/Biophysics in Milano in 1998. After two postdoc periods in Germany and Italy, she got a permanent position at the CNR. In 2006 she moved to the University of Groningen and since 2011 she is Professor of Biophysics/Photosynthesis at VU Amsterdam. Her research focuses on the molecular mechanisms of photosynthesis, using an integrated approach including molecular biology, biochemistry and ultrafast spectroscopy. She has published more than 150 papers. She is a member of the Royal Holland Society of Science and Humanity and recipient of several personal research grants.

ABSTRACT

Breaking the red-limit: driving oxygenic photosynthesis with far-red light

Roberta Croce
Department of Physics and Astronomy and LaserLab Amsterdam, Faculty of Science, Vrije Universiteit Amsterdam

The capacity of photosynthetic organisms to harvest light is a crucial factor in the photosynthetic process, especially in light-limited conditions, which occur in greenhouses and canopies. It was believed that only visible light could drive oxygenic photosynthesis, but the discovery of cyanobacteria species containing chlorophyll d and f, which absorb in the far-red region of the spectrum, has shown that this is not the case. However, due to their different energetics, chlorophyll d and f are expected to alter the excited state dynamics of the photosynthetic units and, ultimately, their performances. How can cyanobacteria use far-red light for efficient photochemistry? To answer this question, we use a combination of biochemistry and spectroscopic measurements on intact cells and isolated complexes. We show that chlorophyll f insertion marginally affects the charge separation efficiency of Photosystem I but decreases significantly that of Photosystem II. The difference between the two photosystems, the response of plants to far-red light, and the possibility of extending the photosynthetic active radiation in crops will be discussed.

ROBERT FURBANK

Australian National University

Robert heads the Furbank Lab – Improving photosynthesis and crop yield, and is the Centre Director of Translational Photosynthesis, and a Program Leader in the ARC COE for Translational Photosynthesis.
Robert was awarded a Bachelor of Science (first class Honours) from the University of Wollongong, in 1979. He completed his PhD at The Australian National University, in 1982.
Robert is internationally known for his research into aspects of photosynthesis and carbon allocation/transport in crop plants, and understanding and manipulating C4 photosynthesis. He is also part of the CIMMYT led International Wheat Yield Consortium and the IRRI Global Rice Initiative Science Partnership.
He was awarded the Queen Elizabeth II research fellowship in 1987 and two ACT ICT Innovation Awards in 2013 and the CSIRO Plant Industry Leadership Award in 2014.

ABSTRACT

C4 Rice: the challenge of turbocharging photosynthesis

Robert T. Furbank
Research School of Biology Centre of Excellence for Translational Photosynthesis, Australian National University, Canberra, Australia

Feeding a burgeoning global population in the face of climate change and decreasing arable land area is indeed a grand challenge. A step change in genetics and agronomy will be required to even get close to meeting world demand for nutritious food. Improving food production through superior genetics and yield in our cereal crops has long been a priority and more recently, improving photosynthesis through molecular genetics and synthetic biology has become a major target. This presentation tells the story of one of the longest running and most ambitious crop engineering “Moon Shot” programs ever attempted: The C4 Rice Project. This project, funded by the Bill and Melinda Gates Foundation in 2008 and now in the fourth phase of its life, aims to increase rice yields by 50% by installing the C4 photosynthetic pathway into the world number one cereal crop which uses C3 photosynthesis. The story begins with the Australian discovery of a whole new photosynthetic pathway in 1966, and then how 40 years later, 16 labs in 11 countries got together to assemble the genetic parts to turbocharge photosynthesis, using Synbio principles before they were even labelled as such. Recent progress installing the metabolic components of the C4 pathway, remaining challenges, the outlook and likelihood of success will be presented and discussed.

PLENARY SPEAKER

MANAJIT HAYER-HARTL

Max-Planck-Institute of Biochemistry
Research Group "Chaperonin-assisted Protein Folding"
Protein Folding and Assembly, Rubisco, GroEL and GroES, Mass Spectrometry Proteins can only fulfill their tasks in their correct three-dimensional structure. Cellular chaperones are responsible for the correct folding in the cell. However, they can also help scientists to artificially produce important proteins in the test tube. Manajit Hayer-Hartl and her research group "Chaperonin-mediated protein folding" have already succeeded in doing this for the key protein of photosynthesis "RuBisCO". The complex structure of RuBisCO (16 subunits) made it impossible to produce a functional version of the protein in the test tube. Using chaperones and helper proteins, the researchers have succeeded in producing a functional RuBisCO complex.

ABSTRACT

Multiprotein condensate formation in b-carboxysome assembly

M. Hayer-Hartl
Max Planck Institute of Biochemistry, Department of Cellular Biochemistry, Martinsried, Germany.

Photosynthesis is a fundamental process in biology as it converts solar energy into chemical energy and thus, directly or indirectly, fuels all life on earth. The chemical energy is used to fix atmospheric CO_2 and produce reduced carbon compounds in the Calvin-Benson-Bassham cycle. The key enzyme for this process in all photosynthetic organisms is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is responsible for the conversion of an estimated amount of ~1011 tons of CO_2 per annum into organic material. Rubisco is the most abundant enzyme in nature, owing in part to its low catalytic turnover rate and limited specificity for CO_2 versus O_2. Moreover, Rubisco is prone to inactivation by inhibitory sugar phosphates and requires the hexadecameric AAA+ chaperone Rubisco activase (Rca) for conformational repair.

To avoid the reaction with O_2 , b-cyanobacteria have evolved proteinaceous microcompartments called b-carboxysomes, in which the enzymes Rubisco and carbonic anhydrase (CA) are enclosed. Dissolved CO_2 in the form of HCO_2 diffuses through the proteinaceous carboxysome shell and is converted to CO_2 by CA, generating a high concentration of CO_2 for carbon fixation by Rubisco – the so-called CO_2 - concentrating mechanism (CCM). The shell also prevents access to reducing agents, generating an oxidizing environment inside the carboxysome. Assembly of the b-carboxysome first involves the aggregation of Rubisco, CA and Rca followed by shell formation. Recent advances have shown that early in the process of pro-carboxysome assembly, a specialized scaffolding protein called CcmM initiates phase-separation of both Rubisco and CA into biomolecular condensates. While the recruitment of Rca is mediated by its interaction with Rubisco. In my presentation I will describe our present understanding of the complex multivalent interactions that result in the sequestration of the various essential proteins during pro-carboxysome assembly. Understanding b-carboxysome biogenesis will be important for efforts to engineer a CO_2 - concentrating mechanism into C3 crop plants.

PLENARY SPEAKER

STEPHEN LONG

University of Illinois
Dr Long's research bioengineers the photosynthesis process in crops to achieve higher productivity, sustainability, and adaptation to climate change. He heads an international project to improve the crops that feed many of the poorest in the world, which has led to the discovery of a way to engineer photosynthesis that resulted in a 20% increase in crop productivity.

ABSTRACT

Enhancing and adapting photosynthesis for food security under climate change

Steve Long
Departments of Plant Biology and Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL; Lancaster Environment Centre, Lancaster University, UK

After several years in which global food supply has improved, shortages are now re-emerging. This increases the probability of repetition of the high food prices of 2007/8 that triggered food riots in many poor countries and possibly the Arab Spring. The United Nations Food & Agricultural Organization project a worsening situation with global demand for our major crops rising 60% by 2050. This is at a time when the steady increases in yield seen over the second half of the last century are stagnating, or even reversing, under global climate change. The approaches of the Green Revolution are approaching their biological limits, and new innovations are urgently needed if we are to insure against future shortages. It will be shown that improvement of photosynthetic efficiency is the largest remaining opportunity to increase genetic crop yield potential. Its efficiency in crops falls well below the theoretical maximum and has been improved little by centuries of selection and breeding; the reasons for which will be explained. Today photosynthesis is the best understood of all plant processes, allowing us to describe each of its 100+ steps mathematically. Using this as the basis of in silico engineering using high-performance computing we have identified a number of points at different levels of organization from metabolism to organization of leaves in field crops where efficiency could be improved. This includes both adaptation to rising temperature and changing water availability. Bioengineering has begun to validate a number of these suggested improvements with substantially greater crop productivity demonstrated in replicated field trials. This will be illustrated with some specific examples, including improvement of the speed with which crop leaves relax non-photochemical quenching (NPQ) during the frequent sun to shade transitions that occur in crop canopies. Our analyses suggest that such engineering could lead to a >50% sustainable improvement in crop yield potential so providing insurance against future food shortage and avoiding yet further agricultural expansion and destruction of natural areas.

PLENARY SPEAKER

WILLIAM MARTIN

William Martin is an evolutionary biologist with an active interest in biochemistry. He completed an undergraduate degree in Biology at the University of Hannover, and a PhD in Genetics under the wise and generous supervision of Heinz Saedler at the Max Plank Institute for Breeding Research in Cologne. On completing his PhD, Bill returned to Rüdiger Cerff's group, then at the University of Braunschweig, and worked there for 10 happy and productive years. In 1999, Bill became a Professor at Heinrich-Heine-Universität Düsseldorf. William Martin's group is at the Institute for Molecular Evolution. The group pursues research on the biochemistry and evolution of chloroplasts, mitochondria (including their anaerobic forms, hydrogenosomes), and eukaryotes. To pursue these questions and to probe even earlier phases of evolution, going back to life's origin, the group uses laboratory experiments and bioinformatic techniques, with the help of gene and genome comparisons.

ABSTRACT

PLENARY SPEAKER

ÜLO NIINEMETS

Professor Ülo Niinemets is Head of the Department of Crop Science and Plant Biology at the Estonian University of Life Sciences and member of the Estonian Academy of Sciences. He has got BSc, MSc, and PhD from the University of Tartu, Estonia and carried out research in more than 20 countries including postdoctoral positions at University of Bayreuth, Germany, University of Antwerp, Belgium and Centro di Ecologia Alpina, Italy, and professor positions at Canterbury University, New Zealand (Erskine fellow, 2002), University of Hawaii, USA (G. P. Wilder Chair, 2006-2007) and Utrecht University, The Netherlands (F. C. Donders Chair, 2007).

ABSTRACT

Within-canopy photosynthetic acclimation: importance for photosynthesis in current and future climates

Ülo Niinemets
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51006, Estonia

Within plant canopies, daily average integrated light intensity (Qint) varies typically 10- to 50-fold, and this long-term variation in light availability results in major differences in daily photosynthetic production of leaves in different canopy positions. To enhance daily photosynthetic production, foliage structural, chemical and physiological characteristics acclimate to canopy light availability, resulting in a positive correlation between photosynthetic capacity per area (Aarea) and Qint. A worldwide meta-analysis of 831 within-canopy gradients with standardized light estimates for 304 species demonstrates that Amax plasticity to light is similar in different plant functional types, but different combinations of underlying traits are responsible for Amax plasticity in different plant functional types. In particular, in species with low leaf life span such as herbs and fast-growing woody species, Amax changes mainly due to variation in leaf nitrogen content per dry mass, whereas in species with slow leaf turnover, including most woody species, Amax change mainly results from light-dependent modifications in leaf dry mass per unit area. There is limited information of how global change alters canopy light gradients and Amax plasticity. All else being equal, vegetation under greater [CO_2] is expected to support greater leaf area due to enhanced carbon availability in CO_2-enriched atmospheres and greater water use efficiency. Denser canopies and higher atmospheric [CO_2] would imply stronger light gradients and overall greater limitation of canopy photosynthesis by low light availability. An analysis of relatively short-term experimental studies, from months to ca. 10 years, provides some support to these hypotheses, but longer-term experimental data are currently missing. On the other hand, elevated [CO_2] effects on whole canopy photosynthesis also depend on modifications in light-dependent plasticity. A meta-analysis of effects of elevated growth [CO_2] on Amax plasticity, indicates that the [CO_2] effects are variable with enhanced plasticity observed in some studies, and similar or even negative plasticity in other cases, and analogous variability is evident for other underlying leaf structural and chemical traits. Overall, light limitation of photosynthesis is expected to become more important in future [CO_2]-enriched atmospheres, and prediction of vegetation productivity in future conditions requires long-term datasets of canopy dynamics and quantitative understanding of the extent to which the capacity of foliage to acclimate canopy light conditions is modified by elevated [CO_2].

PLENARY SPEAKER

BARRY POGSON

Australian National University
Barry Pogson completed his PhD at Macquarie University. He moved to the USA in 1994 working as a postdoc with Dean DellaPenna before taking an Assistant Professorship at Arizona State University in 1997. He moved to ANU late 1999.
Pogson is Deputy Director of the ARC Centre of Excellence in Plant Energy Biology (www.plantenergy.edu.au) and lead CI on an International Wheat Yield Partnership grant to Improve Wheat Yeild by Optimising Energy Use Efficiency (iwyp.org).

ABSTRACT

Learning the languages of cells across time and space

Barry J Pogson
ARC Centre for Future Crops, Division of Plant Sciences, Research School of Biology, Australian National University

Abiotic stress such as excess-light and drought cause significant crop losses by reducing photosynthetic efficiency, growth and yield. Key to limiting crop losses in harsh and variable climates is an understanding about how cells perceive and communicate environmental perturbations. Chloroplasts, the site of photosynthesis, can be viewed as an environmental sensor for the cell as they dynamically respond to light, temperature and drought. How do they communicate their status to the rest of the cell and how does the plant respond? Has this varied across evolutionary time scales and can we identify and regulate the system for better crops?

Our strategy has been a holistic one spanning from the lab to the field and moving from gene to signal to whole plant, alongside a consideration of evolutionary drivers and consequences. Our key contributions were to elucidate how chloroplasts communicate their photosynthetic status to the nucleus, which is a process known as retrograde signaling. We have uncovered communication networks, gained insights into how the photosynthetic apparatus is protected and how plants capture and use energy. The journey has provided the foundation for industry-focused projects, such as developing higher yielding wheat lines. The seminar will focus on some highlights of this journey and reflect on where the challenges remain.

Chan et al. (2016) Ann Rev Plant Biology 67:25-53
Chan et al. (2016) PNAS 113: E4567-E4576
Pornsiriwong et al. (2017) eLIFE 6: e23361
Crisp et al. 2016) Science Advances 2: e1501340
Zhao et al. (2019) PNAS 116: 5015-5020

PLENARY SPEAKER

SHARON ROBINSON

University of Wollongong
Sharon Robinson researches how Antarctic plants respond to climate change. She uses radiocarbon signatures, left behind in the atmosphere by nuclear testing, to date mosses and track environmental change around the coast of Antarctica. Her group identifies the sunscreens plants make to protect themselves from elevated UV-B radiation due to ozone depletion. She is also applying new technologies, including the use of drones in Antarctica, to monitor plant health and productivity, and developing novel sensors that will help to track crop and forest health in future.

ABSTRACT

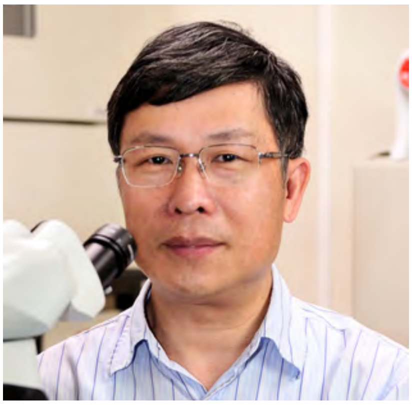
Basking in the sun: How mosses photosynthesise and survive the coldest continent on Earth

Sharon A. Robinson[1,2], Alicia Perera Castro [2,3], Krystal Randal [1,2] and Melinda J. Waterman[1,2]
[1] Securing Antarctica's Environmental Future, University of Wollongong, Wollongong, NSW, Australia;
[2] Centre for Sustainable Ecosystem Solutions, University of Wollongong, Wollongong, NSW, 2522, Australia;
[3] Universitat de les Illes Balears, Mallorca, Spain

Antarctica is the coldest, highest, driest, windiest continent on Earth. The region has experienced major changes in temperature, wind speed and stratospheric ozone levels over the last 50 years. Whilst West Antarctica and the peninsula have shown rapid warming and consequent ecosystem change, at first East Antarctica appeared to be little impacted by climate warming and thus biological changes were predicted to be relatively slow. Recently, however, Antarctica has experienced extreme climatic events in the form of summer heatwaves in 2019/20 [Robinson et al. (2020) Glob. Change Biol. 26: 3178-3180] and an autumn heatwave in March 2022.
Terrestrial plants are limited to the peninsula region and mosses are the dominant plants for most of the frozen continent. Mosses are restricted to sparse ice-free areas and grow where melt water is available through the summer. Soils are generally poor and nutrient sources often come from marine sources, including ancient penguin colonies. Even in the summer, Antarctic mosses and lichens battle sub-zero temperatures, extreme winds and reduced water availability; all influencing their ability to survive and grow. However, in summer, Antarctic moss canopy temperatures can be well above air temperature. At midday, canopy temperatures can exceed 15°C, depending on moss turf water content [Perera Castro et al. (2020) Front. Plant Sci. 11 1178]. How do these tiny plants protect themselves in such harsh conditions?

This talk will address how mosses photosynthesise on the cold continent, how they protect themselves from excess light and ultraviolet (UV) radiation [Waterman et al. (2017) J. Nat. Prod. 80: 2224-2231; Waterman et al. (2018) Biol. Res. 51: 49] and what a changing climate might mean for their survival and health

PLENARY SPEAKER



JIAN-REN SHEN

Faculty of Science Okayama University
Professor, Research Institute for Interdisciplinary Science, Okayama University, Japan/Institute of Botany, Chinese Academy of Sciences, China Professor Jian-Ren Shen's research focuses on elucidation of the structures of photosynthetic membrane-protein complexes involved in water oxidation and light energy harvesting and transfer. He and his colleagues solved the first high-resolution crystal structure of photosystem II (PSII) which revealed the organization of the catalyst for water oxidation. His group further solved the damage-free structure and intermediate state structures of PSII by X-ray free electron lasers, which provided critical information regarding the mechanism of water oxidation. His group also solved the structure of PSI from high plants and other organisms, and PSII in complex with its light-harvesting antennas from other eukaryotic algae. These results provide important clues on the arrangement of pigments and energy transfer pathways in PSI and PSII. Prof. Shen has received a number of national and international awards including the Gregori Aminoff Prize from the Royal Swedish Academy of Sciences.

ABSTRACT

Structural studies of photosystem II water oxidation and other photosystem-light harvesting protein supercomplexes from different organisms

Jian-Ren Shen
Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan. Institute of Botany, CAS, Beijing, China

Water oxidation by photosystem II (PSII) is catalyzed by a Mn_4CaO_4 cluster through an S-state cycle with Si=0-4. By using the method of pump-probe X-ray free electron laser (XFEL) structural analysis, we have demonstrated that a new oxygen atom called O6 is inserted into the Mn_4CaO_4 cluster at a position close to O5 during S2-S3 transition, enabling the two oxygen atoms to form O=O bond through an oxyl/oxo coupling mechanism. Time-resolved pump-probe XFEL analysis further show a number of structural changes at both water molecules and amino acid residues following 1 or 2 flashes illumination in a short time range (nsec-µsec), reflecting transit structural dynamics accompanying electron and proton transfer in this time range. These structural changes finally converged to the changes seen in the meta-stable S2 or S3 states. Among them, an important change is seen in a water molecule close to the Ca^{2+} ion, which appeared after 2 flashes in µsec time range but disappeared later. Accompanying the disappearance of this water molecule, the electron density of O6 is appeared and became stronger in the S3-state. This led us to speculate that this water molecule is the origin of O6. We also used cryo-electron microscopy (cryo-EM) to study the structures of reaction center (RC)-light harvesting (LH) supercomplexes, PSII-LHCII and PSI-LHCI supercomplexes from a range of different organisms. Among them, the structure of FMO-RC from a green sulfur bacterium provides important insights into the evolution of photosystems, and the structures of PSII-FCPII, PSI-FCPI supercomplexes from diatoms provide a surprising diversity of the light-harvesting complexes as a result of adaptation to various light environments that each organism occupies.

PLENARY SPEAKER

SUSANNE VON CAEMMERER

ARC Centre of Excellence for Translational Photosynthesis
Professor Susanne von Caemmerer research focusses on understanding and improving C3 and C4 photosynthesis, genetically manipulating the photosynthetic processes, and developing better leaf and plant photosynthetic models. She co-developed one of the most widely used biochemical models in plant biology – the Farquhar, von Caemmerer and Berry model of C3 photosynthesis and identified the key physiological measurements needed to apply the model. She developed an equally widely used model of C4 photosynthesis. Her current research is resolving critical issues of CO2 diffusion inside leaves. She is a fellow of the Australian Academy of Science, The Royal Society of London and the German Academy of Science, Leopoldina.

ABSTRACT

Novel insights into the regulation of C4 Photosynthesis

Susanne von Caemmerer
Research School of Biological Science, Australian National University, Canberra Australia

Recent activities to improve photosynthetic performance in crop plants have focused primarily on C3 photosynthesis where there are clear identified targets such as improving Rubisco kinetics, installation of a CO_2 concentrating mechanism and alleviating limitations in chloroplast electron transport. However, C4 plants that utilise the C4 photosynthetic pathway also play a key role in world agriculture. For example, C4 crops such as maize and sorghum are major contributors to both first and third world food production and the C4 grasses sugarcane; miscanthus and switch grass are major plant sources of bioenergy. Understanding the regulation of C4 photosynthesis is essential to manipulate C4 photosynthesis for agricultural benefit.

The C4 photosynthetic pathway is a biochemical CO_2 concentrating mechanism that requires the coordinated functioning of mesophyll and bundle sheath cells of leaves and species have evolved a complex blend of anatomy and biochemistry to achieve this. The limitations to photosynthetic flux are not as well studied in C4 plants, but our work with transgenic *Flaveria bidentis*, a transformable model C4 dicot, has provided gene candidates for improvement of carbon metabolism. We are now using the model monocot C4 species *Setaria viridis* (green foxtail millet) to generate transgenic plants with altered C4 photosynthetic metabolism to study the regulation of C4 photosynthesis. Targets include CO_2 diffusion in the mesophyll and bundle sheath, chloroplast electron transport and Calvin Cycle activity.

PLENARY SPEAKER

JUNKO YANO

Joint Center for Artificial Photosynthesis (JCAP)

Junko Yano received a BA degree in Applied Biological Science from Hiroshima University, Japan, MSc from Graduate School of Biosphere Science, Hiroshima University, Japan, and earned her PhD in Physical Chemistry from Osaka University, Japan in 1999. She has received several awards including the Golda Meir Research Fellowship from Hebrew University in Jerusalem, and the Robin Hill Award by the International Society for Photosynthesis Research. She is currently a Senior Scientist at the Molecular Biophysics and Integrated Bioimaging Division at Lawrence Berkeley National Laboratory. She is also a PI of the Joint Center for Artificial Photosynthesis at Berkeley. Her research areas include the water oxidation in natural photosynthesis and artificial photosynthesis, catalytic reactions in metalloenzymes, application of synchrotron X-ray radiation and X-ray free electron laser techniques to biological and inorganic systems.

ABSTRACT

Capturing sequence of events during the water oxidation reaction in natural photosynthesis

Junko Yano
Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

The water oxidation reaction in nature is carried out by Photosystem II (PS II), a multi subunit membrane protein complex. This light-driven reaction is made possible by a spatially separated, yet temporally connected series of cofactors along the electron transfer chain of PS II over 40 Å, through the donor (the Mn_4CaO_5 catalytic center), the reaction center chlorophylls, to the mobile quinone electron acceptors. Such chemical architecture provides an ideal platform to investigate how to control multi-electron/proton chemistry, using the flexibility of metal redox states, in coordination with the protein and the water network. Understanding the insights from nature's design provides inspiration for how to build artificial photosynthetic devices, where the controlled accumulation of charge and high-selectivity of products is currently challenging. To solve the mechanism of the water oxidation reaction in PS II, our strategy has been to follow the structural and chemical sequence of all events in the enzyme that lead to O-O bond formation, and to understand the cycle of the catalytic reaction at the atomic and electronic structure level. The questions include, what structural parameters control reaction kinetics? And how does the environment (protein and hydrogen-bonding network) change to accommodate a dynamically evolving energy landscape during the multielectron/multiproton chemistry, while adjusting local pH and redox potential to drive chemistry? To answer these questions, we have developed and applied new methods in crystallography and X-ray spectroscopy at the X-ray free electron lasers (XFELs). Our progress is based on the more than decade-long experience developing XFEL-based X-ray methods, which is coupled with improved PS II sample preparation and lab-based characterization methods. Our studies have started revealing the details of how water is oxidized by PS II and the role of the protein environment in the process, and how the catalytic cycle is reset [Kern et al. (2013) Science 340:491-495; Young et al. (2016) Nature 540:453-457; Fuller et al. (2017) Nat. Methods 14:443-449; Kern et al. (2018) Nature 563:421-425; Ibrahim et al. (2020) Proc. Natl. Acad. Sci. USA 117:12624-12635; Hussein et al. (2021) Nat. Commun.12:6531]. We will discuss the interplay of structural changes of the overall protein and the chemical changes occurring at the metal-site as PS II cycles through the enzymatic reaction.

PLENARY SPEAKER

JINDONG ZHAO

College of Life Science, Peking University

Dr Zhao obtained his BSc degree in biology from Southwest University, China. He was admitted to the graduate school of Chinese Academy of Sciences (CAS) for study of ecology of algae. He received a fellowship from CAS and studied in University of Texas at Austin in the field of cyanobacterial photosynthesis as a graduate student. He completed his PhD in Plant Physiology in 1990.

Dr Zhao then went to Penn State University as a postdoctoral scholar and continued study on photosynthetic electron transfer of cyanobacteria. After postdoctoral research Dr Zhao joined Applied Biosystems in California as a research scientist briefly before he returned to China and became an associated professor in Peking University in 1994. He was promoted to professor in 1998. From 2007 to 2018, Dr Zhao served as director of Institute of Hydrobiology, CAS, which was established in 1930. Dr Zhao was elected a member of CAS in 2007 and a member of TWAS in 2010.

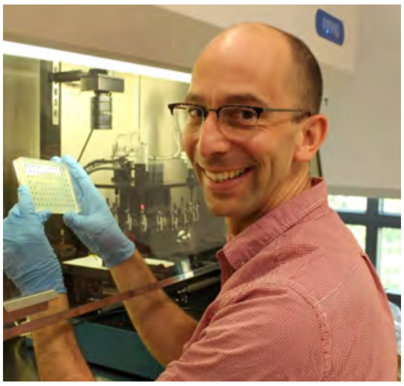
ABSTRACT

Phycobilisome light harvesting and its regulation in Cyanobacteria

Jindong Zhao
College of Life Sciences, Peking University

Phycobilisomes (PBS) are the major light-harvesting complexes for photosynthesis in cyanobacteria and red algae and they have a hierarchical structure of a core and peripheral rods with both consisting of phycobiliproteins and linker proteins. There are four morphological types of PBS: the hemidiscoidal, the hemiellipsoidal, block-type and bundle shape PBS. The cryo-EM structures of first three PBS have been determined while the structure of the bundle-shape PBS remains to be solved. The cryo-EM structures of PBS from the cyanobacteria and red algae revealed that (i) all PBS share a similar core structure, (ii) the distances among the bilins within a PBS are in general between 30 to 40 Å, and (iii) both phycobiliproteins and linker proteins contribute tyrosine residues for tyrosine web formation in facilitating energy transfer within PBS.

The distribution of PBS-absorbed light energy between PSII and PSI is critical to photosynthetic efficiency, and it is regulated by a process called state transitions. The distribution is dependent upon the redox state of the interphotosystems. While it is known that cytochrome *b6f* complex (Cyt*b6f*) is the site sensing the redox change of the interphotosystems, the mechanism of sensing the redox state of the interphotosystems in cyanobacteria remain uncertain. Here we report that the construction of a Cytb6f mutant of *Nostoc* sp. PCC 7120. The mutant destabilized Cyt*b6f*, leading to a 95% loss of Cytb6f complex as judged with immunoblotting. Immunoblotting after blue-native electrophoresis could no longer detect the Cyt*b6f* complex from the mutant. The oxygen evolution activity of the mutant was only 10-20% of that from the wild-type and became partially insensitive to DBMIB. Although the PQ pool was largely reduced under normal light conditions, the mutant had a significantly higher PSII/PSI ratio than the wild-type. State transitions of the mutant was absent as revealed by 77K fluorescence spectra and modulated room temperature fluorescence induction. The electron carrier TMPD, which accepts electrons from PQ pool and bypasses Cyt*b6f*, increased the rate of oxygen evolution several folds but could not induce state transitions in the mutant. These results strongly suggest that, like in chloroplasts of green plants, the Cyt*b6f* complex is required for state transitions in the cyanobacteria.



THE CALVIN-BENSON AWARD WINNER

Structure, biogenesis, and engineering of the pyrenoid

Martin Jonikas
Princeton University, Princeton, New Jersey, USA

Approximately one-third of global CO_2 fixation occurs in an overlooked algal organelle called the pyrenoid. The pyrenoid enhances CO_2 fixation by supplying the CO_2-fixing enzyme Rubisco with a high concentration of its substrate. The molecular structure and biogenesis of this ecologically fundamental organelle have remained enigmatic. Our laboratory is developing and using systems biology tools in the model alga *Chlamydomonas reinhardtii* to identify novel components of the pyrenoid. By characterizing these components, we are gaining a molecular understanding of pyrenoid structure, biogenesis, and function. With our collaborators in the Combining Algal and Plant Photosynthesis project, we aim to transfer algal pyrenoid components into land plants to enhance crop yields.

THE HILL AWARD WINNER

Structural studies of the oxygen-evolving complex by using x-ray free electron lasers

Michi Suga
Okayama University, Okayama, Japan

The water oxidation and oxygen evolving reaction is catalyzed at the catalytic center of PSII, the oxygen evolving complex (OEC), through four oxidation steps of the Kok cycle (Si-state cycle i=0-4). In 2011, the high-resolution crystal structure of PSII revealed the architecture of the Mn_4CaO_5 -cluster. However, the structure of the Mn_4CaO_5 cluster has been damaged by the strong irradiation of the synchrotron X-rays used for the structural analysis. Ultra-brilliant pulses of femtosecond X-ray free-electron lasers (XFEL) have a potential to avoid this issue by the diffraction data collection before the onset of radiation damage. We have used XFEL to analyze the "radiation damage-free" structure of the Mn_4CaO_5 cluster in the dark-stable S1 state at 1.95-Å resolution [Suga M. et al. (2015) Nature 517:99-103]. We also utilized XFEL to analyze the PSII structures in the intermediate S2 and S3 states [Suga M. et al. (2017) Nature 543:131-135; Suga M. et al. (2019) Science 366:334-338] and to analyze its structural dynamics [Li et al. (2021) IUCrJ 8:431-443]. These XFEL structures obtained have provided a basis for the vital understanding of the relationship between the structure of the Mn_4CaO_5 cluster and its physical properties.

THE JAN ANDERSON AWARD WINNER

Inspirations from Jan Anderson: Lateral heterogeneity in the age of cryo-electron tomography

Benjamin Engel
Biozentrum, University of Basel, Basel, Switzerland

In this talk, I will describe some of our group's work that has been heavily inspired by the landmark findings of Jan Anderson. Dr Andersson help establish the fundamental principle that thylakoid membranes are subdivided into discrete domains: in vascular plants, PSII sits in the appressed grana stacks, while PSI is restricted to the non-appressed stroma lamellae. Our group has pioneered the use of cryo-electron tomography to visualize photosynthetic complexes embedded within native thylakoid membranes inside intact cells. Using this revolutionary technique, we have been able to build on Dr Anderson's work by studying the molecular organization of native thylakoids in cyanobacteria, the green alga *Chlamydomonas*, and spinach. I will describe our cryo-ET findings and compare to previous landmark studies of lateral heterogeneity. I will then present a future prospective on our plans to study thylakoid molecular organization in diverse marine algae, during thylakoid biogenesis, and under changing environmental conditions.

References

Gupta T.K., Klumpe S., Gries K., Heinz S., Wietrzynski W., Ohnishi N., Niemeyer J., Spaniol B., Schaffer M., Rast A., Ostermeier M., Strauss M., Plitzko J.M., Baumeister W., Rudack T., Sakamoto W., Nickelsen J., Schuller J.M., Schroda M., Engel B.D. (2021). Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. Cell184:3643-3659.e23

Wietrzynski W., Schaffer M., Tegunov D., Albert S., Kanazawa A., Plitzko J.M., Baumeister W., Engel B.D. (2020). Charting the native architecture of *Chlamydomonas* thylakoid membranes with single-molecule precision. eLife 9: e53740

Rast A, Schaffer M, Albert S, Wan W, Pfeffer S, Beck F, Plitzko JM, Nickelsen J, Engel BD (2019). Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. Nat. Plants 5:436-446

THE JALAL ALIYEV AWARD WINNER

Don Ort is the Robert Emerson Professor of Plant Biology and Crop Sciences at the University of Illinois. His BS degree is in biology/chemistry from Wake Forest University and he earned his PhD in plant biochemistry from Michigan State University. He served as President of the American Society of Plant Biology, President of the International Society of Photosynthesis Research and as Editor-in-Chief of Plant Physiology. He is an ASPB Kettering Award recipient, ASPB Fellow, AAAS Fellow, ARS Science Hall of Fame, 2016-19 ISI Highly Cited Author and Member of the National Academy of Sciences. He is a Theme Leader in the Institute for Genomic Biology, Director of Research for the DOE Center for Advanced Bioengergy & Bioproducts Innovation and Deputy Director of the Gates Foundation RIPE program at the University of Illinois. His laboratory is engaged in three lines of research: i) Redesigning photosynthesis for improved efficiency; ii) Molecular and biochemical basis of environmental interactions with crop plants; iii) Ecological genomics: interactive effects of CO_2, temperature and drought on plant, plant canopy and plant ecosystem performance.

MITIGATING THE IMPACT OF A WARMING CLIMATE ON PHOTOSYNTHETIC CARBON GAIN WITH AN ALTERNATIVE PHOTORESPIRATORY PATHWAY

Donald R. Ort
Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL

Agricultural production faces numerous global change-related abiotic stresses, including rising temperatures, which pose a threat to global food production and sustainability. Global temperatures are rising, and higher rates of temperature increase are projected over land areas that encompass the globe's major agricultural regions. In addition to increased growing season temperatures, heat waves are predicted to become more common and severe. Among the physiological processes that underlie crop yield that are susceptible to high temperatures, photosynthetic carbon gain is among the most important where the effects high temperature can interact with other climate change factors. Strategies to adapt photosynthesis to the warming climate and episodes of extreme high temperature will be critical to sustaining productivity much less to increase productivity to meet anticipated increasing agricultural demand. There are numerous points of high temperature sensitivity in photosynthesis that could be targeted including photorespiration. Photorespiration is a very large energetic cost to C3 plants and its rate increases with temperature, increasing the overall energetic cost and lowering net photosynthesis. We have investigated installing more energetically efficient non-native pathways that substitute for the native pathway to mediate the impact of increasing temperature on net carbon gain with promising results.

CLIMATE SESSION SPEAKERS

OLAF MORGENSTERN

National Institute of Water and Atmospheric Research
Dr Olaf Morgenstern holds a diploma in physics from the University of Freiburg, Germany, and a PhD in meteorology from ETH Zurich, Switzerland. His PhD thesis focused on the interaction of severe precipitation with high topography. After completing his degree he has twice worked at the University of Cambridge, UK, interspersed with a stint at the Max-Planck-Institute of Meteorology in Hamburg, on the development of atmospheric chemistry and chemistry-climate models. He is currently leading the NIWA Climate Variability and Change Programme, which focusses on using global and regional climate models and a hydrology model to predict climate globally and for the New Zealand region, ranging from seasonal to centennial timescales

CATE MACINNIC-NG

The University of Auckland
Associate Professor Cate Macinnis-Ng is a plant ecophysiologist in the School of Biological Sciences at Waipapa Taumata Rau, the University of Auckland. Her research focusses on impacts of climatic conditions on water and carbon fluxes of forests with a particular focus on extreme events. In 2015, she was awarded a Rutherford Discovery Fellowship to explore the influence of drought on water relations of kauri. Much of Cate's work uses field-based methods but she also uses glasshouse experiments and modelling approaches. She is interested in biodiversity responses to climate change and has co-authored two recent papers on climate change in ecosystems of Aotearoa New Zealand. Cate was a contributing co-author for Chapter 11 in the recent Working Group II report for the IPCC Assessment Round Six. Cate lives in Auckland with her husband, two teenage children and two guinea pigs.

GENERAL INFORMATION

CAR PARKING

Paid parking is available in the three car park buildings in Moray Place standard hourly rates are approximately $2.00/hour

COVID 19 HYGIENE / HEALTH & SAFETY PROTOCOL

We need to keep everyone as safe as possible under the current New Zealand Government Covid19 Orange Light Setting. However, as there are currently no mandates or restrictions at indoor events, we can only give recommendations to delegates. Our recommendations follow the NZ Ministry of Health Guidelines for conferences./ congresses

But, we would like to encourage delegates to wear a face mask where possible as social distancing is difficult to maintain. Hand sanitiser will be provided. We encourage delegates to follow the general public advice about staying home if not well, to get tested immediately and inform the Congress organisers 027 474 9887.

MOBILE PHONES/DEVICES/LAPTOPS

The organising committee respectfully requests that you turn your devices off (or on vibrate or silent) as a courtesy to the speakers and other delegates attending the sessions. If you forget to turn off your phone and it rings please do not answer it in the session - please take your call outside.

NAME BADGES

For security reasons, all delegates, speakers and visitors must wear their conference name badge at all times during the conference. Access to the sessions, morning, afternoon teas and lunch and the social functions will not be permitted without a name badge.

PROGRAMME

Should any changes occur to the programme during the conference they will be announced in the welcome & housekeeping sessions each morning.

REGISTRATION & INFORMATION DESK

The registration desk will be open during the following times:

- Sunday 31 July 12.00pm - 8.00pm
- Monday 1 August 7.30am - 7.00pm
- Tuesday 2 August 7.30am - 7.00pm
- Wednesday 3 August 12.00pm - 4.00pm
- Thursday 4 August 7.30am - 5.00pm
- Friday 5 August 7.30am - 3.00pm

SMOKING

The Dunedin City Council buildings and all public building's in New Zealand are Smoke Free this includes hotels, motels, cafes etc, Smoking is not allowed inside or within 6 metres of the buildings. If you must smoke please cross the road and smoke in the carpark opposite the main entrance of the Dunedin Centre.

SOCIAL FUNCTIONS

Sunday 31 July Opening Reception 6.00pm - 7.30pm Fullwood Foyer
Tuesday 2 August - 7.30 - 9.30pm Pizza and pints Poster Session
Thursday 4 August Conference Dinner 7.00pm - 11.30pm Glenroy Auditorium - Harrop Street entrance

SPECIAL DIETARY REQUIREMENTS

If you have advised the conference organisers of any special dietary requirements, these will have been forwarded to the caterers, and will be managed by them. The conference dinner is a plated meal please make yourself known to the wait staff and your special dietary need.

TRANSPORT and AIRPORT SHUTTLE AND TAXIS

We recommend booking your airport transfers well in advance.

Airport Shuttles Dunedin Ltd: Phone: 0800 477 800 OR www.airportshuttlesdunedin.co.nz
Kiwi Shuttles Phone: 0800 365 494 OR www.kiwishuttles.co.nz
SuperShuttle: Phone: 0800 748 885 OR 09 522 5100 OR www.supershuttle.co.nz download the app SuperShuttleNZ
Dunedin Taxis: Phone: +64 3 477 7777 OR www.dunedintaxis.co.nz
Green Cabs: www.greencabs.co.nz OR download the app Green Cabs Go Green

VIRTUAL PLATFORM ACCESSS

Before July 30, 2022 all delegates will receive a email with, either information for the in person app or the virtual access information. After the congress, all delegates will be emailed a link to access all presentations and videos for a month after the congress closed.

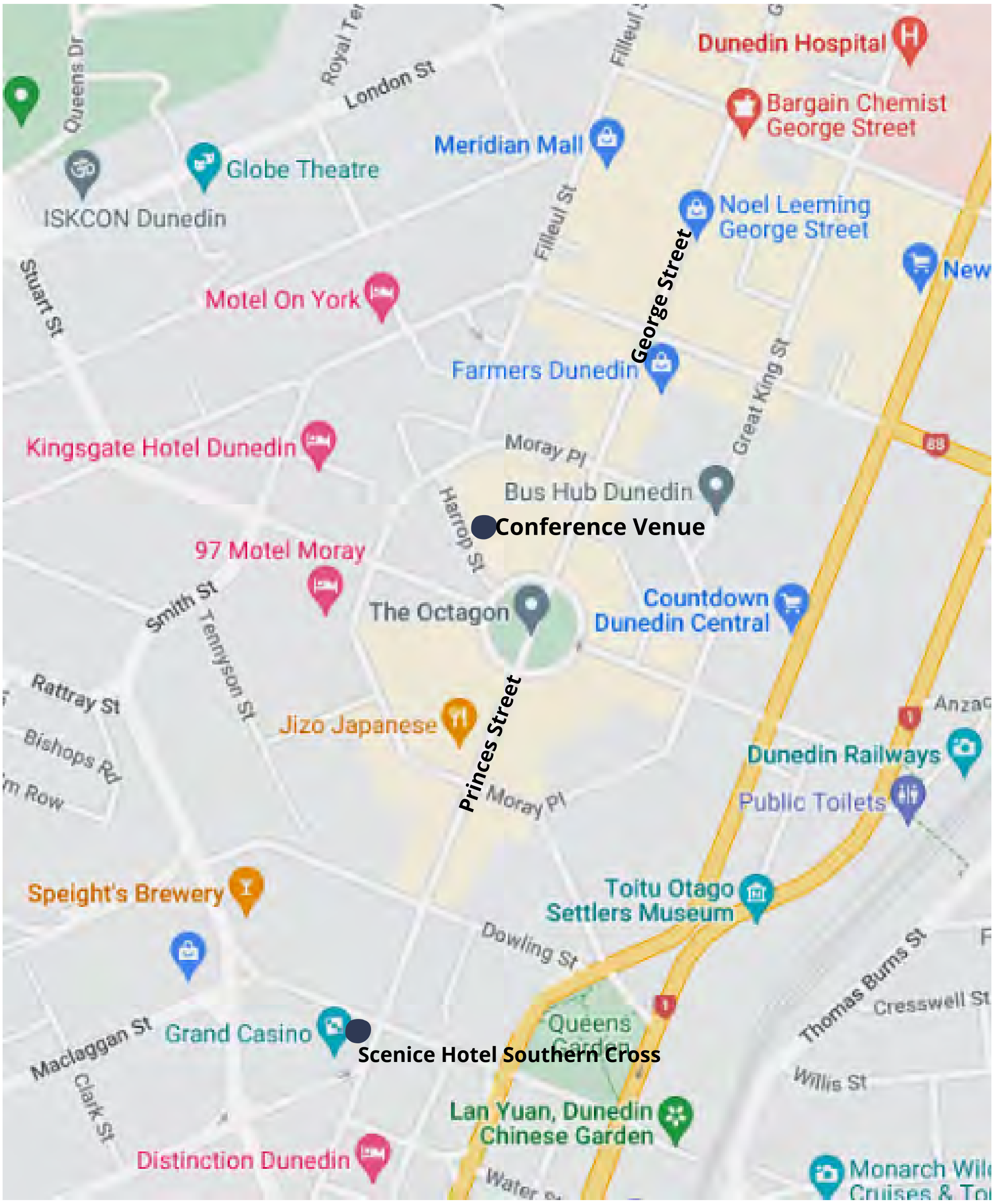
WIFI PASSWORD

Network: DVML_Guest_Network
Password: Guest@DV

MAPS

clickable link

[Dunedin Google Map]



SCIENTIFIC PROGRAMME

18th International Congress on Photosynthesis Research

July 31 – August 5, 2022

SUNDAY 31 July

15:30 – 17:00	Registration
17:30 – 17:45	**Opening Ceremony Fullwood Room**
17:45 – 18:45	**Opening Plenary Presentations - *Chair Wim Vermaas*** **Enhancing and adapting photosynthesis for food security under climate change** Stephen Long *(University of Illinois, USA)* - Pre Recorded **Emerging Approaches for PSI-Based Bio-hybrid Devices** Barry Bruce *(University of Tennessee, USA)* – In Person
18:45 – 20:30	Welcome Reception – Fullwood foyer

MONDAY 1 August

08:30 – 09:00	**Plenary Session - *Chair Grant Pearce*** **Phycobilisome light harvesting and its regulation in Cyanobacteria** Jindong Zhao *(Peking University, China)* – Pre Recorded	
09:00 – 09:30	**Novel insights into the regulation of C4 Photosynthesis** Susanne von Caemmerer *(Australian National University, Australia)* – In Person	
09:30 – 10:00	Coffee Break	
	PARALLEL SYMPOSIA	
ROOM	**FULLWOOD**	**CONFERENCE 1**
10:00 – 12:15	**Electron and Proton Transfer in Photosynthesis** *Chair / Discussion Leader:* Marilyn Gunner *(City College of New York, CUNY, USA)* - Pre Recorded Introduction	**Rubisco and Photorespiration** *Chair / Discussion Leader:* Spencer Whitney *(Australian National University, Australia)* - In Person Introduction
10:10 – 10:30	253 IP - John Golbeck *(Penn State University, USA)*	251 IP - Florian Busch *(University of Birmingham, UK)*
10:30 – 10:50	257 V - Hiroshi Ishikita *(University of Tokyo, Japan)*	264 V - Maureen Hanson *(Cornell University, USA)*
10:50 – 11:05	44 V - Shinji Masuda	59 IP - Oliver Mueller-Cajar
11:05 – 11:20	146 IP - Greg Tira	63 IP - Stavros Azinas
11:20 – 11:35	199 V - Jingcheng Huang	104 IP - Yi-Chin Candace Tsai
11:35 – 11:50	214 V - Andrea Fantuzzi	171 V - Shin-Ichi Miyazawa
11:50 – 12:15	Discussion	Discussion
12:15 – 13:30	Lunch Break and Poster Viewing	
13:30 – 14:00	**Plenary Session – *Chair Julian Eaton-Rye*** **Capturing sequence of events during the water oxidation reaction in natural photosynthesis** Junko Yano *(Lawrence Berkeley National Laboratory, USA)* - Pre Recorded	
14:00 – 14:30	**Within-canopy photosynthetic acclimation: importance for photosynthesis in current and future climates** Ülo Niinemets *(Estonian University of Life Sciences, Estonia)* - Pre Recorded	
14:30 – 14:50	Coffee Break	

PARALLEL SYMPOSIA		
ROOM	**FULLWOOD**	**CONFERENCE 1**
14:50 – 17:25	**Regulation of Electron Transfer and Alternative Electron Transport Pathways** *Chair / Discussion Leader:* Marc Nowaczyk *(Ruhr University Bochum, Germany)* - Pre Recorded Introduction	**Multiscale Modelling of Photosynthesis** *Chair / Discussion Leader:* Xinguang Zhu *(Shanghai Institutes for Biological Sciences, CAS, China)* Pre Recorded Introduction
15:00 –15:20	243 IP - Rob Burnap *(Oklahoma State University, USA)*	39 V - Chandra Bellasio *(University of Balearic Islands, Spain)*
15:20 – 15:40	252 V - Yagut Allahverdiyeve-Rinne *(University of Turku, Finland)*	276 IP - Alex Wu *(University of Queensland, Australia)*
15:40 – 15:55	5 IP - Christine Lewis	68 IP - Jun Tominaga
15:55 – 16:10	17 IP - Nina Maryn	154 IP - Eduardo Gorron
16:10 – 16:25	47 V - Adrien Burlacot	195 V - Daipayan Sarkar
16:25– 16:40	177 V - Guang-Ye Han	209 V - Muhamed Amin
16:40 – 17:05	Discussion	Discussion
17:05 – 17:25	Rest Break	

PARALLEL SYMPOSIA		
ROOM	**FULLWOOD**	**CONFERENCE 1**
17:25 – 19:30	**Membrane Ultrastructure and Dynamics** *Chair / Discussion Leader:* Matthew Johnson *(University of Sheffield, UK)* - Pre Recorded Introduction	**Leaf Anatomy and Photosynthetic Performance** *Chair / Discussion Leader:* Ichiro Terashima *(Tokyo University, Japan)*- In Person Introduction
17:35 – 18:00	245 V - Wojciech Wietrzynski	145 V - Eiji Gotoh *(Kyushu University, Japan)*
18:00 – 18:25	262 V - Luning Liu	188 V- Danny Tholen *(University of Natural Resources and Life Sciences, Austria)*
18:25 – 18:45	216 V - Helmut Kirchhoff	7 IP - Florence Danila
18:45 – 19:05	248 V - Andrew Hitchcock	172 V - Yuko Hanba
19:05 – 19:30	Discussion end of day	Discussion end of day

TUESDAY 2 August

08:30 – 09:00	**Plenary Session – *Chair Laura Gunn*** **Rubisco regulation in crops** Elizabete Carmo-Silva *(Lancaster University, UK)* - Pre Recorded	
09:00 – 09:30	**Multiprotein condensate formation in b-carboxysome assembly** Manajit Hayer-Hartl *(Max-Planck-Institute of Biochemistry, Germany)* – In Person	
09:30 – 10:00	Coffee Break	
	PARALLEL SYMPOSIA	
ROOM	**FULLWOOD**	**CONFERENCE 1**
10:00 – 12:15	**Light Harvesting and its Regulation in Eukaryotic Systems** *Chair / Discussion Leader:* Herbert van Amerongen *(Wageningen University, the Netherlands)* - Pre Recorded Introduction	**Regulation of Carbon Assimilation in C3 and C4 Systems** *Chair / Discussion Leader:* Martha Ludwig *(University of Western Australia, Australia)* – In Person Introduction
10:10 – 10:30	247 V - Xiaowei Pan *(Capital Normal University, China*	258 V - Asaph Cousins *(Washington State University, USA)*
10:30 – 10:50	249 IP - Howe Siang Tan *(Nanyang Technological University, Singapore)*	VP - Stéphanie Arrivault (*Max Planck Institute of Molecular Plant Physiology, Germany)*
10:50 – 11:05	29 IP - Collin Steen	3 IP - Florian Galbier
11:05 – 11:20	45 V - Olli Virtanen	8 V - Yuan Xu
11:20 – 11:35	46 IP - Julia Walter	87 V - Petar Mohorovic
11:35 – 11:50	169 V - Ryouichi Tanaka	116 IP - Robert Sharwood
11:50 – 12:15	Discussion	Discussion
12:15 – 13:15	Lunch Break	
	PARALLEL SYMPOSIA	
ROOM	**FULLWOOD**	**CONFERENCE 1**
13:15 – 15:30	**Reaction Centres: Structure and Function** *Chair / Discussion Leader:* Kevin Redding *(Arizona State University, USA)* - Pre Recorded Introduction	**Ecophysiology and Biodiversity in Natural and Managed Systems** *Chair / Discussion Leader:* Elizabeth Ainsworth *(University of Illinois, USA)* - Pre Recorded Introduction
13:25 –13:45	254 V - Po-Lin Chiu *(Arizona State University, USA)*	118 IP - Demi Sargent
13`:45 – 14:05	278 V - Christopher Gisriel *(Yale University, USA)*	273 - Sam Taylor
14:05 – 14:20	6 IP - Gary Hastings	40 V - Quentin Charras
14:20 – 14:35	34 V - Jing-Hua Chen	103 IP - Shoko Tsuji
14:35 – 14:50	129 V - Shigeru Itoh	179 V - Francesco Bellamoli

14:50 – 15:05	130 V - Michael Hippler	80 V- Oula Ghannoum
15:05 – 15:30	Discussion	279 V Chandra Bellasio *(Extra 15min talk)* Discussion
15:30 – 15:50	Coffee Break	
15:50 – 16:20	**Plenary Session - *Chair Tina Summerfield*** **Structural studies of photosystem II water oxidation and other photosystem-light harvesting protein supercomplexes from different organisms** Jian-Ren Shen *(Okayama University, Japan)* - Pre Recorded	
16:20 – 16:50	**Breaking the red-limit: driving oxygenic photosynthesis with far-red light** Roberta Croce *(VU Amsterdam, the Netherlands)* - Pre Recorded	
16:50 – 17:00	Short Break	
	PARALLEL SYMPOSIA	
ROOM	**FULLWOOD**	**CONFERENCE 1**
17:00 – 19:15	**Synthetic Biology for Food, Fuel and Chemical Production** *Chair / Discussion Leader:* Himadri Pakrasi *(Washington University St Louis, USA)* - Pre Recorded Introduction	**Photosynthetic Phenomics and Crop Improvement** *Chair / Discussion Leader:* Christine Raines *(University of Essex, UK)* - Pre Recorded Introduction with talk 273 V - Christine Raines *(University of Essex, UK) (note time 17:00 – 17: 25)*
17:10 –17:30	205 V - Graham Peers *(Colorado State University, USA)*	
17:30 – 17:50	246 V - Claudia Vickers *(CSIRO and Queensland University of Technology, Australia*	261 V - Caitlin Moore *(University of Western Australia, Australia)* (17:25 – 17:45)
17:50 – 18:05	150 IP - Prem Pritam	270 V - Mark Aarts *(Wageningen University, the Netherlands)* (17:45 – 18:05)
18:05 – 18:20	155 IP - Wim Vermaas	11 IP - Liana Acevedo-Siaca
18:20 – 18:35	197 V - Annesha Sengupta	212 V - Yin Wang
18:35 – 18:50	229 IP - Paul Hudson *(KTH Royal Institute of Technology, Sweden) [extra 20-min talk]*	219 V - Oliver Knopf
18:50 – 19:15	Discussion	Discussion
19:15 – 21:30	Poster Session (Pizza with NZ Wine and Craft Beers)	

WEDNESDAY 3 August

08:30 – 09:00	**Awards Talks – *Chair Wim Vermaas*** **The Robin Hill Award Talk** Michi Suga - In person
09:00 – 09:30	**The Melvin Calvin-Andrew Benson Award Talk** Martin Jonikas Pre Recorded
09:30 – 10:00	Coffee Break

PARALLEL SYMPOSIA

ROOM	FULLWOOD	CONFERENCE 1
10:00 – 12:15	**Light Harvesting and its Regulation in Prokaryotic Systems** *Chair / Discussion Leader:* Bob Blankenship *(Washington University, St Louis, USA)* - Pre Recorded Introduction	**Chloroplast Development and Assembly of the Photosynthetic Apparatus** *Chair / Discussion Leader:* Peter Nixon *(Imperial College London, UK)* - Pre Recorded Wataru Sakamoto *(Okayama University, Japan)* - Pre Recorded Introduction
10:10 – 10:30	190 V - Tina Dominguez Martin *(University of Cordoba, Spain)*	61 V - Josef Komenda *(Institute of Microbiology, Centre Alagatech and University of South Bohemia, Czech Republic)*
10:30 – 10:50	250 V - Michal Koblizek *(Institute of Microbiology, Centre Alagatech, Czech Republic)*	267 V - Ben Engel *(Max Planck Institute of Biochemistry, Germany)*
10:50 – 11:05	4 V - Olga Chukhutsina	27 V - Monique Liebers
11:05 – 11:20	83 V - Michal Gwizdala	65 V - Lianyong Wang
11:20 – 11:35	98 V - Dvir Harris	71 IP - Haruhiko Jimbo
11:35 – 11:50	162 IP - Takehisa Dewa	192 V - Marc Nowaczyk
11:50 – 12:15	Discussion	Discussion
12:15	Free Time	

THURSDAY 4 August

08:30 – 09:00	**Plenary Session - *Yulia Pushkar*** **The Tom Wydrzynski Lecture on Water Oxidation** Gary Brudvig *(Yale University, USA)* - In Person
09:00 – 09:30	**Basking in the sun: How mosses photosynthesise and survive the coldest continent on Earth** Sharon Robinson *(University of Wollongong, Australia)*
09:30 – 09:50	Coffee Break
09:50 – 10:20	**Jan Anderson Lecture Award Talk - *Chair Wim Vermaas*** Ben Engel - Pre Recorded
10:20 – 10:30	Short Break

PARALLEL SYMPOSIA

ROOM	FULLWOOD	CONFERENCE 1
10:30 – 12:45	**The Tom Wydrzynski Water Oxidation Session** *Chair / Discussion Leader:* Johannes Messinger *(Uppsala University Sweden)* - Pre Recorded Introduction	**CO_2 Diffusion, Transport and Concentration Mechanisms** *Chair / Discussion Leader:* David Hanson *(University of New Mexico, USA)* – In Person Introduction
10:40 – 11:00	263 V - Dimitrios Pantazis *(Max Planck Institute for Coal Research, Germany)*	148 V - Roberto Salomon *(Polytechnic University of Madrid, Spain)*
11:00 – 11:20	240 V - Holger Dau *(Free University, Berlin, Germany)*	170 IP - Mina Momayyezi *(University of California, Davis, USA)*
11:20 – 11:35	136 IP - David Vinyard	33 IP - David Savage
11:35 – 11:50	183 V - Isabel Bogacz	52 IP - Justin Lau
11:50 – 12:05	184 IP - Yulia Pushkar	53 V - Genki Horiguchi
12:05 – 12:20	152 V - Felix Rummel	92 IP - Alicia V Perera-Castro
12:20 – 12:45	193 Kizashi Yamaguchi (12:20 – 12:35) Discussion *(may extend to 13:00)*	Discussion
12:45 – 13:30	Lunch Break	

PARALLEL SYMPOSIA		
ROOM	**FULLWOOD**	
13:30 – 15:45	**Special Symposia** Olle Björkman Ecophysiology of Photosynthesis Session *Chair / Discussion Leader:* Joe Berry *(Carnegie Institution for Science, Stanford, USA)* Jen Johnson *(Carnegie Institution for Science, Stanford, USA)* - Pre Recorded Introduction	Additional Time for In House and Pre-Recorded Poster Viewing
13:40 –14:00	235 V - Rowan Sage *(University of Toronto, Canada)*	
14:00 – 14:20	269 V - Krishna Niyogi *(University of California Berkeley)*	
14:20 – 14:35	163 V - Barbara Demmig-Adams	
14:35 – 14:50	198 V - David Kramer	
14:50 – 15:05	143 IP - Jen Johnson	
15:05 – 15:20	112 V - Wu Sun	
15:20 – 15:45	Discussion	
15:45 – 16:15	Coffee Break	
16:30 – 18:30	**Special Climate Session** ***Chair: John Troughton*** (*Consultant, Sydney, Australia)* - In Person Olaf Morgenstern *(National Institute of Water and Atmospheric Research, New Zealand)* – In Person Cate Macinnis-Ng *(Auckland University, New Zealand* – In Person Sharon Robinson *(University of Wollongong, Australia)* – In Person *Open to the Public and all Conference Delegates*	
18:30 – 19:00	Rest Break	
19:00 – 24:00	**Conference Dinner – Glenroy Auditorium from 19:00**	

FRIDAY 5 August

8:30 – 9:00	**Plenary Session – *Chair Barry Bruce*** William Martin *(Heinrich Heine University, Germany)* - Pre Recorded	
9:00 – 9:30	**Learning the languages of cells across time and space** Barry Pogson *(Australian National University, Australia)* – In Person	
9:30 – 10:00	Coffee Break	
	PARALLEL SYMPOSIA	
ROOM	**FULLWOOD**	**CONFERENCE 1**
10:00 – 12:15	**Evolution of Photosynthesis** *Chair / Discussion Leader:* Anthony Larkum *(University of Sydney and University of Technology, Sydney, Australia)* – In Person Introduction	**Acclimation of the Photosynthetic Apparatus** *Chair / Discussion Leader:* Joanna Kargul *(University of Warsaw, Poland)* - Pre Recorded Introduction
10:10 – 10:30	182 IP - Martha Ludwig *(University of Western Australia, Australia)*	156 V - Stanisław Karpiński *(Warsaw University of Life Sciences, Poland)*
10:30 – 10:50	228 V - Tanai Cardona *(Imperial College London, UK)*	259 V - Tomas Morosinotto *(University of Padova, Italy)*
10:50 – 11:05	50 V - José Navarro	15 IP - Marten Moore
11:05 – 11:20	144 IP - Beverley Green	25 V - Emilie Wientjes
11:20 – 11:35	167 V - Nancy Kiang	30 V - Taina Tyystjärvi
11:35 – 11:50	206 V - Ryan Wessendorf	51 V - Xiaobo Li
11:50 – 12:15	Discussion	Discussion
12:15 – 13:00	Lunch Break	
13 00 – 15:00	**Translational Photosynthesis: Lab to Field Plant Biology** ***Co-Chairs Robert Furbank and Spencer Whitney*** **C4 Rice: the challenge of turbocharging photosynthesis** Plenary - Robert Furbank *(Australian National University, Australia)* - Pre Recorded **The Jalal Aliyev Award Lecture** Don Ort *(University of Illinois, USA)* - Pre Recorded 239 Spencer Whitney *(Australian National University, Australia)* – In Person 12 Maria Ermakova *(Australian National University, Australia)* – In Person	
15:00 – 15:30	**Closing Ceremony, Poster Prizes and Announcement of Next Congress**	

SUBMISSION INFORMATION

Submissions are listed in order of the submission number, regardless if the submission has been accepted for an oral or poser presentation.

The submission list, lists the presenter, the style and the type of presentation.

Full abstracts follow with details of the presenter and authors.

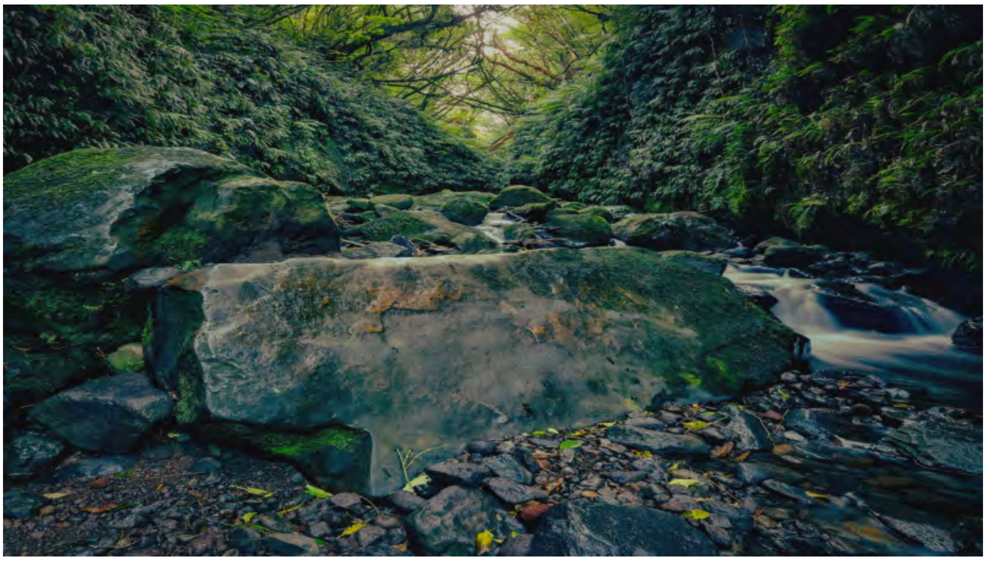


Image: Clive Copeman Photography

SUBMISSIONS

NUMBER	PRESENTER	PRESENTATION	
		STYLE	TYPE
1	Jonathan Lindsey	Pre Record	Poster
2	Harvey Hou	Pre Record	Poster
3	Florian Galbier	In Person	15 Minute Oral
4	Olga Chukhutsina	In Person	15 Minute Oral
5	Christine Lewis	In Person	15 Minute Oral
6	Gary Hastings	In Person	15 Minute Oral
7	Florence Danila	In Person	15 Minute Oral
8	Yuan Xu	Pre Record	15 Minute Oral
9	Yan Wang	Pre Record	Poster
10	Dhilippan M. Panneerselvam	Pre Record	Poster
11	Liana Acevedo-Siaca	In Person	15 Minute Oral
12	Maria Ermakova	In Person	Poster
13	Audrey Short	In Person	Poster
14	Denis Fabre	In Person	Poster
15	Marten Moore	In Person	15 Minute Oral
16	Csaba Éva	In Person	Poster
17	Nina Maryn	In Person	15 Minute Oral
18	Marcos Bacarin	In Person	Poster
19	Diolina Silva	In Person	Poster
20	Patricia Soares	In Person	Poster
21	Andrea Moura	In Person	Poster
22	Noam Adir	Pre Record	Poster
23	Jie He	In Person	Poster
24	Sunil Tiwari	Pre Record	Poster
25	Emilie Wientjes	Pre Record	15 Minute Oral
26	Hisashi Ito	Pre Record	Poster
27	Monique Liebers	Pre Record	15 Minute Oral
28	Barry Osmond	In Person	Poster
29	Collin Steen	In Person	15 Minute Oral
30	Taina Tyystjärvi	Pre Record	15 Minute Oral
31	Christopher Duffy	In Person	Poster
32	Esa Tyystjarvi	Pre Record	Poster
33	David Savage	In Person	15 Minute Oral
34	Jing-Hua Chen	Pre Record	15 Minute Oral
35	Ting-Shuo Nien	Pre Record	Poster
36	Ming-Yang Ho	Pre Record	Poster

37	Hajime Fujii	Pre Record	Poster
38	Pierrick Bru	In Person	Poster
39	Chandra Bellasio	Pre Record	20 Minute Oral
40	Quentin Charras	Pre Record	15 Minute Oral
41	Peter Bos	Pre Record	Poster
42	Shannon Loriaux	In Person	Poster
43	Ting-So Liu	Pre Record	Poster
44	Shinji Masuda	Pre Record	15 Minute Oral
45	Olli Virtanen	Pre Record	15 Minute Oral
46	Julia Walter	In Person	15 Minute Oral
47	Adrien Burlacot	Pre Record	15 Minute Oral
48	Han-Yi Fu	Pre Record	Poster
50	José A. Navarro	Pre Record	15 Minute Oral
51	Xiaobo Li	Pre Record	15 Minute Oral
52	Justin Lau	In Person	15 Minute Oral
53	Genki Horiguchi	Pre Record	15 Minute Oral
54	Yuki Okegawa	Pre Record	Poster
55	Peter Adams	Pre Record	Poster
56	Collin Steen	In Person	Poster
57	Michal Koblizek	In Person	Poster
58	Hongjie Li	Pre Record	Poster
59	Oliver Mueller-Cajar	In Person	15 Minute Oral
60	Eunchul Kim	Pre Record	Poster
61	Josef Komenda	Pre Record	20 Minute Oral
62	Yoshiki Nakajima	Pre Record	Poster
63	Stavros Azinas	In Person	15 Minute Oral
64	Yu Ogawa	Pre Record	Poster
65	Lianyong Wang	Pre Record	15 Minute Oral
67	Maria Paola Puggioni	Pre Record	Poster
68	Jun Tominaga	In Person	15 Minute Oral
69	Paul Curmi	In Person	Poster
70	Ko Imaizumi	In Person	Poster
71	Haruhiko Jimbo	In Person	15 Minute Oral
72	Afshan Begum	In Person	Poster
73	Willem Marulanda Valencia	Pre Record	Poster
74	Anouska van Troost	Pre Record	Poster
75	Laura Díaz Jiménez	In Person	Poster
76	Minoru Kumazawa	In Person	Poster
77	Anjali Pandit	Pre Record	Poster

78	Tomomi Inagaki	In Person	Poster
79	Mitsuo Shoji	In Person	Poster
80	Oula Ghannoum	In Person	Poster
81	Aurélie Crepin	Pre Record	Poster
82	Michal Gwizdala	Pre Record	Poster
83	Michal Gwizdala	Pre Record	15 Minute Oral
84	Yuxi Niu	Pre Record	Poster
85	Tjaart Krüger	Pre Record	Poster
86	Ladislav Nedbal	Pre Record	Poster
87	Petar Mohorovic	Pre Record	15 Minute Oral
88	Gabriella Benko	In Person	Poster
89	Orkun Aydın	Pre Record	Poster
90	Alicia Perera	In Person	Poster
91	Thien Crisanto	In Person	Poster
92	Alicia V Perera-Castro	In Person	15 Minute Oral
93	Sanchali Nanda	Pre Record	Poster
94	Tomas Malina	In Person	Poster
95	Johan Nothling	Pre Record	Poster
96	Shin-Ichiro Ozawa	Pre Record	Poster
97	Kaori Kohzuma	In Person	Poster
98	Dvir Harris	Pre Record	15 Minute Oral
99	Ichiro Terashima	In Person	Poster
100	Timothy Rhodes	In Person	Poster
101	Tanya Skinner	In Person	Poster
102	Kentaro Ifuku	In Person	Poster
103	Shoko Tsuji	In Person	15 Minute Oral
104	Yi-Chin Candace Tsai	In Person	15 Minute Oral
105	Hirozo Oh-oka	Pre Record	Poster
106	Lily Chen	In Person	Poster
107	Toru Kondo	Pre Record	Poster
108	Ryo Nagao	Pre Record	Poster
109	Sinjini Bhattacharjee	Pre Record	Poster
110	Soichiro Seki	In Person	Poster
111	Milan Szabo	Pre Record	Poster
112	Wu Sun	Pre Record	15 Minute Oral
113	Naoki Tsuboshita	Pre Record	Poster
114	Priyanka Patil	Pre Record	Poster
115	Sabit Mohammad Aslam	Pre Record	Poster
116	Robert Sharwood	In Person	15 Minute Oral

117	Paul Greife	In Person	Poster
118	Demi Sargent	In Person	20 Minute Oral
119	Janosch Brandhorst	Pre Record	Poster
120	Sarah Mäusle	In Person	Poster
121	Aleksandra Urban	In Person	Poster
122	Francesca Marchetto	Pre Record	Poster
123	XianJun Zhang	Pre Record	Poster
124	Sergio Santaeufemia Sánchez	Pre Record	Poster
125	Maria Drosou	Pre Record	Poster
126	XianJun Zhang	Pre Record	Poster
127	Mohamd Yahia Dekmak	Pre Record	Poster
128	Lennart Ramakers	Pre Record	Poster
129	Shigeru Itoh	Pre Record	15 Minute Oral
130	Michael Hippler	Pre Record	15 Minute Oral
131	Rin Taniguchi	In Person	Poster
132	Ashley Hancock	Pre Record	Poster
133	Aliya Tychengulova	Pre Record	Poster
134	Jun Minagawa	Pre Record	Poster
135	Wojciech Nawrocki	Pre Record	Poster
136	David Vinyard	In Person	15 Minute Oral
138	Mae Mercado	Pre Record	Poster
139	Krzysztof M. Tokarz	Pre Record	Poster
140	Audrey Norris	Pre Record	Poster
141	Madeline Hoffmann	Pre Record	Poster
142	Julia Kirpich	Pre Record	Poster
143	Jen Johnson	Pre Record	15 Minute Oral
144	Beverley Green	In Person	15 Minute Oral
145	Eiji Gotoh	Pre Record	20 Minute Oral
146	Greg Tira	In Person	15 Minute Oral
147	Sam Wilson	In Person	Poster
148	Roberto Salomon	Pre Record	20 Minute Oral
149	Chen Hu	Pre Record	Poster
150	Prem Pritam	In Person	15 Minute Oral
151	Amit Kumar Chaturvedi	In Person	Poster
152	Felix Rummel	Pre Record	15 Minute Oral
153	Keshav Dahal	Pre Record	Poster
154	Eduardo Gorron	In Person	15 Minute Oral
155	Wim Vermaas	In Person	15 Minute Oral
156	Stanisław Karpiński	Pre Record	20 Minute Oral

157	Tyler Chapman	Pre Record	Poster
158	Man QI	Pre Record	Poster
159	Jingfang Hao	Pre Record	Poster
160	Wasim Iqbal	Pre Record	Poster
161	Anne-Christin Pohland	Pre Record	Poster
162	Takehisa Dewa	In Person	15 Minute Oral
163	Barbara Demmig-Adams	Pre Record	15 Minute Oral
165	Claudia Büchel	Pre Record	Poster
166	Tom Dongmin Kim	In Person	Poster
167	Nancy Kiang	Pre Record	15 Minute Oral
168	Hiroyuki Mino	In Person	Poster
169	Ryouichi Tanaka	Pre Record	15 Minute Oral
170	Mina Momayyezi	In Person	20 Minute Oral
171	Shin-Ichi Miyazawa	Pre Record	15 Minute Oral
172	Hanba Yuko	Pre Record	15 Minute Oral
173	Callum Gray	In Person	Poster
174	Peter Nixon	Pre Record	Poster
175	Shahniyar Bayramov	Pre Record	Poster
176	Irada Huseynova	Pre Record	Poster
177	Guang-Ye Han	Pre Record	15 Minute Oral
178	Armida Gjindali	Pre Record	Poster
179	Francesco Bellamoli	Pre Record	15 Minute Oral
180	Stefano Cazzaniga	Pre Record	Poster
181	Neva Agarwala	Pre Record	Poster
182	Martha Ludwig	In Person	20 Minute Oral
183	Isabel Bogacz	Pre Record	15 Minute Oral
184	Yulia Pushkar	In Person	Poster
185	Wenda Wang	Pre Record	Poster
186	Matteo Ballottari	Pre Record	Poster
187	Eckhard Hofmann	Pre Record	Poster
188	Daniel Tholen	Pre Record	20 Minute Oral
189	Federico Perozeni	Pre Record	Poster
190	Maria Agustina Dominguez Martin	Pre Record	20 Minute Oral
191	Nico Betterle	Pre Record	Poster
192	Marc Nowaczyk	Pre Record	15 Minute Oral
193	Kizashi Yamaguchi	In Person	15 Minute Oral
194	Mitsuo Shoji	In Person	Poster
195	Daipayan Sarkar	Pre Record	15 Minute Oral
196	Donghee Hoh	Pre Record	Poster

197	Annesha Sengupta	Pre Record	15 Minute Oral
198	David Kramer	Pre Record	15 Minute Oral
199	Jingcheng Huang	Pre Record	15 Minute Oral
200	Tim Jeffers	In Person	Poster
201	Jack Forsman	In Person	Poster
202	Asmit Bhowmick	Pre Record	Poster
203	Patricia Walker	Pre Record	Poster
204	Michael Nelson	Pre Record	Poster
205	Graham Peers	Pre Record	20 Minute Oral
206	Ryan Wessendorf	Pre Record	15 Minute Oral
207	Takashi Kawakami	Pre Record	Poster
208	Philipp Simon	Pre Record	Poster
209	Muhamed Amin	Pre Record	15 Minute Oral
210	K. V. Lakshmi	Pre Record	Poster
211	Chunhua Lv	Pre Record	Poster
212	Yin Wang	Pre Record	15 Minute Oral
213	Charles Chen	Pre Record	Poster
214	Andrea Fantuzzi	Pre Record	15 Minute Oral
215	Dan-Hong Li	In Person	Poster
216	Helmut Kirchhoff	Pre Record	20 Minute Oral
217	Prabha Rai Kalal	Pre Record	Poster
219	Oliver Knopf	Pre Record	15 Minute Oral
220	Hui Min Oung	Pre Record	Poster
221	Kevin Sheridan	In Person	Poster
222	Faiza Arshad	In Person	Poster
223	Ei Phyo Khaing	In Person	Poster
224	Fikret Mamedov	Pre Record	Poster
225	Alice Goyal	Pre Record	Poster
226	Min Chen	Pre Record	Poster
227	Min Chen	Pre Record	Poster
228	Tanai Cardona	Pre Record	20 Minute Oral
229	Paul Hudson	In Person	20 Minute Oral
230	Oliver Craig	In Person	Poster
231	Adrian Smith-Beech	In Person	Poster
232	Victor Zhong	In Person	Poster
233	Elisabet Romero	Pre Record	Poster
234	Alain Boussac	Pre Record	Poster
235	Rowan Sage	Pre Record	20 Minute Oral
236	Thekla von Bismarck	Pre Record	Poster

237	Thomas Oliver	Pre Record	Poster
238	Bharat Kumar Majhi	Pre Record	Poster
239	Spencer Whitney	In Person	Pending
240	Holger Dau	Pre Record	20 Minute Oral
241	Toby Brown	In Person	Poster
242	Dimitri Tolleter	Pre Record	Poster
243	Robert Burnap	In Person	20 Minute Oral
244	Roberta Croce	Pre Record	20 Minute Oral
245	Wojciech Wietrzynski	Pre Record	20 Minute Oral
246	Claudia Vickers	Pre Record	20 Minute Oral
247	Xiaowei Pan	Pre Record	20 Minute Oral
248	Andrew Hitchcock	Pre Record	20 Minute Oral
249	Howe-Siang Tan	In Person	20 Minute Oral
250	Michal Koblizek	Pre Record	20 Minute Oral
251	Florian Busch	In Person	20 Minute Oral
252	Yagut Allahverdiyeva-Rinne	Pre Record	20 Minute Oral
253	John Golbeck	In Person	20 Minute Oral
254	Po-Lin Chiu	Pre Record	20 Minute Oral
255	Margaret Doyle	Pre Record	Poster
256	Richard Debus	Pre Record	Poster
257	Hiroshi Ishikita	Pre Record	20 Minute Oral
258	Asaph Cousins	Pre Record	20 Minute Oral
259	Tomas Morosinotto	Pre Record	20 Minute Oral
260	Jay-How Yang	In Person	Poster
261	Caitlin Moore	Pre Record	20 Minute Oral
262	Luning Liu	Pre Record	20 Minute Oral
263	Dimitrios Pantazis	Pre Record	20 Minute Oral
264	Maureen Hanson	Pre Record	20 Minute Oral
265	Kamil Woronowicz	Pre Record	Poster
266	Hiroki Makita	Pre Record	Poster
267	Benjamin Engel	Pre Record	20 Minute Oral
269	Krishna Niyogi	Pre Record	20 Minute Oral
270	Mark Aarts	Pre Record	20 Minute Oral
271	Briony Smith	In Person	Poster
272	Tina Summerfield	In Person	Poster
273	Sam Taylor	Pre Record	20 Minute Oral
274	Christine Raines	Pre Record	20 Minute Oral
275	Stephanie Arrivault	Pre Record	20 Minute Oral
276	Alex Wu	In Person	20 Minute Oral

277	Atsushi Takabayashi,	Pre Record	Poster
278	Christopher J. Gisriel	Pre Record	20 Minute Oral
279	Chandra Bellasio	Pre Record	20 Minute Oral

1 Pre Recorded Poster

Chemical synthesis of chlorophylls and bacteriochlorophylls

Khiem Chau Nguyen, Duy Chung, Kathy-Uyen Nguyen, Jonathan Lindsey

North Carolina State University, Raleigh, North Carolina, USA

Chemical synthesis has provided an immensely versatile platform in support of diverse scientific fields, with the photosynthetic sciences as a significant exception. We are working to develop efficient synthetic routes to the native photosynthetic pigments (e.g., chlorophyll *a*, bacteriochlorophyll *a*) and thereby open the door to address physiological, photophysical, and evolutionary questions in the plant sciences. Sweeping synthetic advances have accrued since Woodward's magisterial – yet incomplete – campaign toward chlorophyll a in 1960 [Liu et al. (2018) Nat. Prod. Rep. 35: 879-901]. One advance entails a new route to form the skeleton of bacteriochlorophylls and chlorophylls by joining AD and BC halves via (i) Knoevenagel condensation and (ii) Nazarov cyclization, S_EAr, and MeOH elimination to form ring E and the aromatic macrocycle. A second advance, relying on a new route for constructing dihydrodipyrrins, installs *trans*-dialkyl substituents of ring D (and B for bacteriochlorophylls) at the outset of the synthesis [Chau Nguyen et al. (2021) New J. Chem. 45: 569-581]. To date we have prepared the BC half, rings A and D, and shown that stereochemically defined substituents can be carried over the entire course of the synthesis. We welcome collaborations in diverse plant sciences arenas where such synthetic capabilities (derivatives, isotopologues, surface attachment features, ample quantities of rare chlorophylls, etc.) may be of use. This work is supported by the NSF (CHE-2054497).

2 Pre Recorded Poster

Photoacoustic spectroscopic analysis: Photosynthesis and forensic Science

Harvey J. M. Hou

Alabama State University, Montgomery, Alabama, USA

The kinetics and the structures of membrane protein complexes in photosynthesis have made significant progress. However, the thermodynamics of photosynthetic reactions is limited. Photoacoustic spectroscopy is able to measure the critical thermodynamic information including energy efficiency, volume change, and enthalpy of reactions in photosynthesis [Hou HJM and Mauzerall C (2011) J. Photochem. Photobiol. B, 104:357-365; Hou HJM and Sakmar TP (2010) Sensors, 10:5642-5667]. In forensic science, profiling analysis of forensic evidence is an exceptional tool and has made ground-breaking contributions. Recent literature has shown that profiling of controlled substances methamphetamine and heroin can provide specific information of their manufactured sources and distribution routes. However, there is no report on the profiling analysis of drugs in the determination of the drug time intervals (DTI) in the literature. In this work the methodology of photoacoustic spectroscopy is used in understanding the thermodynamic mechanism of energy conversion in *menG* mutant and in DTI analysis using profiles of cocaine compounds in forensic science. We found that although free energy for the electron transfer from the foreign quinone Q anion to F_X in *menG* mutants is small (+0.15 eV), the apparent entropy for this reaction is quite large (+0.49 eV). This indicates that the electron transfer from P_{700} to Q is mainly enthalpy driven; the electron transfer from Q to F_A/F_B is nearly entropy driven. In terms of forensic analysis, we found the appearance of three GC peaks caused by the disappear of norcocaine peak. The degradation components of norcocaine were identified. Next, we will acquire the photoacoustic profile of cocaine compounds and construct a DTI model of cocaine profiles by chemometrics. Because of its unique detection principle and ability, photoacoustic spectroscopy may open the new route in forensic analysis and reveal more valuable thermodynamic information in photosynthesis.

3 In Person Oral

Substrate and catalytic promiscuity of chloroplast fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase in *Arabidopsis thaliana*

Florian Galbier, Martina Zanella, Barbara Pfister, Michaela Fischer-Stettler, Samuel C. Zeeman

ETH Zürich, Zürich, Switzerland

Arabidopsis thaliana mutants of the two Calvin-Benson-Bassham cycle (CBBC) bisphosphatases – fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase) grow poorly, but are viable. Metabolite profiling and enzyme activity assays of *fbp* and *sbp* plant extracts, as well as assays of recombinant FBPase and SBPase suggest that both enzymes exhibit substrate promiscuity, each acting on the other's canonical substrate. Therefore, we conclude that FBPase can compensate to some extent for the loss of SBPase, and vice versa, explaining the mutants' viability. Consistent with this, the *fbp sbp* double mutant is seedling lethal. Unexpectedly, both enzymes also show catalytic promiscuity, producing the non-canonical 1-phosphate forms (i.e., fructose 1-phosphate (F1P) and sedoheptulose 1-phosphate (S1P)). Recombinant FBPase catalyses the production of fructose 6-phosphate (F6P), but simultaneously also the production of F1P, albeit at rates several orders of magnitude lower. However, this catalytic promiscuity is pronounced when the enzymes act on their non-canonical substrate and high levels of F1P are found in the *fbp* mutant, where SBPase metabolises fructose 1,6-bisphosphate (FBP). Analogously, high levels of S1P are found in the *sbp* mutant, when FBPase metabolises sedoheptulose 1,7-bisphosphate (SBP). We questioned whether these non-canonical metabolites negatively affect plant metabolism, contributing to the poor growth of the mutants. Therefore, Arabidopsis plants were created that express a heterologous, chloroplast-targeted ketohexokinase, producing high levels of F1P in the chloroplast. These plants grew normally, revealing no deleterious effect of elevated F1P on metabolism and growth. We conclude that the observed growth retardation in the *fbp* and *sbp* mutants is primarily due to changes in metabolic flux of the CBBC rather than the accumulation of our newly discovered non-canonical metabolites F1P and S1P. Nevertheless, the presence of these compounds in wild-type plants, and their active turnover, leaves unanswered questions about the pathways by which they are metabolised.

4 In Person Oral

Light activation of orange carotenoid protein

Volha Chukhutsina, J. M. Baxter, A. Fadini, K. Maghlaoui, J. J. van Thor

Department of Life Sciences, Imperial College London, London SW7 2AZ, London, United Kingdom

Orange Carotenoid protein (OCP) is the only known photoreceptor which uses carotenoid for its activation[1,2]. It is found exclusively in cyanobacteria, where it functions to control light-harvesting of the photosynthetic machinery. However, the photochemical reactions and structural dynamics of this unique photosensing process are not yet resolved. We present time-resolved crystal structures at second-to-minute delays under bright illumination, capturing the early photoproduct and structures of the subsequent reaction intermediates. The first stable photoproduct shows carotenoid trans/cis isomerization at the C7'-C8'double bond and structural changes in the N-terminal domain with minute timescale kinetics. These are followed by a thermally driven cis/trans isomerization that recovers to the dark state carotenoid configuration. Structural changes propagate to the C-terminal domain, resulting, at later time, in the -bond rupture of the carotenoid keto group with protein residues. The isomerization and its transient nature are confirmed in OCP crystals and solution by FTIR and UV/Vis spectroscopy. This study reveals the isomerization of the carotenoid and subsequent thermal structural reactions as the basis of OCP photoreception. Understanding and potentially controlling the OCP dynamics offers the prospect of novel applications in biomass engineering[3] as well as in optogenetics and bioimaging.

1. Kerfeld, C. A. et al. (2003) The crystal structure of a cyanobacterial water-soluble carotenoid binding protein. Structure 11:55–65

2. Leverenz, R. L. et al. (2015) A 12 Å carotenoid translocation in a photoswitch associated with cyanobacterial photoprotection. Science 348:1463–1466

5 In Person Oral

Electrochemically driven photosynthetic electron transport in cyanobacteria lacking Photosystem II

Christine Lewis[1,2], Cesar Torres[3,4], Thomas Moore, Ana Moore[5,6], Justin Flory, Petra Fromme[5,7], Bruce Rittmann[8,4]

[1]Arizona State University, Tempe, Az, USA. [2]Biodesign Institute Center for Applied Structural Discovery, Tempe, Az, USA. [3]Arizona State University School for Engineering of Matter, Transport and Energy, Tempe, Az, USA. [4]Biodesign Swette Center for Environmental Biotechnology, Tempe, Az, USA. [5]Arizona State University School of Molecular Sciences, Tempe, Az, USA. [6]Julie Ann Wrigley Global Institute of Sustainability and Innovation, Tempe, Az, USA. [7]Biodesign Center for Applied Structural Discovery, Tempe, Az, USA. [8]Arizona State University School for Sutainable Engineering and the Built Environment, Tempe, Az, USA

Light-activated photosystem II (PSII) carries out the critical step of splitting water in photosynthesis. However, PSII is susceptible to light-induced damage. Here, results are presented from a novel microbial electro-photosynthetic system (MEPS) that uses redox mediators in conjunction with an electrode to drive electron transport in live *Synechocystis* (Δ*psbB*) cells lacking PSII. MEPS-generated, light-dependent current increased with light intensity up to 2050 µmol photons m^{-2} s^{-1}, which yielded a delivery rate of 113 µmol $electrons^{-1}$ $mg\text{-}chl^{-1}$ and an average current density of 150 A m^{-2} s^{-1} $mg\text{-}chl^{-1}$. $P700^{+}$ re-reduction kinetics demonstrated that initial rates exceeded wildtype PSII-driven electron delivery. The electron delivery occurs ahead of the cytochrome b_6f complex to enable both NADPH and ATP production. This work demonstrates an electrochemical system that can drive photosynthetic electron transport, provides a platform for photosynthetic foundational studies, and has the potential for improving photosynthetic performance at high light intensities.

6 In Person Oral

Time-resolved FTIR difference spectroscopy for the study of A_1 in Photosystem I

Gary Hastings

Georgia State University, Atlanta, GA, USA

FTIR difference spectroscopy is widely used to study the properties of the electron transfer cofactors in photosynthetic proteins. The technique is molecular-specific and sensitive to small changes in pigment-protein interactions that may occur during the course of a chemical reaction.

Here I will discuss work we have undertaken to probe the properties of the phylloquinone (PhQ, a.k.a. Vitamin K1) molecule that occupies the A_1 binding site in photosystem I. PhQ is a disubstituted naphthoquinone and is involved in several interactions with the surrounding protein. To investigate these molecular interactions, we have been using time-resolved FTIR difference spectroscopy to study altered photosystem I samples where either the native PhQ has been replaced with a closely (or distantly) related analogue, or important pigment-protein interactions are modified via site directed mutagenesis.

FTIR difference spectra can often be difficult to interpret (assign bands to molecular bonds). To aid in this endeavor we have developed QM/MM density functional theory-based methods for vibrational frequency calculations. We show that through comparison of calculated and experimental spectra we can assess not only the specific orientation of pigments (and their molecular specific interactions) in protein binding sites, but also the conformation of the sidechains of the bound pigments.

7 In Person Oral

Tunnels and walls: importance of plasmodesmata and suberin lamellae in C_4 photosynthesis

Florence Danila, Hong Ting Tsang, Susanne von Caemmerer, Robert Furbank

Australian National University, Canberra, ACT, Australia

Plants performing C_4 photosynthesis are more efficient in converting CO_2 and light into food than their C_3 counterpart. The secret lies in their ability to concentrate CO_2 near the site of Rubisco reducing energy-wasteful photorespiration. In the majority of C_4 plants, photosynthesis is distributed between the mesophyll and bundle sheath cells of the leaf. This cooperative process allows CO_2 to be initially fixed in the mesophyll cells into C_4 acids which then diffuse to the bundle sheath cells, where CO_2 is regenerated for fixation by Rubisco. Our 3D quantification studies showed that the efficiency of the C_4 pathway relies on the rapid exchange of the metabolites between the mesophyll and bundle sheath cells facilitated by the numerous nanotunnels called plasmodesmata. While metabolites must freely move between cell types, CO_2 leakage from the bundle sheath must be minimized. A *Setaria viridis* mutant lacking suberin allowed us to demonstrate the importance of the suberin lamellae around the bundle sheath in reducing this CO_2 leakage. Together with the modified biochemistry, these leaf ultrastructural features give C_4 plants the photosynthetic advantage over C_3 plants. The aim now is to identify the genes that control these C_4 leaf ultrastructural features in view of improving the yield of agronomically important C_3 crops like rice, wheat, and barley through improvement of photosynthesis.

8 Pre Recorded Oral

Metabolic fluxes in photosynthesizing leaves: new insights into old puzzles

Yuan Xu, Xinyu Fu, Berkley Walker, Thomas Sharkey, Yair Shachar-Hill

Michigan State University, East lansing, MI, USA

Respiration in the light (RL) lowers net carbon fixation, understanding it could help improve plant carbon-use efficiency and modeling of crop photosynthesis. To identify reactions contributing to RL, we developed an improved 13C isotopically nonstationary metabolic flux analysis (INST-MFA) system for fast quenching (<0.5 s) during labeling, which is important for measuring Calvin–Benson cycle (CBC) intermediates with extremely fast turnover rate. We increased the number of metabolites followed by developing five mass spectrometer methods to expand biochemical network coverage in CBC, photorespiration, sugar pathways, starch synthesis pathway, tricarboxylic acid (TCA) cycle, and oxidative pentose phosphate pathway (OPPP).

The flux analysis indicated that ≤10% of RL results from TCA cycle reactions, which are widely considered to dominate RL. Further analysis of the results indicated that oxidation of glucose 6-phosphate to pentose phosphate via 6-phosphogluconate (the G6P/OPP shunt) can account for >93% of CO2 released by RL. Quantitative estimates of synthesis and turnover of sucrose and hexose better integrates CBC into cellular central metabolism and explains long-standing questions about CBC labeling kinetics that can be rapidly labeled initially, but then labeling slows considerably. In showing the interconnection of three compartments, we have drawn a more complete picture of how carbon moves through photosynthetic metabolism in a way that integrates the CBC, cytosolic sugar pools, glucose-6-phosphate shunt, and vacuolar sugars into a single system.

The methods established in this study are being applied in the broader research to mapping leaf carbohydrate turnover in plants grown in long days and short days. The flux analysis indicated increased flux in sucrose and starch synthesis pathways, but decreased OPPP in short day plants compared to long day plants. Our ongoing and future directions are to further improve the dynamic modeling of leaf photosynthetic metabolism and apply it to questions of regulation and transgenic interventions.

9 Pre Recorded Poster

Bilin-dependent regulation of chlorophyll biosynthesis and photoacclimation in *Chlamydomonas*

Yan Wang[1], Weiqing Zhang[1], Robert Willows[2], J. Clark Lagarias[3], Deqiang Duanmu[1]

[1]Huazhong Agricultural University, Wuhan, China. [2]Macquarie University, Sydney, Australia. [3]University of California Davis, Davis, CA, USA

Biosyntheses of chlorophyll and heme in oxygenic phototrophs share a common trunk pathway that diverges with insertion of magnesium or iron into the last common intermediate, protoporphyrin IX. Since both tetrapyrroles are pro-oxidants, it is essential that their metabolism is tightly regulated. Here, we establish that heme-derived linear tetrapyrroles (bilins) function to stimulate the enzymatic activity of magnesium chelatase (MgCh) via their interaction with GENOMES UNCOUPLED 4 (GUN4) in the model green alga *Chlamydomonas reinhardtii*. A key tetrapyrrole-binding component of MgCh found in all oxygenic photosynthetic species, CrGUN4, also stabilizes the bilin-dependent accumulation of protoporphyrin IX-binding CrCHLH1 subunit of MgCh in light-grown *C. reinhardtii* cells by preventing its photooxidative inactivation. Exogenous application of biliverdin IXα reverses the loss of CrCHLH1 in the bilin-deficient heme oxygenase (*hmox1*) mutant, but not in the *gun4* mutant. We propose that these dual regulatory roles of GUN4:bilin complexes are responsible for the retention of bilin biosynthesis in all photosynthetic eukaryotes, which sustains chlorophyll biosynthesis in an illuminated oxic environment.

We also observed reduced accumulation of photosystem I (PSI) reaction centers, and the loss of PSI and photosystem II antennae complexes during photoacclimation in *hmox1* mutant. The *hmox1* mutant can be rescued by exogenous biliverdin IXa, the bilin produced by HMOX1. This rescue is independent of photosynthesis and is strongly dependent on blue light. RNA-seq analyses reveal that tetrapyrrole biosynthesis and known photoreceptor and photosynthesis-related genes are not impacted in the *hmox1* mutant at the transcript level. We propose that a bilin-based, blue light-sensing system within plastids evolved together with a bilin-based retrograde signaling pathway to ensure that a robust photosynthetic apparatus is sustained in light-grown Chlamydomonas.

10 Pre Recorded Poster

Transport modeling of microphotosynthetic cell

Soroush Rahimi, Dhilippan M. Panneerselvam, Muthukumaran Packirisamy

Concordia University, Montreal, QC, Canada

Environmentally friendly, reduced carbon footprints and more reasonable availability are the quantifying factors for any alternative fuels over the carbon-based fossil fuels. Over the past decades, enormous research resulted in Lab-on-chip micro-photosynthetic power cells (μ-PSC) producing electricity from photosynthesis. The maturity of this research resulted in an Open Circuit Voltage (OCV) of ~800mV and Short Circuit Current (SCC) of ~1mA from organic bio-organisms. However, the electron transport physics in this μ -PSC are assumed to be correlated with photosynthesis. This paper presents an initial investigation in modelling the μ - PSC as a hydrogen fuel cell to unravel the electron transport physics. Our investigation exposed that the photosynthesis electron chain constitutes leaky electrons constitute the energy harvesting in a μ-PSC.

Fossil fuels sustain the global energy demand due to the extreme exploration, availability and cost-effectiveness. Nevertheless, fossil fuel burning results in environmental degradation. An estimate provides that fossil fuel utilization will peak by 2050 and start to deplete after that; Thus, there is an urgency to explore any alternative fuel which compensates for the global energy demand. A μ-PSC is a perfect candidate for offsetting global energy demand and preserving the environment through their by-product, oxygen. Our group researched the various possible μ-PSC configurations using freshwater algal cells (*Chlamydomonas reinhardtii)* powering ultra-low and low power rating sensors. However, there is no validation to verify the electron transport physics in this μ-PSC. In this paper, assuming the μ-PSC to be a hydrogen fuel cell, we have solved the chemical species and electrical charge transport equations and extracted the localized current density at the electrode terminals. Any analytical assumptions are eliminated, and the solution relies only on the initial and boundary conditions. The electron transport physics on μ-PSC and its correlation with the natural photosynthesis process will be reported.

11 In Person Oral

Assessing NPQ kinetics in wheat spikes under yield potential and heat-stress conditions to identify avenues for photosynthetic improvement

Liana Acevedo-Siaca, Matthew Reynolds

Global Wheat Program, CIMMYT, El Batan, Mexico, Mexico

Most of our understanding of photosynthesis is within the context of steady-state conditions. Instead, plants in an agricultural or natural ecosystem experience constantly changing light conditions due to changes in cloud cover, wind, and self-shading within the plant canopy. These abrupt changes in irradiance reduce overall CO_2 assimilation in wheat as leaves must undergo induction or relaxation of concurrent photosynthetic processes during each light transition. Non-photochemical quenching (NPQ) helps to protect the photosynthetic apparatus in plants by dissipating excess energy from light as heat. Previously, the slow relaxation of NPQ from high to low light conditions was identified as a target for improvement to increase crop productivity. However, little is known about chlorophyll fluorescence-based parameters in wheat spikes, such as NPQ induction and relaxation, NPQ components, and the quantum yield of photosystem II – especially in non-steady-state conditions. This is despite wheat spikes contributing up to 20% to wheat yields [Zhang et al., (2020) Field Crops Research 257: 107931]. These parameters were evaluated in 15 field-grown wheat genotypes under yield-potential and heat-stress conditions. Measurements aiming to characterize non-steady-state photosynthetic performance at both the leaf and whole-spike level were performed to better understand NPQ in spikes, identify inefficiencies in chlorophyll fluorescence kinetics, and evaluate the effect of heat stress on these parameters. Additionally, this work explores how measurements of complex traits related to photosynthesis can be observed over time through chlorophyll fluorescence imaging. Preliminarily, significant differences were found between environments and phenological stage, with NPQ induction and relaxation being significantly slower under heat environments and later in plant development. Additionally, plants with faster NPQ induction response were more likely to have a faster NPQ relaxation response. These results may help us understand how NPQ and other chlorophyll fluorescence parameters may be improved in the future from existing germplasm.

12 In Person Poster

Improving C_4 photosynthesis for food and energy security

<u>Maria Ermakova</u>[1], Srinivas Belide[2], Robert Furbank[1], Susanne von Caemmerer[1]

[1]Australian National University, Canberra, ACT, Australia. [2]CSIRO Agriculture & Food, Canberra, ACT, Australia

C_4 crops are becoming increasingly important for food and bioenergy security. The global production of maize often surpasses the two key C_3 cereals wheat and rice, whilst miscanthus and switchgrass are two leading biomass crops. C_4 plants have a special type of photosynthesis that includes a metabolic C_4 cycle acting as a biochemical carbon concentrating mechanism and allowing Rubisco to operate close to maximum efficiency. Although C_4 plants already have high CO_2 assimilation rates, improvements to C_4 photosynthesis were projected to further increase crop yield [Wu et al. (2019) Nature Plants 5:380–388]. We have demonstrated this, using a model plant *Setaria viridis* and a multipurpose crop *Sorghum bicolor*.

In *Setaria*, engineering plasma membranes of mesophyll cells with the CO_2 permeable aquaporins enhanced mesophyll conductance and allowed higher carboxylation efficiency at low CO_2 [Ermakova et al. (2021) Elife 10:e70095]. This modification could potentially improve crop productivity in conditions when stomatal conductance is limited, *e.g.*, under drought, and will be tested in future. Increasing content of SBPase, known to regulate carbon flux through Calvin cycle in C_3 plants, did not affect C_4 photosynthesis [Ermakova et al. (2022) bioRxiv doi:10.1101/2022.05.09.491242]. Increasing the rate of electron transport through overexpression of the Rieske subunit of Cytochrome b_6f complex stimulated CO_2 assimilation at non-limiting CO_2 and high light [Ermakova et al. (2019) Comm. Biol. 2:314], which could have a positive impact on crop productivity.

To identify the effects of Rieske overexpression on crop yield, we created transgenic *Sorghum* with increased Rieske content. Although the steady-state rates of electron transport and assimilation were not changed, *Sorghum* with higher Rieske abundance grown in a glasshouse showed faster induction of photosynthesis, accumulated more biomass and produced more seeds. Our results demonstrate that improving C_4 photosynthesis can increase crop yield and help sustain the growing food and energy demands.

13 In Person Poster

Xanthophyll-cycle based model of the rapid photoprotection of *Nannochloropsis oceanica* in response to regular and irregular illumination sequences

Audrey Short, Thomas Fay, Thien Crisanto, Krishna Niyogi, Graham Fleming

University of California, Berkeley, Berkeley, CA, USA

Utilizing time-correlated single photon counting, we explore the photoprotection dynamics of *Nannochloropsis oceanica* under regular and irregular high light (HL) illumination sequences. The varying light sequences mimic natural conditions, allowing us to probe the real-time response of non-photochemical quenching (NPQ) pathways. Durations of fluctuating light exposure during a fixed total experimental time and prior growth conditions of the algae are both found to have a profound effect on NPQ. These observations are rationalized with a quantitative model based on the xanthophyll cycle and the pH-gradient across the thylakoid membrane. The model can accurately describe the dynamics of non-photochemical quenching across a variety of light sequences. The combined model and observations suggest that the accumulation of a quenching complex is responsible for the gradual rise in NPQ. Additionally, the model makes specific predictions for the light sequence dependence of xanthophyll concentrations that are in reasonable agreement with independent high-performance liquid chromatography (HPLC) [Short et al. (2022) J Chem Phys 156]. From the HPLC data, the intermediate carotenoid in the xanthophyll cycle, antheraxanthin (A), seems to play an important role. By looking at the HPLC data for the rapid periodic response (1 min HL-1 min dark) we see that 1 minute of HL is not long enough to go from violaxanthin (V) through A to zeaxanthin (Z) and leads to the suggestion that A may be used as a stop gap quencher. It is, however, not as efficient a quencher as Z which leads to slower overall kinetics compared to sequences with longer high light periods. Through a continuation of HPLC experiment and adding A to our existing model, we can explore the role it plays in quenching as well as estimate rate of conversion between the xanthophyll carotenoids.

14 In Person Poster

Optimizing C source-sink balance can improve rice photosynthesis and yield in a CO_2 elevation context

Denis Fabre[1], Xinyou Yin[2], Michael Dingkuhn[1], Delphine Luquet[1]

[1]CIRAD, Montpellier, France. [2]Wageningen University, Wageningen, Netherlands

The increase in atmospheric CO_2 concentration (e-CO_2) will significantly impact agricultural crop productivity. Varieties of C3-type cereals vary greatly in their photosynthetic response to e-CO_2, in large part because of a variable capacity to adjust sink capacity to an increased C source. Optimizing C source-sink relationships might provide gains in photosynthesis response to e-CO_2 and thereby increase biomass and yield. These relationships are complex as they involve plant, organ and process levels. Understanding the link between these scales and unraveling effects on photosynthesis is challenging.

An experiment was carried out on IR64 rice to study the diurnal dynamic of photosynthetic parameters at mid grain-filling stage under modified of C source-sink balance. For this purpose, control plants were compared to panicle-pruned plants (sink limitation) at two CO_2 levels: ambient (400ppm) and e-CO_2 (800ppm for 14d from heading) (source boosting). Flag leaf and internode NSC (starch, sucrose and hexose concentrations) were measured, as well as photosynthetic parameters on the flag leaf of the main stem.

TPU (triose-phosphate utilization) was identified as the main biochemical driver of photosynthesis down-regulation by sink limitation, occurring predominantly in the afternoon. A negative correlation was found between TPU and markers of sink limitations: leaf [sucrose] and the local C source-sink ratio (LSSR), computed as the ratio between flag leaf area and grain number of the adjacent panicle on the main stem.

A second experiment was carried out in order to confirm these results among 5 indica genotypes having constitutive variation of LSSR. Plants were compared under two continuous CO_2 treatments, 400 and 800 ppm. A negative relationship between genotypic LSSR and photosynthetic capacity under e-CO_2 was confirmed. Plant biomass and grain yield response to e-CO_2 were negatively correlated with LSSR as well, suggesting a key role of C sink capacity in enhancing C3 plant productivity under e-CO_2.

15 In Person Oral

Retrograde control of cytosolic translation: Synchronising photosynthetic protein production and nuclear high-light acclimation

Marten Moore[1], Barry Pogson[1], Karl-Josef Dietz[2]

[1]The Australian National University, Canberra, ACT, Australia. [2]Bielefeld University, Bielefeld, NRW, Germany

Canonical retrograde signalling comprises information transmission from organelles to the nucleus and in particular controls gene expression for organellar proteins. The need to re-assess this paradigm was suggested by discrepancies between protein and transcript abundance in response to excess light. Here we uncover main components of a translation-dependent retrograde signalling pathway that first impacts translation and then gene expression. The response realization depends on the kinases, MPK6 and SnRK1 subunit, AKIN10. Global ribosome foot-printing revealed differential ribosome association of 951 transcripts within 10 min after transfer from low to high light. About one third of these transcripts, including glyceraldehyde-3-phosphate dehydrogenase (GAPC) and Stress Associated Proteins (SAP) 2 and 3, share regulatory motifs in their 5`-UTR that act as binding sites for light shift-responsive RNA binding proteins (RBPs). SAP2 and 3 are both translationally regulated and were shown to interact with the calcium sensor Calmodulin-like 49, and thereby relocating to the nucleus to co-regulate a translation-dependent nuclear response. Thus, translation-dependent retrograde signalling bifurcates to directly activate a translational circuit and to initiate a translation-reliant nuclear circuit synchronizing retrograde and anterograde response pathways, serving as rapid mechanism for functional acclimation of the chloroplast.

16 In Person Poster

Aquaporin channels and anion transporters could take part in root-based inorganic carbon uptake of barley and Arabidopsis

Csaba Éva, Liesel Gamarra Reinoso, Imre Majláth, Mihály Dernovics, Ervin Balázs

Agricultural Institute, ELKH Centre for Agricultural Research, Martonvásár, Hungary

Root-based inorganic carbon uptake of land plants may serve as supplementing the photosynthesis with extra carbon and maintaining it under osmotic stress with less stomatal conductance. However its mechanism remains elusive. Here we successfully optimized hydroponics-based growth-promoting $NaHCO_3$-treatments for barley and Arabidopsis. In case of barley, the 2 mM $NaHCO_3$ treatment was beneficial at pH=5.6 in 1/4 strength Hoagland, but for Arabidopsis only the pH=7.2 Hoagland treatment worked, containing 2 or 3 mM $NaHCO_3$. We also tried 1/4 MS-based hydroponics, and in this case 2 mM $NaHCO_3$ treatment at low pH of 5.6 boosted growth of Arabidopsis. The beneficial treatments increased fresh weight and improved photosynthetic parameters, and also mitigated the effects of osmotic stress. Based on the literature, we hypothesized that at low pH (CO_2 being the dominant form of inorganic carbon) the inorganic carbon crosses into the root symplast through carbon-dioxide permeable aquaporin channels, while at high pH, plants take up bicarbonate ion (dominant form at high pH) through secondary function of anion transporters. In Arabidopsis, gene expression of the PIP1;2 aquaporin channel indeed increased at low pH and the PIP1;2 mutant showed less growth promotion. However increase of ^{13}C-labelled aspartate could also be detected in xylem sap, which could indicate PEPC-based fixation of bicarbonate ion at low pH, and the PEPC3 mutant also showed less growth promotion. At high pH, anion transporter genes activated. In barley, gene expression of aquaporin genes did activate at low pH, nonetheless many anion channels showed higher fold gene expression. The results might argue for the role of carbon-dioxide permeable aquaporin channels in root-based carbon uptake at low pH and the role of borate, nitrate, phosphate and sulfate anion channels in the uptake at both low and high pH.

The project is supported by grants NKFI FK 134874 and ATK-MATE no. 0205K0036P.

17 In Person Oral

Harnessing natural variation of photoprotection: Rapid NPQ (non-photochemical quenching) kinetics in ferns

Nina Maryn[1], Audrey Short[1], Masakzu Iwai[2], Graham Fleming[3], Krishna Niyogi[1]

[1]UC Berkeley, Berkeley, California, USA. [2]LBNL, Berkeley, California, USA. [3]UC Berkeley, Berkeley, CA, USA

Photosynthesis is a precarious process that brings excited chlorophylls into close proximity with molecular oxygen, which can generate reactive oxygen species. Photosynthetic light harvesting must be continuously regulated to adjust to changes in ambient light conditions in natural habitats and crop canopies. High light exposure rapidly induces non-photochemical quenching (NPQ) mechanisms that are important for photoprotection, but NPQ competes with photochemistry and can require several minutes to relax after transitions back to low light. It has been shown that accelerating the relaxation kinetics of NPQ can improve photosynthesis and biomass productivity in a model crop. A pulse amplitude modulated (PAM) fluorometric survey of over 100 species of land plants was conducted to identify species with rapid (<1 min) NPQ relaxation. Three fern species were identified that exhibit nearly instantaneous induction and relaxation of NPQ under fluctuating light conditions. They also have higher maximum NPQ capacity than the model angiosperms Arabidopsis thaliana and Nicotiana benthamiana. These results were corroborated by time-correlated single photon counting spectroscopy using similar light regimes. High-performance liquid chromatography was used to analyze the levels of chlorophyll and carotenoid pigments during dark acclimation, high light treatment, and recovery in the dark. The fern species have 2-3x higher carotenoid content than leaves of model angiosperms, and they specifically retain the photoprotective xanthophyll zeaxanthin in the dark, prior to NPQ induction. This combined evidence suggests that the ferns are primed to induce pH-dependent NPQ, known as qE, but relaxation is not dependent on the conversion of zeaxanthin back to violaxanthin in the dark. These species, therefore, are excellent candidates for discovery of new mechanisms of rapid NPQ relaxation that could lead to increases in crop productivity.

18 In Person Poster

The analysis of photosynthesis in *Ipomoea imperati* (Vahl) Griseb in the coastal zone of Espírito Santo State (Brazil) affected by Fundão Dam collapse.

Marcos Bacarin[1], Mariela Silva[2], Namir Lopes[2], Romario Silva Jr[2], Diolina Silva[3]

[1]Botany Department, UFPEl, PELOTAS, RS, Brazil. [2]Graduate Program in Plant Biology, UFES, VITORIA, ES, Brazil. [3]Biological Sciences Department, UFES, VITORIA, ES, Brazil

The Fundão Dam collapse occurred in Minas Gerais, Brazil, in 2015, is characterized as a worst environmental disaster of mining industry, both in terms of the volume of tailings dumped and the magnitude of the damage. After the Fundão dam failure, 35 million m^3 of iron ore tailings that went into the environment continue to cause environmental damage, polluting approximately 700 km of watercourses from the Doce River and the coastal zone of Espírito Santo State. During 2019 we measured the transient fluorescence (Handy Pea – Hansatech Instruments Ltd.) and exchange gases (LCApro T - ADC BioScientific Ltd.) in *Ipomoea imperati* plants in two sampling stations with spatial distribution that covered the area closest the mouth (E6 - Cacimbas) and other further from the Doce River mouth (E1 - Environmental Protection Area of Conceição da Barra). The maximum quantum yield of photosystem II (F_V/F_M) didn't present normal standard during the sample period, but we observed that in sampling station E1 higher values were measured in February and December 2019 (wet season). At the ending of the dry season and the beginning of the wet season (between July and November) we observed that in the sampling station closest to Doce River mouth (E6) the performance index was higher than in station E1. The net photosynthesis rate (*A*) in E1 showed behavior similar of a negative parabola opens upward, the minimum vertex was observed in August. In the station E6 we observed the highest *A* value in November. We conclude that the photosynthesis process in of *I. imperati* plants was influenced by the wet or dry season and seems be affected by the distance of the Doce River mouth.

19 In Person Poster

Can the viability of pollen grains be correlated with the chlorophyll fluorescence in *Canavalia rosea* (Sw.) DC on Restinga affect by the collapse of Fundão dam?

Geovane Gudin[1], Pedro Damasceno Jr.[2], Thaís Santos[1], Marcos Bacarin[3], Diolina Silva[4]

[1]Graduate Program in Plant Biology, Federal University of Espirito Santo, Vitória, Espírito Santo, Brazil. [2]Department of Plant Science, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil. [3]Botany Department, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil. [4]Biological Sciences Department, Federal University of Espirito Santo, Vitória, Espirito Santo, Brazil

The collapse of the Fundão dam, in Mariana, Minas Gerais State (Brazil), in November 2015, released about 35 million m^3 of iron ore tailings into the environment. These iron ore mining tailings were transported into the Doce River, reaching the southeast coast of Brazil. This coastal zone is formed by fragments of Restinga. In this work, in April 2019, we evaluated the transient chlorophyll fluorescence and the viability of pollen grains from the flowers of *Canavalia rosea* plants in four sampling stations (SS) with spatial distribution that covered an area along the coastline to north and to south of the Doce River mouth. In these SS we measured: a) chemical elements in leaves; b) chlorophyll fluorescence with HandyPEA (Hansatech Instruments); c) the viability/fertility of the pollen grains using Alexander's stain (method for differential staining of aborted and non-aborted pollen grains). In the SS (E6-E7), nearest to mouth of the Doce River mouth, we observed higher content of S and Zn, and lower of Mn in leaves than was observed at SS farther from the mouth (E1). In plants of SS E3 content of Cr and Ni were the highest and the parameters associated with the photosystem I (PSI) activity (δR_0 and φE_0) and PI_{total}, calculated by JIP-test, were lower than the measured in the SS E1 (more distant of the Rio Doce mouth). The lowest viability pollen grain in the different environments studied were found in plants from SS E3 (51.9%). In this station, the pollen grains were sticky inside the anthers. This stickiness was confirmed after cutting the anthers and subsequent observation of unstained pollen grains under an optical microscope. So, we propose that the viability of pollen grains can be correlated with the transient chlorophyll fluorescence, mainly parameters associated with PSI.

20 In Person Poster

Parameters and indicators of environmental quality in Restiga vegetation impacted by the rupture of the Fundão Dam: Case of photosynthesis

Patricia Soares[1], Sabrina Broetto[2], Marcos Bacarin[3], Eustaquio Castro[4], Diolina Silva[5]

[1]Graduate Program in Plant Biotechnology, Federal University of Espirito Santo, Vitória, Espírito Santo, Brazil. [2]Graduate Program in Plant Biology, Federal University of Espirito Santo, Vitória, Espírito Santo, Brazil. [3]Botany Department, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil. [4]Chemistry Department, Federal University of Espirito Santo, Vitória, Espírito Santo, Brazil. [5]Biological Sciences Department, Federal University of Espirito Santo, Vitória, Espírito Santo, Brazil

The Fundão dam was one megastructure of the mining complex, located in the municipality of Mariana, Minas Gerais, southeastern Brazil. In November 2015, the dam collapse carried over 35 million m^3 of iron ore tailings into the Doce river and caused enormous environmental impacts, reaching the southeast coast of Brazil. This coastal zone is formed by fragments of Restinga vegetation. In 2016, the Renova Foundation (https:// .fundacaorenova.org/en/the-foundation/) was created to repair and to compensate the damages caused by the dam rupture. After its creation, numerous projects were structured, among which one was designed aiming to monitor the costal environment of Espirito Santo State, including the analysis of the effect of the mine ore tailings on Restinga vegetation. In 2018, the researchers in our group started monthly measurements of floristics, phytosociology and ecophysiology, in addition to chemical analyzes of the mineral content in leaves and in soil and soil microbiota. Analysis has been done in herbaceous, shrubs, tree plants, at various points along the coast. With all results, the scientists proposed some parameters and indicators of environmental quality and biodiversity in the monitored area. For each biotic indicator, three risk categories were determined: High, Moderate and Low; according to the relation between the physiological alteration, stress caused and metals level. These analyses still are in progress, the results so far show that performance index and net photosynthesis rate could be good indicators of the injury and recovery of Restinga plants, as observed by the high correlation between these parameters and concentrations metal(loid)s quantified in soil and in leaves. However, we could yet observe that the adaptation and acclimation are strategies of plants to cope with climate changes and they consist of genotypic and phenotypic adjustments that allow plants to grow and reproduce successfully in a stressful environment.

21 In Person Poster

The impact of Fundão Dam collapse on soil bacterial population and trail photosynthetic in *Guapira pernambucensis* (Casar.) Lundell plants growing in Restiga of coastal zone of Espírito Santo State, Brazil

Andrea B. Moura[1], Vanessa N. Soares[1], Marcos A. Bacarin[2], Gislaine C. O. Silvares[3], Diolina M. Silva[4]

[1]Crop Protection Department, UFPel, PELOTAS, RS, Brazil. [2]Botany Department, UFPel, PELOTAS, RS, Brazil. [3]Graduate Program in Plant Biology, UFES, VITORIA, ES, Brazil. [4]Biological Sciences Department, UFES, VITORIA, ES, Brazil

The Fundão dam was one of the megastructures of the mining complex, located at Mariana, Minas Gerais, southeastern Brazil. The total collapse of the Fundão tailings dam took place in November 2015, and about 35 million m^3 of tailings were released, causing severe environmental injury to hundreds of watercourses in the basin of the Doce River and associated ecosystems. The mud reached the Atlantic Ocean, expanding the impacts to the fragile, yet diverse estuarine and coastal region of Espírito Santo State, where the principal type of vegetation is the Restinga. In 2019 April, we collected soil under the dossel projection of *G. pernambucensis* in diverse sampling stations (SS) with spatial distribution that covered an area along the coastline to north and to south of the Doce River mouth. In this soil we estimated the bacterial colony forming units (CFU) by plating the sample serially diluted in sterile saline, and the number of visibly growing colonies was count after 72 of incubation. In seven SS we measured transient fluorescence with Handy Pea (Hansatech) and exchange gases in two stations (E1 and E6) with LCApro T (ADC). The CFU after 72 hours showed the lowest value in SS closer to of the Doce River mouth (E6 and E7). We observed that the SS more distant (E2) also presented a low CFU. The maximum quantum yield of PSII and index performance had demonstrated the same behavior observed to CFU. The net photosynthesis rate in plants of the E1 (more distant) had the same value measured in plants at station E6 (closer). Therefore, we could suggest that in SS closer the Doce River mouth there was reduction in CFU and in JIP-test parameters (F_V/F_M, indexes performance), but the net photosynthesis rate was not altered.

22 Pre Recorded Poster

Live cell and hybrid material-based bio-photoelectrochemical cells for clean solar energy conversion

Noam Adir, Yaniv Shlosberg, Hagit Shoyhet, Dvir Harris, Gadir Schuster

Technion, Haifa, Israel

Photosynthetic organisms and complexes are attractive materials for solar energy conversion (SEC). We describe here how the remarkable photocatalytic activity of the photosynthetic apparatus can provide significant electrical current and hydrogen evolution in Bio-Photo-Electro-Chemical (BPEC) cells via the simplest and cleanest of processes whether in the absence of added electron transport molecules. We show that we can obtain significant electrical current using live cyanobacteria [Saper et al. (2018) Nat. Comm.9, 2168; Shlosberg et al. (2020) iScience 24, 101892], microalgae [Shlosberg et al. (2021) Catalysts 11, 1120], macroalgae (seaweeds) [Shlosberg et al. (2021) Biosens. Bioelectron. 198, 113824] or higher plants [Shlosberg et al. (2022) submitted]. A different strategy is to use isolated photosynthetic complexes and chemically connect them to the electrochemical cell. A problem with this strategy is the small number of light absorbing chromophores connected to the complex, lowering the photochemical efficiency. This can be overcome by connection of the reaction centers to either biological light harvesting (LH) complexes or nanoparticles (NPs). Photosystem II (PSII) is the only enzyme that catalyzes light-induced water oxidation being the basis for its application as a biophotoanode in various bio-photovoltaics and photo-bioelectrochemical cells. The absorption spectrum of PSII limits the quantum efficiency in the range of visible light, due to a gap in the green absorption region of chlorophylls (500 - 600 nm). To overcome this limitation, we have used two strategies: stabilizing the interaction between PSII and Phycobilisomes LH complex within an Os-complex-modified hydrogel on macro-porous indium tin oxide electrodes (MP-ITO) resulting in notably improved, wavelength dependent, incident photon-to-electron conversion efficiencies [Hartmann et al. (2020) J. Mat. Chem. A 8, 14463]. With the Au-NPs, record currents were obtained, with strong improvement in the SPR range of wavelengths that are weakly absorbed by chlorophylls [Shoyhet et al. (2021) J. Mat. Chem. A 9, 17231].

23 In Person Poster

Deficit irrigation impacts on photosynthetic performance, productivity and nutritional quality of aeroponically grown Tuscan Kale in a tropical greenhouse

Jie He, Crystalbelle, Jingxuan Chang (Zeng), Lin Qin

National Institute of Education, Nanyang Technological University, Singapore, Singapore, Singapore

Deficit irrigation, defined as the application of water below the evapotranspiration requirements, not only saves irrigation water, but it can also be used to control vegetative growth without compromising yield. This study aimed to investigate the photosynthetic performance, productivity, and nutritional quality of nutritious Tuscan Kale (Brassica oleracea var. acephala 'Lacinato') under deficit irrigation. Seedlings were grown for four weeks in 5-, 30- and 60-min nutrient spraying intervals, NSI (defined as 5minNSI, 30minNSI, and 60minNSI). After four weeks of transplanting, some 5minNSI plants were transferred to different aeroponic systems with 60 and 90 min NSI to induce deficit irrigation. The light-saturated rate of photosynthesis, A_{sat} of 30minNSI plants was not significantly different from 5 and 60minNSI plants, but the A_{sat} of 5minNSI plants were significantly higher than that of 60minNSI plants. No significant differences were observed for light-saturated stomatal conductance, $g_{s\ sat}$, internal CO_2 concentration, C_i, and transpiration rate, T_r of plants under various NSI treatments. Tuscan Kale reduced its photosynthetic light use efficiency and increased its energy dissipation measured by chlorophyll fluorescence, with longer NSI to protect against drought stress. Higher nitrate accumulation was observed in longer NSI plants which had higher Rubisco protein contents compared to 5minNSI plants. Plants with longer NSI of showed a reduction in shoot and root biomass accumulation compared to 5minNSI, indicating a negative effect on growth and productivity. Overall enhancement in nutritional quality through deficit irrigation during growth or pre-harvest was measured by total soluble sugar, proline, ascorbic acid, and total phenolic compounds. In conclusion, it is better to grow Tuscan Kale under 5minNSI for four weeks followed by one week under 60minNSI before harvest to reduce agricultural water use, penalty on yield and enhance nutritional quality.

24 Pre Recorded Poster

Exploring phycocyanin from different cyanobacteria: Phycocyanin content, extraction, purification, and stability

Sunil Tiwari, Wim Vermaas

Arizona State University, Tempe, Arizona, USA

Phycocyanin is a major light-harvesting antenna protein of cyanobacteria. It is used in nutraceuticals, cosmetics, and medicine, but it needs to be temperature, pH, and light stable in these applications. We aimed to develop an efficient, industrially viable approach to purify phycocyanin from different species at high yield and purity and perform a quantitative (phycocyanin mg/g dry biomass) and qualitative (heat, light and pH stability) analysis of phycocyanin extracted from three mesophilic (*Synechocystis* sp. PCC 6803, *Spirulina platensis* UTEX LB 2340 and *Synechococcus* sp. PCC 11901) and a thermophilic cyanobacterium (*Thermosynechococcus elongatus* BP-1) providing a direct comparison of their properties. Phycocyanin was extracted from homogenized cells using 45 mM calcium chloride and was purified by a single-step ammonium sulfate precipitation. As expected, phycocyanin content decreased with increasing light intensity (25 - 200 µmoles photons m^{-2} s^{-1}). An increase in temperature (from 25 to 40 °C for mesophiles and from 45 to 60 °C for the thermophile) improved the phycocyanin content in vivo in all four strains. Isolated *Thermosynechococcus* phycocyanin was the most thermostable retaining 95% of phycocyanin absorbance after a four-hour incubation at 60 °C whereas *Spirulina* phycocyanin displayed a rapid loss of 620-nm absorbance at temperatures above 25 °C. *Synechocystis* phycocyanin was stable for several hours at temperatures up to 50 °C. In terms of pH stability, isolated *Synechocystis* phycocyanin was the most pH stable, with only 10 % loss in phycocyanin absorbance over 24 between pH 4 and 9. This work highlights that there is surprisingly much to be explored regarding phycocyanin, and preliminary work indicates that we may be removing main obstacles for broader commercial use of phycocyanin. This work is supported by the US Department of Energy's Office of Energy Efficiency and Renewable Energy (EERE) under the Bioenergy Technology Office award number DE-EE0009274.

25 Pre Recorded Oral

Imaging state transitions in Arabidopsis leaves

Dana Verhoeven, Herbert van Amerongen, Emilie Wientjes

Wageningen University, Wageningen, Netherlands

Photosynthetic organisms use state transitions to balance the excitation-energy distribution between Photosystem I (PSI) and Photosystem II (PSII) under different light environments. This process has shown to be important for plant fitness and has been thoroughly investigated. However, there are still several open questions regarding the functional effect and mechanism of state transitions in plants. One reason for this is that most studies have been performed at the leaf level or on isolated and/or fixed samples, while in vivo studies at the single chloroplast level have not been reported yet.

How state transitions affect the distribution of excitation-energy between photosystems is not yet resolved. In addition, it is unknown how state transitions affect the local energy distribution within the grana and stroma lamellae of the thylakoid membrane. Finally, although state transitions are often associated with a major change in the thylakoid organization, this has never been directly observed in a leaf.

Here we use confocal fluorescence lifetime imaging to study state transitions at the level of single chloroplasts in *Arabidopsis thaliana* leaves. The results show how the excitation-energy is distributed between PSI and PSII for 8 different wavelengths within the photosynthetic active region, and how this is affected by state transitions. Next, the redistribution of excitation-energy is measured within single chloroplasts with sub-micrometer spatial resolution. Finally, our data shows that the overall thylakoid macro-organization is not substantially affected by the process of state transitions, in contrast to what was reported in earlier studies.

26 Pre Recorded Poster

Crystal structure and reaction mechanism of a bacterial Mg-dechelatase homolog

Debayan Dey[1], Masayoshi Nishijima[2], Genji Kurisu[2], Hideaki Tanaka[2], Hisashi Ito[1]

[1]Hokkaido University, Sapporo, Japan. [2]Osaka University, Suita, Osaka, Japan

Chlorophyll degradation plays a myriad of physiological roles in photosynthetic organisms, including acclimation to light environment and nutrient relocalization during senescence. Mg extraction from chlorophyll *a* is the first and committed step of the chlorophyll degradation pathway. This reaction is catalyzed by the Mg-dechelatase enzyme encoded by *Stay-Green* (*SGR*). The reaction mechanism of SGR still remains elusive since metal ion extraction from organic molecules is not a common enzymatic reaction. Additionally, experimentally derived structural information about SGR or its homologs has not yet been reported. In this study, the crystal structure of the SGR homolog from *Anaerolineae* bacterium was determined. Biochemical analysis showed that three residues – H32, D34, and D62 are essential for catalytic activity of the enzyme. Mutation analysis of the D34 residue further highlighted its importance on the functioning of the dechelatase. Docking simulation also revealed interaction between the D34 side chain and central Mg ion of chlorophyll a. Structural analysis showed the arrangement of D34/H32/D62 in the form of a catalytic triad that is generally found in hydrolases. The probable reaction mechanism suggests that deprotonated D34 side chain coordinates and destabilizes Mg, resulting in Mg extraction. Besides, H32 possibly acts as a general base catalyst and D62 facilitates H32 to be a better proton acceptor. Taken together, the dechelation reaction by SGR is implemented in a mechanism that partially overlaps to the one observed in hydrolases.

27 Pre Recorded Oral

Regulation of the photomorphogenesis and chloroplast biogenesis –mediating protein PAP8

Monique Liebers, Thomas Pfannschmidt

Leibniz University Hannover, Hannover, Germany

Light induced transition from etioplasts to chloroplasts involves the assembly of nuclear encoded PEP associated proteins (PAPs) to the plastid encoded RNA polymerase (PEP), forming a transcriptionally active complex that transcribes photosynthesis genes. PAPs are crucial for chloroplast formation demonstrated by albinism if PAP proteins are lacking from the PEP complex [Steiner et al. (2011) Plant Physiology 157:1043–1055; Liebers et al. (2020) EMBO J. 39:e104941]. We demonstrated that PAP8, similar to PAP5, is not only localized to plastids, but localizes also to the nucleus, where it participates in the phytochrome B-dependent degradation of phytochrome-interacting factors (PIFs) and subsequently in the stabilization of elongated hypocotyl 5 (HY5), the expression of the photomorphogenesis regulator golden2-like (GLK) and ultimately the initiation of photomorphogenesis [Liebers et al. (2020) EMBO J. 39:e104941]. Despite the importance of PAPs their regulation remains poorly understood. We demonstrated that in the light, HY5 fine-tunes PAP expression. However, PAPs are already expressed in dark-grown seedlings. Therefore, we describe a genetic screening approach for the identification of the transcription factor responsible for PAP expression in the dark. Furthermore, we showed first evidence that in dark-grown seedlings PAP expression occurs specifically in the epidermis, while illuminated leaves display PAP expression also in mesophyll cells [Liebers et al. (2018) Planta. 248:629-646]. Since PAP proteins are present in epidermal- and mesophyll cells of etiolated seedlings, it is feasible that a non cell-autonomous movement from epidermis to mesophyll cells occurs. Here, we describe an approach to proof potential PAP8 cell-to-cell movement, using a PAP8-GFP construct that will be specifically expressed in the epidermis through the promoter region of *Arabidopsis thaliana* meristem layer 1 (pATML1).

28 In Person Poster

Chasing sun flecks in avocado canopies in the field and on *Alocasia* indoors with 30 ms flash protocols. Non photochemical quenching declined in weak sun flecks; the functional absorption cross-section of open photosystem II centres increased and less light was needed to saturate electron transport.

Barry Osmond[1,2], Fred (Wah Soon) Chow[1], Sharon Robinson[2]

[1]Australian National University, Canberra, ACT 2601, Australia. [2]University of Wollongong, Wollongong, NSW 2522, Australia

We report the remote sensing of PSII antennae and reaction centre functions relevant to photoprotection and monitor dynamic responses in photosynthetic electron transport (ETR) during natural sunflecks on avocado shade canopy leaves in the field and on *Alocasia* shade leaves indoors at 5-6 s intervals. Our data were obtained non-intrusively by averaging four successive ~30 ms light induced chlorophyll fluorescence transients (LIFT) from fast repetition rate (FRR) Q_A flash protocols that were designed to monitor intrinsic PSII and ETR parameters otherwise susceptible to the redox state of the plastoquinone (PQ) pool [Kolber et al. (1998) BBA–Bioenergetics 1367: 88-106; Osmond et al. (2017) Funct. Plant Biol. 44: 985-1006]. In weak sunflecks and cloud flecks both initial and maximum fluorescence increased, but photochemical yield (ϕ'_{PSII}) and regulated non-photochemical quenching (NPQ) declined an unregulated excitation dissipation (Y_{NO}) increased. Measured functional absorption cross-section of PSII (σ'_{PSII}) increased; probably because excitation from closing PSII centres was transferred to remaining open centres, perhaps facilitated by lutein epoxide-rich antennae of shade avocado that promotes light harvesting [Matsubara S et al. (2008) Plant Cell and Environ. 36: 548-561]. Although NPQ increased to high values in strong sunflecks, rapid relaxation in the shade may reflect retention of photoconverted lutein. The time constants for electron transport into and out of the PQ pool increased and the pool became more oxidized. The light intensity estimated to half-saturate ETR (E_k) declined, showing ETR was prioritized over photoprotection in weak sunflecks. Shade grown *Alocasia* displayed a similar parameter response syndrome, with the exception that the PQ pool always became more reduced in sunflecks. Electron microscopy of chloroplasts in such leaves [Chow et al. (1988) Aust. J. Plant Physiol. 15: 107-122.] revealed huge contorted mega-grana (up to 160 stacked thylakoids) in which plastocyanin diffusion may be constrained, potentially allowing PQ to be more readily reduced.

29 In Person Oral

Interplay between LHCSR proteins and state transitions during photoprotection under fluctuating light

Collin Steen[1,2], Adrien Burlacot[3,4], Audrey Short[5,2], Krishna Niyogi[4,2], Graham Fleming[1,2,5]

[1]Department of Chemistry, University of California, Berkeley, Berkeley, CA, USA. [2]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [3]Carnegie Institution for Science, Stanford, CA, USA. [4]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA. [5]Graduate Group in Biophysics, University of California, Berkeley, Berkeley, CA, USA

Photosynthetic organisms use light energy as the primary energy source to fix CO2. However, in the environment, light energy fluctuates rapidly and often exceeds saturating levels for periods ranging from seconds to hours, which can lead to detrimental effects for cells. Safe dissipation of excess light energy occurs primarily by non-photochemical quenching (NPQ) processes. In *Chlamydomonas reinhardtii*, photoprotective NPQ is mostly mediated by pH-sensing light-harvesting complex stress related (LHCSR) proteins and redistribution of light-harvesting complexes between the photosystems (state transitions). Although each mechanism underlying NPQ has been documented, their relative contributions to the dynamic functioning of NPQ under fluctuating light remain unknown. Here, by monitoring NPQ throughout multiple high light-dark cycles with fluctuation periods ranging from 1 to 10 minutes, we show that the dynamics of NPQ depend on the frequency of light fluctuations. Mutants impaired in the accumulation of LHCSRs (*npq4*, *lhcsr1*, and *npq4lhcsr1*) showed significantly less quenching during light phases, demonstrating that LHCSR proteins are responsible for the majority of the NPQ during light fluctuations. Surprisingly, activation of NPQ was also observed in the darkness and this was exacerbated in mutants lacking LHCSRs. By analyzing 77K chlorophyll fluorescence spectra and chlorophyll fluorescence lifetimes and yields in a mutant impaired in state transitions (*stt7*), we show that this phenomenon arises from state transitions. Finally, we quantified the contributions of LHCSRs and state transitions to the overall NPQ response, finding that interactions between STT7 and LHCSR3 during darkness may underlie the larger role of state transitions in NPQ for green algae compared to plants. These results highlight the dynamic functioning of photoprotection under fluctuating light and open a new way to systematically characterize the photosynthetic response to an ever-changing light environment.

30 Pre Recorded Oral

A novel signaling cascade regulates growth of cyanobacteria according to CO_2

Juha Kurkela[1], Otso Turunen[1], Viktoria Reimann[2], Wolfgang R. Hess[2], Taina Tyystjärvi[1]

[1]University of Turku, Turku, Finland. [2]University of Freiburg, Freiburg, Germany

In cyanobacteria, elevated CO_2 is known to enhance photosynthesis and growth, whereas genes encoding carbon-concentrating mechanisms are down-regulated. However, it remains poorly understood how cells actually sense the amount of inorganic carbon and balance cellular functions accordingly. We have revealed a novel signalling cascade connecting the amount of CO_2 and growth in the model cyanobacterium *Synechocystis* sp. PCC 6803. Acclimation of *Synechocystis* cells to high CO_2 was prevented when the small ω subunit of the RNA polymerase (RNAP) core was deleted (ΔrpoZ strain) [Gunnelius et al. (2014) Nucleic Acids Res. 42:4606-4614; Kurkela et al. (2017) Plant Physiol. 174:172-184]. Compared to *Synechocystis* sp. PCC 6803 control strain, many photosynthetic, nitrogen assimilation and cell wall synthesis genes showed low expression in the ΔrpoZ strain. Photosynthesis of ΔrpoZ strain did not acclimate to high CO_2 and low expression of peptidoglycan synthesis genes induced lysis of dividing ΔrpoZ cells. Spontaneously raised secondary mutations in the *ssr1600* gene rescued the high-CO_2-sensitive phenotype of the ΔrpoZ strain. Biochemical analyses showed that the *ssr1600* gene encodes an anti-σ factor antagonist of the group 2 sigma factor SigC. In the control strain, the formation of the growth-restricting RNAP-SigC holoenzyme reduced upon high CO_2 treatment, whereas in ΔrpoZ formation of the RNAP-SigC holoenzyme increased in high CO_2. The drastically reduced Ssr1600 content in the suppressor mutants reduced the formation of the RNAP-SigC holoenzyme to similar level as in the control strain, allowing almost normal transcriptome and growth of suppressor lines in high CO_2. We suggest a partner switch mode of action for the Ssr1600 protein. Formation of the anti-SigC antagonist Ssr1600/anti-SigC complex releases the SigC sigma factor from the SigC/anti-SigC complex and growth restricting RNAP-SigC holoenzymes are formed.

31 In Person Poster

Trivial excitation energy transfer to carotenoids is an unlikely mechanism for non-photochemical quenching in LHCII

Callum Gray[1], Tiejun Wei[1], Tomas Polivka[2], Christopher Duffy[1], Vangelis Daskalakis[3]

[1]Queen Mary University of London, London, United Kingdom. [2]University of South Bohemia, České Budějovice, Czech Republic. [3]Cyprus University of Technilogy, Limassol, Cyprus

Higher plants defend themselves from bursts of intense light via the mechanism of Non-Photochemical Quenching (NPQ). It involves the Photosystem II (PSII) antenna protein (LHCII) adopting a conformation that favors excitation quenching. In recent years several structural models have suggested that quenching proceeds via energy transfer to the optically forbidden and short-lived S1 states of a carotenoid. It was proposed that this pathway was controlled by subtle changes in the relative orientation of a small number of pigments. However, quantum chemical calculations of S1 properties are not trivial and therefore its energy, oscillator strength and lifetime are treated as rather loose parameters. Moreover, the models were based either on a single LHCII crystal structure or Molecular Dynamics (MD) trajectories about a single minimum. Here we try and address these limitations by parameterizing the vibronic structure and relaxation dynamics of lutein in terms of observable quantities, namely its linear absorption (LA), transient absorption (TA) and two-photon excitation (TPE) spectra. We also analyze a number of minima taken from an exhaustive meta-dynamical search of the LHCII free energy surface. We show that trivial, Coulomb-mediated energy transfer to S1 is an unlikely quenching mechanism, with pigment movements insufficiently pronounced to switch the system between quenched and unquenched states. Modulation of S1 energy level as a quenching switch is similarly unlikely. Moreover, the quenching predicted by previous models is possibly an artifact of quantum chemical over-estimation of S1 oscillator strength and the real mechanism likely involves short-range interaction and/or non-trivial inter-molecular states.

32 Pre Recorded Poster

Singlet oxygen production by Photosystem II is caused by misses of the oxygen evolving complex

Heta Mattila, Sujata Mishra, Taina Tyystjärvi, Esa Tyystjärvi

University of Turku, Turku, ., Finland

Contributions of singlet oxygen ($^1O_2$) and Mn ions of the oxygen evolving complex (OEC) to photoinhibition of PSII have not been quantified. Furthermore, $^1O_2$ is known to be produced via recombination reactions but it remains open which pathway(s) produce $^1O_2$. We compared the temperature dependences of $^1O_2$ production, photoinhibition and charge recombination. $^1O_2$ production increased from -2 to +35 °C, ruling out recombination of $P_{680}^+Pheo^-$ as a main contributor. The recombination reactions of $S_2Q_A^-$ and $S_2Q_B^-$, in turn, showed too steep temperature dependences to contribute significantly. However, the temperature dependence of $^1O_2$ production matched that of the misses of the OEC [Isgandarova et al (2003) Biochemistry 42: 8929–8938], indicating that the miss-associated recombination of $P_{680}^+Q_A^-$ is the main producer of $^1O_2$. Photoinhibition *in vitro* and *in vivo* shared the temperature dependence of $^1O_2$ production but the temperature dependence remained similar in the absence of O_2, indicating that photoinhibition is not caused by $^1O_2$ alone. UV photoinhibition had a weaker temperature response.

Analysis of the activation energies (Ea) with methods designed for complex mechanisms [Mao and Campbell (2019) ACS Catalysis 9: 9465-9473] showed that transition to a miss-prone state of the OEC has an Ea of 0.224 eV. Both this transition and the recombination of $P_{680}^+Q_A^-$ control the rate of the reaction series that produces $^1O_2$ and has an apparent Ea of 0.31 eV. The results show that photoinhibition proceeds via three mechanisms, out of which the UV-functional one Mn mechanism [Hakala et al (2005) Biochim Biophys Acta 1706: 68-80], has an Ea of 0.09 eV and 63–68 % contribution in visible light. The contributions of $^1O_2$ and P_{680}^+ dependent mechanisms, with a combined Ea of 0.43 eV, increase with wavelength but their mutual contributions are not known. The contribution of the $^1O_2$-dependent mechanism would increase with temperature.

33 In Person Oral

Functional reconstitution of the bacterial CO_2 concentrating mechanism

David Savage

UC Berkeley, Berkeley, CA, USA

Many autotrophs rely on biophysical CO_2 concentrating mechanisms (CCMs) to assimilate carbon. It is postulated that principles - and perhaps even components – of bacterial CCMs could be used to improve CO_2 assimilation in plants. Surprisingly, defining a systematic 'parts list' of the bacterial CCM remains an open question. To this end, we have carried out a genome-wide barcoded transposon screen to identify essential and CCM-related genes in the ɣ-proteobacterium *H. neapolitanus*. Screening revealed that the CCM comprises at least 17 and likely no more than 25 genes, most of which are encoded in 3 operons. Found within these genes is a new class of inorganic carbon pump which we term the DAB complex, for 'DABs accumulate bicarbonate,' that is widespread amongst prokaryotes and readily expressed in a heterologous fashion. Informed by this information, we have also performed a functional reconstitution of the bacterial CCM in vivo. Using a novel strain of Rubisco-dependent *E. coli*, we have assessed the importance of known and poorly characterized activities of the CCM for achieving efficient CO_2 assimilation and, ultimately, have engineered a strain which is capable of growth due to efficient CO_2 fixation directly from ambient air.

34 Pre Recorded Oral

Cryo-EM structure of the photosynthetic reaction center complex from a green sulfur bacterium

Jing-Hua Chen

College of Life Science, Zhejiang University, Hangzhou, China

Green sulfur bacteria (GSB) are strictly anaerobic phototrophs that can grow under extremely low light intensities by using a unique photosynthetic apparatus consisting of three coupled modules: a large light-harvesting chlorosome, a soluble energy transmitter FMO, and a membrane-embedded type-I photochemical reaction center (GsbRC). In contrast to the near 100% efficiency of excitation energy transfer in other photosynthetic systems, the excitation energy transfer efficiency from FMO to GsbRC remarkably reduced to 35% to 75%. We reported a 2.7-Å structure of the FMO-GsbRC complex from *Chlorobaculum tepidum* by cryo-electron microscopy, and revealed many distinct features of GsbRC compared with other RCs: (1) The GsbRC binds considerably less pigments than those bond in heliobacterial reaction center (HbRC) and photosystem I (PSI). (2) The antenna BChls in GsbRC are located into two separated clusters similar to that in photosystem II (PSII). In contrast, the antenna (B)Chls in each layer within HbRC and PSI are arranged closer and form a closed ring. (3) The conserved linker chlorophylls seen in other reaction centers are absent in GsbRC. Instead, we observed two long carotenoid derivatives between the two BChl layers in GsbRC, and they possibly participate in the light-harvesting or the energy dissipation process for the surrounding BChls. (4) The distance between the BChls of FMO and GsbRC exceeds 22 Å, much longer than the distances between the chlorophylls of the light-harvesting antenna (LHCI) and the photosystem I core of higher plant. We proposed the energy transfer pathways within the FMO-GsbRC complex based on the structural model. The initial evolvement of RCs occurred 3 billion years ago and all of the extant RCs descend from the ancestral protein. GsbRC exhibits features of both type-I and type-II RCs and might provide insight into how these structures are diverged from a common ancestor.

35 Pre Recorded Poster

Use of quartz sand columns to study far-red light photoacclimation (FaRLiP) in cyanobacteria

Ting-Shuo Nien[1], Donald A. Bryant[2], Ming-Yang Ho[1,3]

[1]Department of Life Science, National Taiwan University, Taipei, Taiwan. [2]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA. [3]Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

Some cyanobacteria can perform far-red light photoacclimation (FaRLiP), which allows them to use far-red light (FRL) for oxygenic photosynthesis. Most of the cyanobacteria able to use FRL were discovered in low visible-light (VL; λ= 400 to 700 nm) environments that are also enriched in FRL (λ= 700 to 800 nm). However, these cyanobacteria grow faster in VL than in FRL in laboratory conditions, indicating that FRL is not their preferred light source when VL is available. Therefore, it is interesting to understand why such strains are abundant in FRL-enriched but not VL-enriched environments. To this aim, we established a terrestrial model system with quartz sand to study the distribution and photoacclimation of cyanobacterial strains. A FaRLiP-performing cyanobacterium, *Leptolyngbya* sp. JSC-1, and a VL-utilizing model cyanobacterium, *Synechocystis* sp. PCC 6803, were compared in this study. We found that, although *Leptolyngbya* sp. JSC-1 can grow well in both VL and FRL, *Synechocystis* sp. PCC 6803 grows much faster than *Leptolyngbya* sp. JSC-1 in VL. In addition, the growth rate was faster in liquid co-cultures than in monocultures of *Leptolyngbya* sp. JSC-1 or *Synechocystis* sp. PCC 6803. In an artificial terrestrial model system, *Leptolyngbya* sp. JSC-1 has an advantage when growing in co-culture at greater depths by performing FaRLiP. Therefore, strong competition for VL and slower growth rate are possible reasons why FRL-utilizing cyanobacteria are enriched in environments with low VL intensities. This model system provides a valuable tool for future studies of cyanobacterial ecological niches and interactions in a terrestrial environment.

36 Pre Recorded Poster

Unexpected diversity of cyanobacteria and algae utilizing far-red light for photosynthesis in an urban park

Pa Yu Chen[1], Jui-Tse Ko[1], Ming-Yang Ho[1,2]

[1]Department of Life Science, National Taiwan University, Taipei, Taiwan. [2]Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

Oxygenic photosynthesis is a critical process performed by cyanobacteria, algae, and plants. For a long time, visible light (VL, wavelength = 400-700 nm) has been known to support oxygenic photosynthesis. However, in recent years, some algae and cyanobacteria were identified to be capable of using far-red light (FRL, wavelength = 700-800 nm) for oxygenic photosynthesis. In contrast to extensive studies of FRL-using cyanobacteria, studies on the diversity and distribution of FRL-using algae remain limited. In this study, we have isolated ten algal and four cyanobacterial strains that can grow in FRL from an urban park located in Taipei, Taiwan. Among the isolated algae, only one *Eustigmatophyceae* sp. has been reported capable of using FRL for photosynthesis, but four freshwater green algal genera have not. In VL and FRL, these algae did not show morphological differences under a microscope, but spectroscopy revealed their distinctive features. Compared to the model unicellular green alga, *Chlamydomonas reinhardtii*, the ten algal strains isolated in this study showed noticeable FRL absorbance features beyond 700 nm. However, unlike the four cyanobacterial strains that synthesize chlorophylls *d* and *f* to assist FRL harvesting, these algal strains only produce chlorophylls *a* or *b*. In addition, fluorescence spectroscopy shows an increase in FRL fluorescence emission beyond 700 nm at room temperature, suggesting that additional red-shifted chlorophyll *a* molecules are likely associated with light-harvesting complexes in FRL. These green algae are not phylogenetic unique but closely related to other green algae in the same genera. However, without experimentally culturing these algae in FRL, it was not easy to discover their capability of using FRL, which might be why they were not previously reported capable of growing in FRL. Overall, our results suggest that FRL-using algae may be widespread, and their diversity may be underestimated in the environment.

37 Pre Recorded Poster

Purification, crystallization, and X-ray crystallographic analysis of Photosystem II of the thermophilic cyanobacterium *Thermosynechococcus vulcanus* cultured in the presence of yttrium

Hajime Fujii[1], Yoshiki Nakajima[2], Chunxi Zhang[3], Shen Jian-Ren[4]

[1]Graduate School of Natural Science and Technology, Okayama University, Okayama-shi, Okayama, Japan. [2]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama-shi, Okayama 700-8530, Japan. [3]Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. [4]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama-shi, Okayama, Japan

Photosystem II (PSII) splits water into protons, electrons and molecular oxygen using light energy at its metal center, the Mn_4CaO_5 cluster. The structure of the Mn_4CaO_5 and the Mn_4SrO_5 clusters have been solved at high resolutions; however, no other metal ions have been known to substitute for the calcium ion functionally. In this study, we used yttrium ( ) to replace Ca, and examined liquid culture conditions for *Thermosynechococcus vulcanus* in the presence of without Ca. As a result, we found a suitable concentration for growth, and the cells cultured under this condition, together with the thylakoid membrane, crude-PSII and purified PSII purified from the cells, showed lower oxygen-evolving activities than those cultured under Ca-containing condition and Ca-containing PSII. In order to confirm the replacement of Ca by , we crystallized the PSII dimer isolated from the cells grown in the presence of (-PSII), and collected X-ray diffraction datasets at the X-ray absorption edge wavelength of (0.72Å) and remote wavelength (1.0Å). A weak anomalous difference map was observed at the Ca binding site of the Mn cluster in the -PSII crystal from the 0.72Ådata and the diffraction map was smaller at 1.0Å, and this map was not observed at all in the Ca-PSII crystal collected under the same condition. This suggests that is inserted into a part of the Ca-binding site.

38 In Person Poster

Absence of Lhcb6 partially prevents non photochemical quenching qH to occur

Pierrick Bru[1], Zeno Guardini[2], Roberto Bassi[2], Alizée Malnoë[1]

[1]Umeå Plant science Centre, Department of Plant Physiology, Umeå University, Umeå, Sweden. [2]Department of Biotechnology, University of Verona, Verona, Italy

Non photochemical quenching (NPQ) is a mechanism used by plants to protect themselves against excess of light by reemitting this energy in form of heat to avoid photodamage. NPQ qH is a sustained quenching that is negatively regulated by the suppressor of quenching 1 (SOQ1) and positively regulated by the lipocalin in the plastid (LCNP) that gives protection against long-term environmental changes.

Photosystem II (PSII) is organized with light harvesting complex II (LHCII) surrounding a PSII core complex. LHCII are composed of minor monomeric antennae (Lhcb4-6) and major trimeric antennae (Lhcb1-3) connected to the PSII core. Previously, we showed that qH can occur in the LHC trimer antennae but that it is not dependent on a specific Lhcb1-3 isoform. Here, we investigate the implication of the minor antenna in NPQ qH.

In non-stress conditions in the *soq1* mutants without minor antenna (*NoM*) or without Lhcb6, qH induction is abolished. However, in stress conditions such as cold and high light both mutants display an enhanced NPQ compared to *NoM* or *lhcb6* respectively, but to a lesser extent than *soq1*. The absence of Lhcb4 or Lhcb5 in *soq1* background does not prevent qH to occur.

In *soq1* background LCNP is modified and appears with a higher molecular weight compared to LCNP in wild type. Interestingly, in *soq1 lhcb6* in non-stress conditions, LCNP is not modified. However, after stress conditions, LCNP molecular weight is similar or slightly lower than LCNP in *soq1*. The abolished qH in non-stress conditions in *soq1 lhcb6* could be due to the absence of LCNP modification.

39 Pre Recorded Oral

Mighty C_4 plants are thirsty

Chandra Bellasio[1,2], Hilary Stuart-Williams[1], Graham Farquhar[1], Jaume Flexas[2]

[1]Australian National University, Canberra, ACT, Australia. [2]University of the Balearic Islands, Palma, Balearic Islands, Spain

All plants ultimately operate the same photosynthetic pathway evolved by cyanobacteria some 3.5 billion years ago, termed 'C_3'. The enzyme 'Rubisco' catalyses the key-step of the incorporation of CO_2 into carbohydrates, but a competitive reaction with oxygen leads to wasteful photorespiration, where previously assimilated CO_2 is released. During Earth's history the decreasing concentration of CO_2 and the increasing production of oxygen magnified the burden of photorespiration. This drove some plants to evolve C_4 photosynthesis, a biochemical 'turbocharger' which concentrates CO_2 in the vicinity of Rubisco, thus reducing photorespiration and decreasing unfavourable reactions at high temperatures. As a result, today's C_4 crops attract considerable interest in the face of global warming and rising food demand. However, the feasibility of their widespread adoption is undermined by the inherent complexity of the C_4 system, which leaves it vulnerable to changing environmental conditions. We hypothesize that this higher sensitivity is due to the necessity of exchanging C_4 metabolites through thin cytoplasmic threads called plasmodesmata, which may shrink, for instance under dehydration when intracellular pressure (turgor) decreases. Here we show that, under rapid dehydration, C_4 assimilation decreased faster than C_3, and, after a sharp onset, non-stomatal limitation rose at triple-the-rate in C_4 plants than in C_3. These effects represent two true hydric handicaps for C_4 leaves. However, given enough time, formidable acclimation strategies can make some C_4 plants tolerate drought better than their C_3 counterparts, effectively transforming a weakness into one of the keys of their success. Drought acclimation might have shaped the evolutionary history of C_4 plants, and may be responsible for their dominance of most of today's hotter grasslands. Little is known of the underpinning mechanisms, but any attempt to ameliorate C_4 plants or to turn a C_3 into a C_4 crop will need an understanding of nature's C_4 solution.

40 Pre Recorded Oral

Ecophotobiology of the seagrass *Posidonia oceanica*

Quentin Charras[1,2], Andreas naschberger[3], Alexey Amunt[3], Roberta Croce[4], Colette Jungas[1]

[1]Biotechnology institute of Aix Marseille, Marseille, France. [2]KTH university, Stockholm, Sweden. [3]Scilifelab, Stockholm University, Stockholm, Sweden. [4]Department of Physics and Astronomy and Institute for Lasers, Life and Biophotonics , Faculty of Sciences , Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Seagrasses (angiosperm) are emblematic marine primary producers widely distributed worldwide. In the Mediterranean Sea, seagrasses are mainly represented by *Posidonia oceanica*, concidered as true engineer specie. It colonizes coastal areas up to 40 m depth where light intensity is 20 times less than surface, and only blue light persists. As depth increases, light intensity diminution create an energy deficit from both photosystems. As *P.oceanica* needs a proper tandem operation of both photosystems- how does the plant insure its fitness with depth?

We highlighted profound changes in the organization of the photosynthetic membrane with depth. On that basis, we focused on the structural and functional adaptations mechanisms of both photosystems. Strikingly, *P. oceanica* does not display the same light acclimation mechanisms as terrestrial angiosperms but appear closer to ancestral phylum such as lycophytes and green algae. In *P. oceanica*, regardless of the growing depth, we were able to highlight the presence of a PSI-LHCII supercomplex that differs from terrestrial angiosperms by virtue of a bigger antenna size. Moreover, we showed that in *P.oceanica*, as in green algae, chlorophylls of photosystem I are blue-shifted. Appeared in land angiosperm, the red-shifted chlorophylls of PSI play a role in photoprotection but limit PSI antenna size. As *P.oceanica* lives in a red light deprived environment, the loss of red chlorophylls allows to increase PSI antenna size without penalizing photosystem I. We recently obtained the high resolution structure of the Large-PSI-LHCII by cryo-EM and our recents data point towards the formation of higher order PSI mega complexes.

Similarly to *P.oceanica*, blue shifted antenna and the same Large-PSI-LHCII were also present in *Zostera marina* and *Cymodocea nodosa*, two marine plants growing up to 15m in depth. Therefore, such a supercomplex appears to be a key adaptive trait express by marine angiosperms toward the necessity to optimize light capture.

41 Pre Recorded Poster

Spectral diversity of Photosystem I from flowering plants

Peter Bos, Christo Schiphorst, Emilie Wientjes

Wageningen University & Research, Wageningen, Netherlands

Photosystem II (PSII) and Photosytem I (PSI) work in series to convert solar energy into chemical energy. PSI is a plastocyanin-ferredoxin oxidoreductase that uses light energy to transfer electrons over the thylakoid membrane of oxygenic photosynthetic organisms. In the past decade, the absorption, fluorescence emission and excitation energy trapping characteristics of PSI from various photosynthetic organisms have been extensively studied. However, how the characteristics compare between PSI's from different plants has not been properly investigated. Here we have used a combination of biochemical and spectroscopic methods to compare PSI from 5 species: *Arabidopsis thaliana*, *Spinacia oleracea*, *Zea mays*, *Spathiphyllum wallisii* and *Calathea roseopicta*. These include eudicots and monocots, and sun tolerant and shade tolerant plants. We compared the absorption spectra, fluorescence emission spectra and excitation-energy trapping kinetics and show how the fluorescence emission spectra depend on the temperature. We observed statistically significant differences in the emission maxima of PSI from the different species, however the absorption properties were indistinguishable in the far-red region. Finally, we show for the first time that the emission spectrum and intensity of PSI is affected by changes in temperature in the physiological relevant range (280 K to 298 K).

42 In Person Poster

Validating equilibrium assumptions for steady-state measurements of CO_2 exchange from liquid suspensions

Jason Hupp, Shannon Loriaux

LI-COR Inc., Lincoln, NE, USA

Open flow-through steady-state gas exchange systems are widely used in measurements of CO_2 exchange at the leaf-level. Due to the steady-state nature of the measurement, CO_2 and O_2 concentrations at the sample interface remain constant, minimizing opportunity for physiological response to the measurement technique. Despite this marked advantage, steady-state measurements have seen little application for samples in liquid suspension. Complexities around CO_2 measurement in solution have favored the use of O_2 sensors for liquid measurements. The low precision of many O_2 sensing technologies has driven the use of transient, time derivative, measurement techniques where large changes in O_2 concentration are required. Here we describe a steady-state system for measurements of CO_2 exchange from liquid suspensions capable of resolving sub-ppm signals and demonstrate its use for measurements of photosynthetic CO_2 response of an algal suspension (*Monoraphidium* sp.). The application of any gas exchange system for the measurement of CO_2 response requires that the CO_2 concentration in solution be known. The liquid sample in the system described here, is in constant contact with the measurement air stream, and the headspace concentration above the sample is assumed to represent the equilibrium concentration in solution. This assumption was validated by comparing the total release (or uptake) of CO_2 during transition between two zero-flux states, with the change in dissolved inorganic carbon predicted from theory, and by a fitting approach to estimate a chamber specific mass transfer coefficient (2.55×10^{-4} mol s^{-1}). Application of the derived mass transfer coefficient to measured data, shows that during the CO_2 response measurements presented, the headspace concentration was nearly identical (within 1.03%) to the equilibrium concentration in solution.

43 Pre Recorded Poster

Investigating far-red light-inducible promoters to regulate gene expression in cyanobacteria

Ting-So Liu[1], Ke-Feng Wu[1], Han-Wei Jiang[1], Kai-Wen Chen[1], Donald A. Bryant[2], Ming-Yang Ho[1]

[1]National Taiwan University, Taipei, Taiwan. [2]The Pennsylvania State University, University Park, Pennsylvania, USA

Synthetic biology in cyanobacteria continues to have some limitations, such as the scarcity of inducible promoters and the availability of strains for genetic engineering. Chemically inducible promoter systems may be toxic or incompatible with some cyanobacteria, and external additions of chemicals can increase the cost of industrial production. Instead, light-inducible promoters can regulate genes of interest precisely and effectively. Blue-light, low-light, and green-light induced expression systems have been investigated in cyanobacteria. The promoters, photoreceptors, and signal-transducing proteins in these systems have also been modulized as synthetic biology parts for regulating heterologous gene expression. However, regulated systems with other light sources have not been well-developed.

In some cyanobacteria, during far-red light photoacclimation (FaRLiP), RfpA, RfpB, and RfpC proteins transduce the far-red light signal and activate the transcription of genes required for the cellular response for photosynthesis in far-red light. We were inspired by this system and aimed to construct a far-red light-inducible expression system. We cloned predicted promoter sequences from the FaRLiP strains *Leptolyngbya* sp. JSC-1 and *Synechococcus* sp. PCC 7335 with the EYFP reporter into a FaRLiP strain, *Chlorogloeopsis fritschii* PCC 9212 via conjugative transformation. The activity of a specific promoter, $P_{chlFJSC1}$, was induced up to 30-fold under far-red light exposure. Far-red light intensities controlled the induction level, and the induction was reversible under visible light.

In summary, we have established a far-red light-induced gene expression system in *C. fritschii* PCC 9212, which could serve as a potential light-regulated gene expression platform for industrial applications. We are currently optimizing the expression of RfpA, B, and C in model organisms such as *Escherichia coli* and *Synechococcus* sp. PCC 7002 to investigate the potential portability of the system.

44 Pre Recorded Oral

Characterization of evolutionally conserved mechanism of proton translocation across the chloroplast envelope-membrane and cytoplasmic membrane of cyanobacteria

Akane Echigo[1], Hikaru Tagami[2], Shinji Masuda[2]

[1]Graduate School of Life Science & Technology, Tokyo Institute of Technology, Yokohama, Kanagawa, Japan.
[2]Graduate School of Life Science & Technology, Yokohama, Kanagawa, Japan

Light illumination induces acidification and alkalization of the thylakoid lumen and the chloroplast stroma, respectively, for activation of NPQ and the Calvin-Benson-Bassham cycle. Thus, pH homeostasis in chloroplasts is crucial for photosynthesis control. Despite extensive studies, molecular mechanism of how chloroplast pH can be maintained at proper level are still poorly understood. We have identified various proteins regulating chloroplast pH, and identified novel NPQ-regulatory proteins: Fluctuating-Light Acclimation Protein1 (FLAP1), Day-Length-dependent Delayed Greening1 (DLDG1) and plastidial-encoded Ycf10 (1). These proteins localize in the envelope membranes of chloroplast, whose mutants showed pale-green phenotype under specific light conditions. DLDG1 and Ycf10 are homologs of the cytoplasmic-membrane-localizing cyanobacterial Proton-Exchange A that is required for light-induced $^{+}$ extrusion. The FLAP1 homolog as well as two DLDG1 homologs are conserved in cyanobacteria, whose mutants showed unusual light-induced H^{+} extrusion/uptake across the cytoplasmic membranes (2). These results suggest that the three protein components, FLAP1, DLDG1 and Ycf10, functionally interact to control $^{+}$ release for pH homeostasis in oxygenic phototrophs. We recently transformed the cyanobacterial mutants by the genetically-encoded pH indicator gene, and found that FLAP1, DLDG1 and Ycf10 homologs have crucial role for pH homeostasis in cyanobacteria. We also created *Arabidopsis ycf10* mutant by use of the new plastidial genome-editing technology, and characterized the phenotype. Together with these data, functional and physiological significance of the three novel pH-regulatory proteins, which extrude/uptake $^{+}$ across the envelope membranes for the pH homeostasis will be discussed.

1) Sato et al. (2017) *Plant Cell Physiol.* 58: 1622-1630; Trinh et al. et al. (2019) *Photosynth. Res.* 139: 413-424; Harada et al. (2019) *Plant Cell Physiol.* 60: 2660-2671; Trinh et al. (2021) *Plant Direct* 5: e368.

2) Inago et al. (2020) *Biochim. Biophys. Acta* 1861: 148258

45 Pre Recorded Oral

Bridging plastoquinone pool redox state and state transitions in *Chlamydomonas reinhardtii*: regulation in darkness and under illumination

Olli Virtanen, Esa Tyystjärvi

Department of Biotechnology/Molecular Plant Biology, University of Turku, Turku, Finland

The reduction level of the plastoquinone pool (PQ-pool) of thylakoid membranes acts as a signaling component that induces biochemical feedback mechanisms and regulates gene expression. Recently, we found that state transitions caused by illumination with wavelengths favoring one photosystem (PSII or PSI) depend curvilinearly on the PQ-pool redox state in *Arabidopsis thaliana* [Mattila et al. (2020) The Plant Journal 104:1088-1104]. State transitions have been assumed to function via a similar mechanism in green algae.

The green alga *Chlamydomonas reinhardtii* has similar antenna systems as plants and its chloroplast is therefore expected to respond to wavelengths essentially like plants. We examined how *C. reinhardtii* behaves in wavelengths that strongly reduce or oxidize the PQ-pool of *A. thaliana*. Firstly, in comparison to *A. thaliana*, the PQ-pool redox state was modulated less dramatically, and the PQ-pool remained more reduced under all wavelengths. We suggest that the smaller difference in the Chl *a*/*b* ratios of the antenna systems of the two photosystems in *C. reinhardtii* than in plants make photosynthetic electron flow less wavelength-dependent, thereby dampening the effects of wavelengths on the PQ-pool. Secondly, we found the state transitions to be less dependent on the PQ-pool redox state than in plants. In particular, similar state transitions could be induced either with aerobic and anaerobic dark treatments and by illumination with white light designed to favor one of the photosystems, although the reduction of the PQ-pool was found to be very different after these treatments. Thirdly, the light state of *C. reinhardtii* was found to depend on both intensity and quality of light. Light intensity is the primary signal in *C. reinhardtii* whereas in plants the redox state of the PQ-pool dominates. Regulation mainly by light intensity may suggest that state transitions play a photoprotective role in *C. reinhardtii*.

46 In Person Oral

The minor LHCII antenna protein CP26 is involved in PsbS-independent but ΔpH-dependent NPQ in *Arabidopsis*

Julia Walter[1], Johannes Kromdijk[1,2]

[1]Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom. [2]Carl R Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana-Champaign, Illinois, USA

Under high-light stress, excess excitation energy is harmlessly dissipated as heat in a photoprotective process called non-photochemical quenching (NPQ). The fastest component of NPQ (qE) relies on the PSII subunit S (PsbS) and de-epoxidated xanthophylls, in particular zeaxanthin. PsbS is thought to sense pH changes in the thylakoid lumen and may facilitate rearrangement of the LHCII trimers of PSII-LHCII super-complexes, leading to the formation of major quenching sites in PSII-detached LHCII aggregates. However, the monomeric minor antennae (CP26, CP29 and CP24) may also be involved in the formation of alternative quenching sites upon high light exposure. The minor antennae connect the major LHCII trimers to the PSII core for efficient light-harvesting and initiation of electron transfer between the photosystems. Previous results have shown that CP26 binds zeaxanthin more efficiently than CP29 and CP24, that CP26 undergoes a conformational change into a quenched state upon binding of zeaxanthin only and that *cp26*-deficient *Arabidopsis thaliana* plants lack a more sustained NPQ component. Here, we used a genetic and pharmacological approach to investigate the nature of CP26-associated NPQ. NPQ traces in double mutants deficient in *cp26* with contrasting PsbS levels showed that the *cp26* deficiency led to significantly lower NPQ in the slower phase of NPQ induction but did not affect the impact of PsbS abundance on the amplitude of qE. These results confirm that the mechanism of CP26-associated NPQ is independent from PsbS. Infiltration with specific inhibitors further revealed that CP26-associated NPQ requires a proton gradient across the thylakoid membrane and protonatable residues but surprisingly not the xanthophyll cycle enzyme violaxanthin de-epoxidase.

47 Pre Recorded Oral

Alternative electron pathways of photosynthesis power the algal CO_2 concentrating mechanism

Adrien Burlacot[1,2], Ousmane Dao[2], Pascaline Auroy[2], Stephan Cuiné[2], Yonghua Li-Beisson[2], Gilles Peltier[2]

[1]Carnegie Institution for Science, Stanford, CA, USA. [2]CEA Cadarache, Saint Paul lez Durance, France

Microalgae represent 50% of photosynthetic CO_2 consumption on Earth. The efficiency of aquatic photosynthesis to fix CO_2 relies on a mechanism (CCM) that actively concentrates inorganic carbon (Ci), thus favouring CO_2 fixation by increasing CO_2 concentration at the catalytic site of RuBisCO. While CCM activity requires photosynthetic energy to concentrate Ci, the bioenergetics mechanisms involved in the generation and supply of energy to the various CCM components have been elusive. Here, by measuring photosynthetic CO_2 consumption, O_2 exchange, electrochromic shift of carotenoid and chlorophyll fluorescence in green algae *Chlamydomonas reinhardtii* mutants affected in alternative electron pathways of photosynthesis, we show that the trans-thylakoidal proton gradient generated by the combined action of Flavodiiron proteins and Proton Gradient Regulation Like-1 drives thylakoid-localized CCM transport and transformation steps. We further show that a redox coupling between chloroplast and mitochondrial respiration also contributes to CCM energization, most likely by supplying ATP to non-thylakoid Ci transporters. We propose a global scheme of the CCM energization network, describing how alternative electron pathways of photosynthesis cooperate to efficiently drive both CO_2 concentration and fixation [Burlacot et al. (2022) Nature 605, 366–371]. Our results pave the way towards the use of a synthetic algal CCM to improve crop photosynthesis.

48 Pre Recorded Poster

Assessment of nonphotochemical quenching processes in the extremophilic red alga *Cyanidioschyzon merolae*

Han-Yi Fu, Yu-Hao Chiang, Yu-Jia Huang

Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan

Nonphotochemical quenching (NPQ) is a frontline response to protect photosystem (PS) II from photodamage. Red algal NPQ is uniquely characterized by its strong formation under a series of multi-turnover saturating light pulses. Two NPQ processes, intrinsic fluorescence quenching within PSII and energy spillover from PSII to PSI, were separately proposed for red algae, yet no consensus was reached on which process played the major role in NPQ. Furthermore, the trans-thylakoid pH gradient (ΔpH) appears to regulate NPQ, but it does not satisfactorily explain the residual fluorescence quenching with addition of the protonophore under high illumination. In this work, multiple NPQ processes were assessed in the extremophilic red alga *Cyanidioschyzon merolae* under a series of saturating light pulses or under continuous illumination at 300 μmol photons m^{-2} s^{-1}. Fluorescence emission spectra at 77K were measured after different periods of light treatments, and external fluorophores were added for normalization of the fluorescence level. The phycobilisome (PBS)- and PSII-related NPQ processes were distinguished by light preferentially absorbed by PBSs and PSs, respectively. Both intrinsic fluorescence quenching within PSII and energy spillover from PSII to PSI were identified as the PSII-related NPQ processes under light pulse and continuous illumination conditions. The energy spillover from PSII to PSI was rapidly induced after one light pulse or a short period of illumination and then decreased, whereas the intrinsic fluorescence quenching within PSII was gradually enhanced after a train of pulses or under continuous illumination. In addition, two PBS-related NPQ processes, energetic decoupling of PBSs from PSII and energy spillover from PBSs to PSI, were observed. The effect of various ionophores on the NPQ kinetics suggested that red algal NPQ could be modulated by membrane potential in addition to ΔpH.

50 Pre Recorded Oral

Modelling of the [cytochrome *f*:acceptor] electron transfer complexes of diatoms *Phaeodactylum tricornutum* (containing cytochrome c_6) and *Thalassiosira oceanica* (containing plastocyanin)

Carmen Castell[1], Luis A. Rodríguez-Lumbreras[2], Mercedes Roncel[1], Manuel Hervás[1], Juan Fernández-Recio[2], José A. Navarro[1]

[1]Instituto de Bioquímica Vegetal Fotosíntesis, cicCartuja, CSIC-Universidad de Sevilla, Seville, Spain.
[2]Instituto de Ciencias de la Vid del Vino (ICVV), CSIC-Universidad de La Rioja–Gobierno de La Rioja, Logroño, Spain

In the red lineage of photosynthetic organisms, the coastal diatom *Phaeodactylum tricornutum* contains cytochrome c_6 as the only electron carrier between cytochrome b_6f and photosystem I. However, in the oceanic diatom *Thalassiosira oceanica*, a functional plastocyanin has been described to act as the main electron carrier, replacing cytochrome c_6 and thus decreasing the cell's iron requirements. *T. oceanica* plastocyanin seems to have been acquired by lateral gene transfer from a green alga and, consequently, shows the typical strong negative electrostatics of a "green-type" plastocyanin.

We have carried out docking simulations to study the [cytochrome *f*:acceptor] complex in *P. tricornutum* and *T. oceanica* by using the pyDock docking protocol. The best docking model of the [cytochrome *f*:cytochrome c_6] complex of *P. tricornutum* has a different orientation compared with the equivalent "side-on" complex of green algae, that involves both the large and small domains of cytochrome *f*. Thus, the *P. tricornutum* complex shows a "head-on" orientation, more similar to that described previously in cyanobacteria, in which the small domain of cytochrome *f* does not play a relevant role. In addition, docking models indicate that the [cytochrome *f*:plastocyanin] complex of *T. oceanica* shows a much larger population of low-energy docking orientations compared to the *P. tricornutum* complex, suggesting that the acquired green-type plastocyanin of *T. oceanica* would not have yet achieved a prevalent configuration optimized for electron transfer.

Funding: Grants PID2020-112645GB-I00 (Ministry of Economy, Industry and Competitiveness of Spain) and US-1380339 (Andalusian FEDER Program)

51 Pre Recorded Oral

Functional genomics of eukaryotic photosynthetic acclimation to nitrogen deprivation

Du Cao, Yulong Wang, Penghao Yang, Wenqing Yu, Kangning Guo, Yanlei Feng, Xiaobo Li

Westlake University, Hangzhou, Zhejiang, China

Nitrogen (N) deprivation leads to chlorosis, induction of non-photochemical fluorescence quenching (NPQ), and accumulation of storage lipids in photosynthetic eukaryotes. Regulatory mechanisms behind these processes were poorly understood. In this research, we employed the unicellular green alga *Chlamydomonas reinhardtii* for a large-scale, automated mutant screen, and obtained >500 mutants with accelerated or delayed chlorosis, or altered chlorophyll fluorescence parameters. From these mutants, we identified >200 disrupted loci, covering multiple genes with a known role in acclimation to N deprivation, including genes involved in pigment metabolism, synthesis of the chloroplast alarmon guanosine pentaphosphate (ppGpp), and autophagy. The additional genes identified revealed the importance of photosynthetic electron transport, chloroplast gene expression, nuclear dynamics, RNA metabolism, and redox regulation in acclimation to N deprivation. For 25 of the genes identified, we proved their functions by complementing the original mutant or characterizing additional CRISPR-Cas9 mutant alleles. Phenotypic analyses of Arabidopsis mutants disrupted in homolog genes revealed evolutionarily conserved functions of multiple hits. We next attempted to assign genes into pathways by clustering mutants based on photosynthetic phenotypes across various nutrient and light input conditions, followed by large-scale protein-protein interaction testing and mutation epistasis analyses. These efforts uncovered key proteins that integrate carbon and light availability into metabolic decisions under N deprivation, as well as two distinct pathways that mediate chloroplast-nucleus communications. We further employed the mutants to understand the physiological significance of chlorosis and NPQ induction and demonstrated their essential roles in cell survival under N deprivation. Finally, we employed the knowledge from this screen to design background strains with higher lipid productivity under N deprivation. The mutant collection yielded from this study will be made available to the community through a website under development.

52 In Person Oral

Unravelling the proteome of the liquid-liquid phase separated pyrenoid using proximity proteomics

Justin Lau, Adam Dowle, Philipp Girr, Luke Mackinder

University of York, North Yorkshire, United Kingdom

Chlamydomonas reinhardtii operates a biophysical CO_2 concentrating mechanism (CCM) to boost photosynthesis. At the heart of the CCM lies a liquid-like organelle, the pyrenoid, which is comprised of the phase-separated CO_2-fixing enzyme Rubisco. The pyrenoid can dissolve, recondense and split by fission during the *Chlamydomonas* cell cycle and in response to fluctuations in external CO_2 levels. To understand the pyrenoid biology and the components which govern its dynamic behaviour, it is important to establish the pyrenoid proteome. However, determining the pyrenoid proteome is made difficult by the weak multi-valent interactions which underpin its assembly. In this study, we developed TurboID proximity labelling in *Chlamydomonas* where the promiscuous biotin ligase, TurboID, was fused to known major pyrenoid constituents: RBCS2 and EPYC1. This allowed in vivo biotin labelling of neighbouring proteins in the pyrenoid which were purified via streptavidin interactions and analysed via mass spectrometry. Here we show that this approach can accurately identify the proteome of a liquid-liquid phase separated organelle, with the data identifying many known pyrenoid components and discovering multiple new proteins-of-interest that could play a part in pyrenoid structure and function.

53 Pre Recorded Oral

Cooperation of external carbonic anhydrase and HCO_3^- transporter provides underwater photosynthesis in submerged leaves of the amphibious plant

Genki Horiguchi[1,2,3], Ryoma Oyama[4], Tatsuki Akabane[3], Nobuhiro Suzuki[5], Etsuko Katoh[6,5], Yusuke Mizokami[1], Ko Noguchi[1], Naoki Hirotsu[3,4]

[1]School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan. [2]Japan Society for the Promotion of Science, Chiyoda, Tokyo, Japan. [3]Graduate School of Life Sciences, Toyo University, Itakura, Gunma, Japan. [4]Faculty of Life Sciences, Toyo University, Itakura, Gunma, Japan. [5]Advanced Analysis Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan. [6]Faculty of Food and Nutritional Sciences Life Sciences, Toyo University, Itakura, Gunma, Japan

Hygrophila difformis, a heterophyllous amphibious plant, can use HCO_3^- for photosynthesis under submerged condition. Our previous study showed that the submerged leaves of *H. difformis* are sensitive to internal carbonic anhydrase (CA) inhibitor, ethoxzolamide [Horiguchi et al. (2019) AoB Plants 11:plz009]. However, the mechanism for HCO_3^- utilization is largely unknown. In this study, we evaluated the sensitivities to external CA inhibitor, acetazolamide, and anion exchanger protein inhibitor, 4,4´–diisothiocyanostilbene–2,2´–disulfonic acid (DIDS). Underwater photosynthesis of the submerged leaves of . *difformis* was decreased by each inhibitor, but no additive effect was observed. To identify the gene involved in HCO_3^- utilization, we performed a comparative transcriptomic analysis between terrestrial and submerged leaves. A total of 75,579 genes were identified, and the differentially expressed genes were 4,566 between the terrestrial and submerged leaves. However, genes of well-known HCO_3^- transporters in photoautotrophs were not detected. In submerged leaves, the expression levels of the two putative *α–CA* genes, *Hdα–CA1* and *Hdα–CA2*, and putative *β-CA* gene, *Hdβ–CA1*, increased. From putative amino acid sequences, proteins encoded by *Hdα–CA1* and *Hdα–CA2* genes were predicted to be localized in the apoplastic region, whereas protein encoded by *Hdβ–CA1* gene was predicted to be localized in the cytosol. Recombinant Hdα–CA1 and Hdβ–CA1 showed dominant CO_2 hydration activity over HCO_3^- dehydration activity. We propose the model that HCO_3^- use for photosynthesis in the submerged leaves of *H. difformis* is driven by the cooperation of external CA, Hdα–CA1, and DIDS–sensitive HCO_3^- transporter.

54 Pre Recorded Poster

Impaired PGR5-dependent photosystem I cyclic electron transport alleviates the growth defects in the *ntrc* mutant by redirecting electron distribution from ferredoxin

Yuki Okegawa[1], Ken Motohashi[2], Wataru Sakamoto[1]

[1]Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama, Japan. [2]Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Kyoto, Japan

Photosystem I cyclic electron transport (PSI-CET) is important for photosynthesis and induction of photoprotective mechanism. In *Arabidopsis thaliana*, two partially redundant pathways exist: the PROTON GRADIENT REGULATION5 (PGR5)-dependent pathway is the main one and the NADH dehydrogenase like (NDH) complex-dependent pathway is the minor one. Although our knowledge of physiological function of PSI-CET is accumulating, the contribution of PSI-CET to total electron transport is not well known. Because PSI-CET shares part of its pathway with electron transport from water to $NADP^+$ (linear electron transport), determining its activity alone is difficult.

Here, we showed that the activity of PGR5-dependent pathway is high enough to affect electron distribution from Fd to thioredoxin (Trx) system. In chloroplasts, Trx system plays an important role in reducing and activating various enzymes involved in photosynthesis and metabolic reactions. The Fd/Trx pathway accepts electrons from Fd light-dependently, while NADPH-Trx reductase C (NTRC) pathway accepts electrons from NADPH. A mutant defective in NTRC exhibits growth retardation due to a decrease in the Fd/Trx pathway-dependent activation of the Calvin-Benson-Bassham cycle enzymes. We discovered that the growth defects of the *ntrc* mutant were suppressed by the *pgr5* mutation. In the *ntrc pgr5* double mutant, the reduction level of the Calvin-Benson-Bassham cycle enzymes was partially restored. This indicated that deficiency of PGR5-dependent pathway resulted in an increased supply of reducing equivalents from Fd to the Fd/Trx pathway. We also found that overexpression of PGR5, which leads to enhancement of PGR5-dependent PSI-CET activity, lowered light-dependent activation of the Calvin-Benson-Bassham cycle enzymes. These results suggest that the PGR5-dependent PSI-CET needs to be properly regulated so as not to limit electron distribution from Fd to other metabolic reactions. The proportion of PGR5-dependent pathway to total electron transport may be more significant than expected.

Okegawa et al. (2022) Plant Cell Physiol. 63: 92-103

55 Pre Recorded Poster

Organization and photophysics of light-harvesting protein complexes studied using model lipid membranes assembled from natural plant thylakoids into 2D microarray patterns

Sophie Meredith[1], Takuro Yoneda[2], Ashley Hancock[1], Yuka Kusunoki[2], Simon Connell[1], Stephen Evans[1], Kenichi Morigaki[2], Peter Adams[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Kobe University, Kobe, Japan

The natural thylakoid membranes found in the chloroplasts of green plants contain a large network of light-harvesting (LH) pigment-protein complexes. Delineating the relationship between membrane architecture and energy transfer pathways is crucial for understanding photosynthesis and may inform the development of future nanomaterials and biophotovoltaic devices. One challenge for studying thylakoid membranes is that they are heterogeneous and unstable once extracted from chloroplasts. Other researchers have developed in vitro model systems for the study of LH membranes, but most require extensive purification, chemical alteration or removal of the protein complexes from their native membrane environment. In this presentation, I will describe the development and application of a model system termed "hybrid membranes" which provide a native-like membrane environment. Hybrid membranes are formed by self-assembly of a combination of extracted thylakoid membranes and synthetic lipid vesicles into a microscale 2D-array pattern on glass coverslips. We used these hybrid membranes as a platform to interrogate the behaviour of LH protein complexes at micro- and nanometre length scales [Meredith et al. (2021) Small 17: 2006608]. A combination of fluorescence lifetime imaging and atomic force microscopy revealed the photophysical state and lateral organization of the model membranes. The resulting model membranes were high-quality supported lipid bilayers that incorporated laterally mobile LH protein complexes. Photosynthetic activity was assessed in the hybrid membranes as compared to proteoliposomes, revealing that commonly used photochemical assays to test the electron transfer activity of photosystem II may actually produce false-positive results. In ongoing work, we are applying electric fields across hybrid membranes in order to induce the electrophoretic migration of lipids and protein complexes laterally within the membrane. This appears to be an excellent method to assess concentration-quenching behaviours. In summary, "hybrid membranes" offer several opportunities as a model system to study the behaviour of photosynthetic membrane proteins.

56 In Person Poster

Monitoring the real-time dynamics of singlet oxygen during photoprotection

Collin Steen[1,2], Jens Niklas[3], Oleg Poluektov[3], Lisa Utschig[3], Krishna Niyogi[4,2], Graham Fleming[1,2]

[1]Department of Chemistry, University of California, Berkeley, Berkeley, CA, USA. [2]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [3]Chemical Sciences and Engineering Division, Argonne National Laboratory, Lemont, IL, USA. [4]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA

The avoidance of singlet oxygen ($^1O_2^*$) and reactive oxygen species is often invoked as a central goal of photoprotective non-photochemical quenching (NPQ). Despite the well-known involvement of $^1O_2^*$ in photodamage and cell signaling, few studies have directly related $^1O_2^*$ formation to NPQ, or lack thereof. Here, we employ spin-trapping electron paramagnetic resonance (EPR) spectroscopy for detection of $^1O_2^*$ produced by photosensitization in dye-based chemical systems and use the established method to monitor the time-resolved dynamics of $^1O_2^*$ in natural chlorophyll (Chl)-containing photosynthetic membranes. We find that the apparent $^1O_2^*$ concentration in spinach thylakoid membranes changes throughout a 1-hour period of illumination. During the membrane's initial response to high light, the concentration of $^1O_2^*$ decreases in parallel with a decrease of the Chl fluorescence lifetime via NPQ. Treatment of the membranes with nigericin, an uncoupler of the transmembrane proton gradient, delayed the response of both NPQ and $^1O_2^*$. However, upon saturation of NPQ, the concentration of $^1O_2^*$ increases in both untreated and nigericin-treated membranes, presumably reflecting the utility of NPQ in mitigating photo-oxidative stress in the short term (i.e. ~15 min of high light). The findings support the conventional model of NPQ in regulating the amount of $^1O_2^*$ produced in the photosynthetic apparatus.

57 In Person Poster

Cryo-EM structure of the double ring RC-dLH core complex from *Gemmatimonas phototrophica*

Pu Qian

Materials and Structural Analysis, Thermo Fisher Scientific, Achtseweg Noord 5, 5651 GG Eindhoven, Netherlands, Eindhoven, Netherlands

Phototrophic Gemmatimonadetes, such as *Gemmatimonas (Gem.) phototrophica* strain AP64, has an ability to grow photosynthetically. This pigmented (bacteriochlorophyll *a* and carotenoid) semi-aerobic strain was initially isolated from a freshwater lake located in Gobi desert of Inner-Mongolia in China. Full genome sequencing revealed that it contains a 42.3-kb–long photosynthesis gene cluster (PGC) in its genome. This PGC was found being transferred horizontally from an ancient phototrophic proteobacterium [Zeng et al (2014) PNAS 111, 7795-7800]. The reaction center light harvesting core complex from *Gem. phototrophica* strain AP64 was isolated and purified using detergent β-DDM. Its 3D structure was determined by the use of cryo-EM single particle analysis. 2.4 Å resolution 3D structure reveals a unique, double-ring complex. The central type-2 reaction center (RC) is encircled by an inner 16-subunit light-harvesting 1 (LH1) ring, which is enclosed by an outer 24-subunit antenna ring (LHh) to form a RC-dLH complex. The complex is stabilized further by two new identified membrane-extrinsic polypeptides, RC-S and RC-U. Four different RC-dLH complexes were modeled. All of them contain RC-S subunit. However, RC-U polypeptide only is attached on the model 1b/2b, not on the model 1a/2a. The LHh ring in the model 1a is rotated 7.5 degree relative to that in the model 2a. Same conformation change was found in the model 1b and model 2b. Femtosecond kinetics revealed the flow of energy within the RC-dLH complex, from the outer LHh ring to LH1 and then to the RC. This structural and functional study shows that *Gem. phototrophica* has a new highly effective architecture for harvesting and trapping solar energy [Qian et al (2022) Sci. Adv. 8 (7), eabk3139].

58 Pre Recorded Poster

Structural change in PSII during Si-state transition analyzed by time-resolved XFEL analysis

Hongjie Li[1], Yoshiki Nakajima[1], Daichi Yamada[2], Kana Hashimoto[1], Minoru Kubo Kubo[2,3], So Iwata[3,4], Michihiro Suga[1], Jian-Ren Shen[1]

[1]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan. [2]Graduate School of Science, University of Hyogo, Hyogo, Japan. [3]RIKEN SPring-8 Center, Hyogo, Japan. [4]Department of Cell Biology, Graduate School of Medicine, Hyogo, Japan

Photosystem II (PSII) catalyzes light-induced water-oxidation through the Si-state cycle. During this process, four electrons and four protons are released from two molecules of water, and one molecule of di-oxygen is evolved. Pump-probe time-resolved serial femtosecond crystallography (TR-SFX) has been used to capture several intermediate states of PSII, deepening our understanding of the water oxidation reaction. Significantly, a newly inserted oxygen O6 (or Ox) in the vicinity of O5 was observed in the S3-state before the di-oxygen formation. However, the origin of the O6 and the mechanism to incorporate it before the progress of the S3-state remain to be elucidated. To address this issue, we analyzed the structural dynamics of PSII during the onset of Si-state transition at various time points from nanoseconds to milliseconds after one or two flash illuminations by using X-ray free electron lasers. We found concert movements of several water molecules in the O1- and O4 channels, protein residues, and even ligands, to complete the electron transfer, proton release, and water delivery process. Among them, water molecules in the O1-channel are quite movable, and some of them were dislodged in the several hundred microseconds to milliseconds time range. In particular, one water molecule close to the Ca^{2+} ion appeared in the fast time range, and disappeared later, with the concomitant increase of the O6 density. We speculate that this water is translocated through the Ca^{2+} binding site and may serve as the origin of O6. These results will provide crucial insights into the molecular mechanism of water oxidation in PSII.

59 In Person Oral

A prion-like protein from the red-type pyrenoid of *Phaeodactylum tricornutum* forms heterotypic Rubisco condensates

Zhen-Guo Oh, Warren Ang, Cheng Wei Poh, Soak-Kuan Lai, Siu Kwan Sze, Hoi-Yeung Li, Shashi Bhushan, Tobias Wunder, Oliver Mueller-Cajar

Nanyang Technological University, Singapore, Singapore

The slow kinetics and poor substrate specificity of the key photosynthetic CO_2-fixing enzyme Rubisco have prompted the repeated evolution of Rubisco-containing biomolecular condensates known as pyrenoids in the majority of eukaryotic microalgae. Diatoms dominate marine photosynthesis, but the interactions underlying their pyrenoids are unknown. Here we identify and characterize the Rubisco-linker protein PYCO1 from *Phaeodactylum tricornutum*. PYCO1 is a prion-like tandem repeat protein that localizes to the pyrenoid. *In vitro*, the protein undergoes homotypic liquid liquid phase separation (LLPS) to form spherical droplets, or condensates that specifically partition diatom Rubisco. Saturation of PYCO1 condensates with Rubisco results in immobilization of droplet components. Cryo-electron microscopy and mutagenesis data revealed the sticker motifs required for homotypic and heterotypic phase separation. Our data indicates that the PYCO1-Rubisco network is cross-linked by PYCO1 stickers that must dimerize to bind to the central solvent channel of the Rubisco holoenzyme. Pyrenoidal Rubisco condensates are highly diverse and tractable models of functional LLPS.

60 Pre Recorded Poster

Investigation into the regulatory factor of the formation of multimeric Photosystem II supercomplexes

Eunchul Kim, Asako Ishii, Chiyo Noda, Jun Minagawa

National Institute for Basic Biology, Okazaki, Japan

Photosystem II (PSII) forms supercomplexes with light-harvesting complex II (LHCII) in green plants. In vascular plants, PSII–LHCII supercomplexes further form a unique architecture called "semi-crystalline PSII array". Recently, we suggested that this semi-crystalline formation increases the light-harvesting capability but decreases the photoprotective capability of PSII–LHCII supercomplexes by investigating multimeric PSII–LHCII supercomplexes which were regarded as a fraction of the semi-crystalline array of PSII–LHCII supercomplexes [Kim and Watanabe et al. (2020) J. Biol. Chem. 295:14537-14545]. It represents that the regulation of the semi-crystalline formation is essential for efficient and safe light-harvesting of PSII–LHCII supercomplexes under various light conditions. However, the regulatory factor of the semi-crystalline formation has not yet been identified. Here, we present an investigation into the effect of LHCII phosphorylation and the presence of PsbS on the formation of multimeric PSII–LHCII supercomplexes by comparing *Arabidopsis thaliana* mutants, *stn7* and *npq4*. In addition, we investigated temperature-dependency of the formation of the multimeric PSII–LHCII supercomplexes. Our results provide insight into the regulation mechanism of the light-harvesting system through interactions between PSII–LHCII supercomplexes.

61 Pre Recorded Oral

Building the charge-separating D1/D2 assembly complexes of Photosystem II in cyanobacteria

Josef Komenda[1,2], Jana Knoppova[1], Roman Sobotka[1], Peter Nixon[3]

[1]1Institute of Microbiology of the Czech Academy of Sciences, Trebon, Czech Republic. [2]2Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic. [3]3Department of Life Sciences, Imperial College, London, United Kingdom

Photosystem II (PSII) is light-driven oxidoreductase that oxidizes water in photosynthetic membranes of plants, algae and cyanobacteria. To investigate the earliest steps of its biogenesis, we used strains of the cyanobacterium *Synechocystis* sp. PCC 6803 expressing affinity-tagged PSII subunits and lacking their early assembly partners to isolate PSII reaction center assembly complexes (RCII) and their precursor D1 and D2 modules (D1mod and D2mod). RCII preparations isolated using either a His-tagged D2 or a FLAG-tagged PsbI subunit consisted of two previously described RCII complexes differing with respect to the presence of the Ycf39 protein and high-light-inducible proteins (Hlips), and a larger complex consisting of RCII and monomeric photosystem I (PSI). This large complex was used to obtain the first detailed structure of an early assembly D1/D2-containing complex with associated Ycf48 assembly factor. All RCII complexes contained the PSII subunits D1, D2, PsbI, PsbE, PsbF, the Ycf48 factor and sub-stoichiometric amounts of rubredoxin A (RubA), PsbN, Slr1470 and the Slr0575 proteins, which all have plant homologues. The RCII preparations also contained prohibitins/stomatins of unknown function. RCII complexes were active in light-induced primary charge separation and bound chlorophylls (Chls), pheophytins, β-carotenes and heme. The characterization of isolated D1mod and D2mod indicate that neighbouring small subunits and most accessory factors bind to D1 and D2 soon after their synthesis. As stably bound chlorophyll was detected in D1mod but not D2mod, formation of RCII appears important for stable binding of most Chls and both pheophytins. We suggest that Chl can be delivered to RCII from either monomeric PSI or Ycf39/Hlip complexes and therefore we propose an existence of two different pathways for RCII formation in cyanobacteria [Knoppová et al. (2022) Plant Physiol. 189:790–804].

62 Pre Recorded Poster

Crystal structure analyses of photosystem II isolated from *Thermosynechococcus elongatus* mutants expressing only *psbA2* or *psbA3* genes as the D1 protein.

Yoshiki Nakajima[1], Natsumi Ugai-Amo[2], Naoki Tone[2], Akiko Nakagawa[3], Masako Iwai[4], Miwa Sugiura[3], Masahiko Ikeuchi[4], Michihiro Suga[1], Jian-Ren Shen[1]

[1]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama, Okayama, Japan. [2]Graduate School of Natural Science and Technology, Okayama University, Okayama, Okayama, Japan. [3]Proteo-Science Research Center, Ehime University, Matuyama, Ehime, Japan. [4]The University of Tokyo Graduate School and College of Arts and Sciences, Komaba, Tokyo, Japan

In the thermophilic cyanobacterium *Thermosynechococcus elongatus*, three psbA genes, *psbA1*, *psbA2*, and *psbA3*, are expressed as D1 proteins, and the expression of each gene is activated in response to changes in the growth environment. To clarify the functional differences of these D1 proteins, photosystem II (PSII) dimers from two mutants expressing only *psbA2* gene (PsbA2-PSII) or only *psbA3* gene (PsbA3-PSII) were purified, crystallized, and their structures were analyzed by X-ray crystallography both at 1.9 Å resolutions. It was shown that the changes of D1-Tyr147 to Phe and D1-Thr286 to Ala in PsbA2-PSII caused loss of one of the hydrogen-bonding partners for $Pheo_{D1}$ and P_{D1} (P_{680}), respectively. Furthermore, the change of D1-Pro173 to Met narrowed the Cl-1 channel close to the Mn_4O_5Ca cluster by pushing out two water molecules originally present in PsbA1-PSII, which may slow the proton transfer in some of the S-state transitions. On the other hand, in PsbA3-PSII, the hydrogen bond distance between a sulfoquinovosyl diacylglycerol (SQDG) molecule and D1-Asn287, one of the amino acids constituting the loop structure covering the Q_B binding region (Q_B loop), was shorter than that of PsbA1 due to the change from D1-Ser270 to Ala. These results suggest that the previously reported delayed S-state transition between S_2->S_3 and S_3->S_0 [Sugiura et al. (2014) *Biochim. Biophys. Acta.* 1837:1922-1931] and the slightly lower oxygen-evolving activity in PsbA2-PSII than those of PsbA1-PSII are due to the narrowing of the Cl-l channel and changes in the hydrogen bonding environment of $Pheo_{D1}$ and P_{680}. On the other hand, the structure of PsbA3-PSII suggests that the amino acid changes in D1-270 indirectly affect the hydrogen bonding environment of the Q_B loop through structural changes in the SQDG molecule, which may be responsible for the enhanced oxygen-evolving activity characteristic of PsbA3-PSII.

63 In Person Oral

Using cryo-EM to reveal a new Rubisco assembly intermediate

Stavros Azinas[1], Frida Bostrom[1], Laura Gunn[2,1]

[1]Uppsala University, Uppsala, Sweden. [2]Cornell University, Ithaca, NY, USA

Ribulose-1,5-biphosphate carboxylase-oxygenase (Rubisco) catalyses the first committed step in photosynthetic carbon fixation. Comprised of 8 large- (LSu) and 8 small-subunits (SSu), Rubisco is an important target for improving photosynthetic efficiency. Rubiscos from land plants have strict assembly requirements, and in addition to chaperonins, their biogenesis requires several chaperones, one of which is called Bundle-sheath defective-2 (BSD2). Recently, functional plant Rubisco has been successfully assembled in *E. coli* resulting in carboxylation kinetics similar to those from Rubiscos purified from plants.

In this study we used one of these systems to express and purify tobacco Rubisco, with two distinct SSu isoforms, that are differentially expressed in the plant, and investigate them via Cryo-EM. The resulting high-resolution electron density maps allowed us to build the models confidently. During our analysis, we were able to distinguish two assembly intermediates for one of the isoforms, one with a cognate BSD2 chaperone, and one intermediate containing both BSD2 and SSus, which has not been seen before. Interestingly, we observe that one of the intermediates could potentially be catalytically active. Comparisons with existing crystallographic structures reveal structural rearrangements, and differences in subunit interface interactions and disulphide bridge formation. These data allow us to expand the existing model for higher plant Rubisco biogenesis, and showcases how increasing cryoEM capabilities can be applied to the carbon-fixation problem.

64 Pre Recorded Poster

The function of the FZL protein, a dynamin-like protein localized to the grana margin, to maintain the effective photosynthesis

Yu Ogawa[1], Megumi Iwano[2], Akihiro Kawamoto[3], Lianwei Peng[4], Fumiyoshi Myouga[5], Genji Kurisu[3], Toshiharu Shikanai[6]

[1]Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama, Japan. [2]Graduate School of Biostudies, Kyoto University, Kyoto, Kyoto, Japan. [3]Institute for Protein Research, Osaka University, Suita, Osaka, Japan. [4]Shanghai Key Laboratory of Plant Molecular Sciences, College of Life Sciences, Shanghai Normal University, Shanghai, Shanghai, China. [5]RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan. [6]Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Kyoto, Japan

To approach the enigmatic question of how the thylakoid membranes are generated and maintained, we focus on one of the membrane-remodeling proteins localized to the chloroplast in Arabidopsis, termed FZO-Like (FZL). FZL is a dynamin-like protein and is considered to mediate the fusion of the lipid membrane, but its precise role in the thylakoid maintenance remains unclear. We performed the sub-fractionation of the chloroplasts and the observation of the GFP fusion protein, and found that FZL is mainly localized to the grana margin of the thylakoid. In the fzl knockout mutant, the thylakoid membrane was almost fully developed, but its architecture was disordered; the grana stacking was staggered and the connections between the grana via the stroma lamella were less clear. This observation led us to hypothesize that FZL may fuse the grana and stroma lamella at the grana margin, to form the rigid thylakoid network. We are now constructing the 3D tomography to further investigate the thylakoid architecture in fzl. Physiologically, fzl was shown to be sensitive to high light stress. The analysis of the photoinhibition and the D1 protein accumulation indicated that the photosystem II (PSII) was more sensitive to the photodamage in the fzl mutant, resulting in the faster D1 degradation. The photosensitivity of the PSII was not explained by the acceptor-side photoinhibition and further studies are underway. When the PSII repair capacity was assessed by the recovery from high light, it was normal in the fzl single mutant, but was synergistically and terribly inhibited in the fzl curt1a double mutant; CURT1 is another protein localized to the grana margin, which is also required to form the proper thylakoid structure. Speculatively, FZL and CURT1 may cooperatively maintain the grana margin, the important region for the repair of the photodamaged PS II

65 Pre Recorded Oral

A chloroplast protein atlas reveals novel structures and spatial organization of biosynthetic pathways

Lianyong Wang[1], Weronika Patena[1], Emily Singer[1], Linqu Han[2], Vivian Chen[1], Jianping Hu[2], Danny Schnell[2], Claire McWhite[1], Martin Jonikas[1]

[1]Princeton University, Princeton, New Jersey, USA. [2]Michigan State University, East Lansing, Michigan, USA

Chloroplasts are eukaryotic photosynthetic organelles that drive the global carbon cycle. Despite their importance, our understanding of their protein composition, function, and spatial organization remains limited. Here, we determined the localizations of 1,032 candidate chloroplast proteins by using fluorescent protein tagging in the model alga *Chlamydomonas reinhardtii*. The localizations provide insights into the functions of hundreds of poorly-characterized proteins, including identifying novel components of nucleoids, plastoglobules, and the pyrenoid. We discovered and further characterized novel organizational features, including eleven chloroplast punctate structures, cytosolic crescent structures, and diverse unexpected spatial distributions of enzymes within the chloroplast. We observed widespread protein targeting to multiple organelles, identifying proteins that likely function in multiple compartments. We also used machine learning to predict the localizations of all Chlamydomonas proteins. We anticipate that the strains, localization atlas, and protein-protein interactions developed here will serve as a resource to enable studies of chloroplast architecture and functions.

67 Pre Recorded Poster

Uncovering novel molecular players of sustained quenching processes induced under persistent excess of light

Maria Paola Puggioni, Jianli Duan, Alizée Malnoë

Umeå Plant Science Centre (UPSC), Department of Plant Physiology, Umeå University, Umeå, Sweden

In natural environments photosynthetic organisms are exposed to dynamic light conditions, which require continuous regulation of light harvesting and electron transport to balance the absorption and the utilization of light energy. Under excess light conditions the excess of excitation energy increases the lifetime of singlet chlorophyll and the production of reactive species, harmful for the photosynthetic machinery and cell components. Multiple photoprotection mechanisms act simultaneously to prevent oxidative stress. Among them, non-photochemical quenching (NPQ) thermally dissipates the excess of absorbed energy. NPQ has multiple components which induction and relaxation correlate with the duration and the intensity of light and the molecular players involved [Jahns et al. (2012), Biochimica et Biophysica Acta 1: 182-193; Malnoë (2018), Environmental and Experimental Botany 154:123-133]. Persistent excess of light triggers sustained quenching processes, which are slowly induced and reversed and their photoprotective nature is not yet well understood. In this work, chemical mutagenesis was performed on *Arabidopsis thaliana* mutant *npq1lut2*, which lacks both VIOLAXANTHIN DE-EPOXIDASE and LYCOPENE-CYCLASE and is therefore deficient in xanthophyll pigments zeaxanthin (Zea) and lutein accumulation [Niyogi et al. (2001), Photosynthesis Research 67:139–145], which are both involved in quenching processes. Since Zea accumulation is required in both energy-dependent quenching (qE) and Zea-dependent (qZ) [Nilkens et al. (2010), Biochimica et Biophysica Acta 4: 466-475] and its retention has been observed also during inhibitory quenching (qI) [Adams et al, (1995), Plant, Cell & Environment 2: 117-127], we aim to identify suppressors and enhancers mutants with mutations in genes involved in alternative quenching mechanisms, which will allow us to better characterize the already known sustained quenching mechanisms and possibly uncover new photoprotection components induced under persistent excess light.

68 In Person Oral

Minimum conductance in leaves—cuticle, leaky stomata, or water vapor saturation?

Jun Tominaga[1,2], Joseph Stinziano[1], David Hanson[1]

[1]University of New Mexico, Albuquerque, New Mexico, USA. [2]Hiroshima University, Hiroshima, Japan

Minimum conductance ($g_{w,min}$) in leaves is important for water relations in land plants. Yet, its regulation is unclear due to measurement constraints. Cuticle conductance to water vapor (g_{cw}) was estimated from the difference between calculated and direct measurement of CO_2 concentration in the leaf airspace (C_i) of amphi-stomatous tobacco and sunflower. We estimated g_{cw} in a series of light and dark experiments, and partitioned $g_{w,min}$ into cuticle and stomatal components. Some leaves were detached to simulate severe drought through desiccation conditions where $g_{w,min}$ is generally determined. Between light and dark experiments each g_{cw} was in close agreement, and successfully corrected the discrepancies of calculations from direct measurements. In the dark, either stomatal or cuticle conductance dominated the $g_{w,min}$, suggesting either of them can control the minimum water loss. In the detached leaves, g_{cw} could not be estimated likely due to unsaturation in the leaf airspace, and $g_{w,min}$ was progressively underestimated. Besides cuticle, leaf water status is a potential pitfall of the standard gas exchange model. Our technique is useful to study the minimal gas exchange as well as to refine the model.

69 In Person Poster

Evolutionary origin of the cryptophyte light harvesting antenna: determining the structures of unsuspected scaffolding proteins amongst the rods of the red algal phycobilisome shows the likely origin of the unique soluble cryptophyte antenna protein

Harry Rathbone[1], Katharine Michie[1], Michael Landsberg[2], Beverley Green[3], Paul Curmi[1]

[1]University of New South Wales, Sydney, NSW, Australia. [2]University of Queensland, St Lucia, QLD, Australia. [3]University of British Columbia, Vancouver, BC, Canada

Photosynthetic organisms have developed diverse antennas composed of chromophorylated proteins to increase photon capture. Cryptophyte algae acquired their photosynthetic organelles (plastids) from a red alga by secondary endosymbiosis. Cryptophytes lost the primary red algal antenna, the red algal phycobilisome, replacing it with a unique antenna composed of αβ protomers, where the β subunit originates from the red algal phycobilisome. The origin of the cryptophyte antenna, particularly the unique α subunit, is unknown. Here we show that the cryptophyte antenna evolved from a complex between a red algal scaffolding protein and phycoerythrin β. Published cryo-EM maps for two red algal phycobilisomes contain clusters of unmodelled density homologous to the cryptophyte-αβ protomer. We modelled these densities, identifying a new family of scaffolding proteins related to red algal phycobilisome linker proteins that possess multiple copies of a cryptophyte-α-like domain. These domains bind to, and stabilise, a conserved hydrophobic surface on phycoerythrin β, which is the same binding site for its primary partner in the red algal phycobilisome, phycoerythrin α. We propose that after the secondary endosymbiosis that generated the first cryptophytes, these scaffolding proteins outcompeted the primary binding partner of phycoerythrin β, resulting in the demise of the red algal phycobilisome and emergence of the cryptophyte antenna.

70 In Person Poster

D139N mutation of PsbP enhances the oxygen-evolving activity of photosystem II through stabilized binding of a chloride ion

Ko Imaizumi[1], Taishi Nishimura[1], Ryo Nagao[2,3], Keisuke Saito[4,5], Takeshi Nakano[1], Hiroshi Ishikita[4,5], Takumi Noguchi[2], Kentaro Ifuku[6]

[1]Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto, Japan. [2]Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan. [3]Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan. [4]Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan. [5]Department of Applied Chemistry, The University of Tokyo, Tokyo, Japan. [6]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

The chloride ion (Cl^-) has long been known as an essential cofactor for the light-driven water-oxidizing reaction by photosystem II (PSII). In the oxygen-evolving center (OEC) of PSII, two Cl^- ions (Cl-1 and Cl-2) specifically bind in the vicinity of the Mn_4CaO_5 cluster. However, while intensive studies have revealed the important roles of Cl-1 (associated with D2-Lys317, D1-Asn181, and D1-Glu333) in facilitating the exit of protons produced by the oxidation of water, the functional roles and importance of the other Cl^- ion, Cl-2 (associated with D1-Asn338, D1-Phe339, and CP43-Glu354), remains unknown. In the recent cryo-electron microscopy structures of green plant PSII, we noticed that a specific loop region, Loop 4, of the PsbP subunit is inserted close to the Cl-2 binding site. PsbP is known to critically support Cl^- retention in green plant PSII, and its Loop 4 region consists of highly conserved residues. Here, we have studied the importance of PsbP-Loop 4 using spinach PSII membranes reconstituted with recombinant spinach PsbP proteins harboring mutations in this region. Mutations in Loop 4 had remarkable effects on the oxygen-evolving activity of PSII. Furthermore, we discovered a specific mutation, D139N, that significantly enhances the oxygen-evolving activity of PSII. Further investigation through reconstitution experiments, light-induced Fourier transform infrared (FTIR) difference spectroscopy, and theoretical calculations suggested that the D139N mutation increased the Cl^- retention ability of PsbP through a unique structural change near the Cl-2 binding site. Considering previous reports and comparing the green plant PSII structure with cyanobacterial, red algal, and diatom PSII structures, PsbP-Loop 4 seems to both spatially and functionally replace the C-terminal region of PsbU. Our work provides insight into the functional significance of Cl-2 in the water-oxidizing reaction.

71 In Person Oral

Lipid turnover accelerates the disassembly of PSII complexes and the D1degradation during the PSII repair

Haruhiko Jimbo[1], Masato Abe[2], Hideto Miyoshi[3], Hajime Wada[1]

[1]Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan. [2]Department of Bioscience, Graduate School of Agriculture, Ehime University, Matsuyama, Japan. [3]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Thylakoid membrane consists of four main classes of glycerolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG). Crystallographic analysis of photosystem II (PSII) dimer from *Thermosynechococcus vulcanus* shows that PSII contains 20 lipid molecules per reaction center [Umena et al., (2011) 473:55–60]; however, the roles of PGs in PSII are still unclear. Many lipids associate with the D1 protein, a reaction center of PSII. D1 is rapidly turned over under strong light, thus lipids in D1 seem to be replaced as well during the repair of damaged PSII. In the present study, we investigated the effects of PG turnover on the photoinhibition of PSII. We employed a pgsA mutant of *Synechocystis* sp. PCC 6803 lacking PG synthesis and applied chemically synthesized ether-linked PGs, which cannot be metabolized in *Synechocystis* cells. One synthesized PG, which includes an ether-bond at the sn-2 position between the fatty acid chain and glycerol backbone, accelerated photoinhibition without any change in the rate of photodamage to PSII. By contrast, another synthesized PG in which the ether bond is at the *sn*-1 position, did not affect photoinhibition or photodamage. In addition, we identified a phospholipase A2 (PLA2) that deacylated fatty acids at sn-2 position in PG molecules and dissociated CP43 from the purified PSII monomer in vitro. D1 degradation is inhibited in *pgsA* cells containing ether-bound PG molecules and the mutant lacking *pla2*. Therefore, PG is degraded at the *sn*-2 position to induce CP43 dissociated from PSII complex to enhance the D1 degradation in the PSII repair. We also identified a galactolipase A1 (GLA1) that deacylates fatty acid at *sn*-1 position of MGDG and DGDG. GLA1 disassembled PSII dimer into monomers and enhanced D1 degradation as well. These results show the importance of lipid turnover as new steps in the PSII repair.

72 In Person Poster

Elucidating the oligomerisation mechanism of *Methanococcoides burtonii* Rubisco (MbR)

Afshan Begum[1], Alexander Nasedkin[1], Laura Helen Gunn[1,2]

[1]Department of Cell and Molecular biophysics, Uppsala University, Sweden, Uppsala, Uppsala, Sweden. [2]Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States, Ithaca, New York, USA

Ribulose-1,5-carboxylase/oxygenase (Rubisco) catalyses the addition of CO_2 (carboxylation) or O_2 (oxygenation) of D-ribulose-1,5-bisphosphate (RuBP), subsequent carbon-carbon cleavage forms two molecules of 3-phospho-D-glycerate (PGA) or one molecule of PGA and one molecule of 2-phospho-glycolate, for carboxylation and oxygenation, respectively. This makes Rubisco both the gatekeeper for carbon entry into the biosphere and a target for functional improvement to enhance photosynthesis and plant growth (1). Rubisco's catalytic inefficiencies often limit plant growth and resource-use-efficiency (2). Improving the performance of the CO_2-fixing enzyme Rubisco has the potential to significantly enhance the efficiency of photosynthetic carbon assimilation.

Strategies to engineer more efficient plant Rubiscos have been hampered by evolutionary constraints, prompting interest in Rubisco isoforms from divergent Rubisco lineages. The methanogenic archaeon *Methanococcoides burtonii* Rubisco (MbR) isoform functions to scavenge the RuBP by-product of purine/pyrimidine metabolism (2). The MbR large (L-) subunits assemble into functional dimers (L2). Further assembly into pentamers of L2 (L10) occurs upon binding of either the natural substrate, RuBP, or the reaction intermediate analogue, 2-carboxy-D-arabinitol 1,5-bisphosphate, is facilitated by the Rubisco Assembly Domain (RAD) (2).

In this study, we probe the oligomerisation mechanism of MbR. We present 6 new high-resolution crystal structures of wild-type MbR and an oligomerisation-incapable mutant, and combine these data with biochemical assays with different metals and ligands, biophysical characterisation, small angle X-ray scattering (SAX), and Molecular Dynamics simulations. These reveal that MbR could break the current rules of Rubisco chemistry and provide insights into the unique MbR oligomerisation mechanism, especially highlighting the link between the Rubisco active site and the RAD. Understanding this mechanism could allow utilisation of the RAD in Rubisco engineering strategies (3).

[Wilson R.H., Hernan A. and Whitney S.M. (2016) Sci. Rep. 6:22284; Gunn L.H. Valegård K. and Andersson I. (2017) J. Biol. Chem. 292:6838-6850; Wachter R.M. (2017) J. Biol. Chem. 292:6851-6852]

73 Pre Recorded Poster

Exploring the pH-sensing mechanism and membrane interactions of the light-stress regulator protein PsbS and in vitro-interaction of PsbS and PsbS site-selective mutants with LHCII

Willem Marulanda Valencia, Anouska van Troost, Anjali Pandit

Biophysical Organic Chemistry/ssNMR dept, Leiden Institute of Chemistry, Leiden University, Einstenweg 55, 2333 CC Leiden, Netherlands

At high light conditions an excess of energy can cause photo-oxidative damage in the plant. The process to dissipate excess energy as heat is called non-photochemical quenching (NPQ). qE, the fastest NPQ mechanism, is carried out by the pH-sensing protein Photosystem II Subunit S (PsbS) and the xanthophyll cycle. Early work demonstrated that PsbS is essential for qE [Li et al. (2000) Nature: 391-395]. A low luminal pH is sensed by Glu69 (E1) and Glu173 (E2) which triggers a change in the structure of the lumen amphipathic helical stretches. It was found that the amphiphatic 3-10 helix containing Glu69 unfolds at high pH while the amphipatic helix containing Glu173 moves from the membrane to the water phase [Krishnan et al. (2021) Nat. Commun. 12; Liguori et al. (2019) J. Phys. Chem. Lett. 10: 1737-1742]. PsbS has been hypothesized to associate with Light-Harvesting Complex II (LHCII) and facilitate fluorescence quenching at low pH. Molecular understanding of the PsbS pH sensing mechanism can help optimize photosynthetic yield.

To investigate the role of PsbS helix movement at low pH, we produced recombinant wildtype PsbS and the mutant E173Q from *Spinacia oleracea* and will investigate its interaction with LHCII in liposome membranes. The E173Q mutant was found to be ‘locked’ in a low-pH conformational state, without pH-dependent movement of the helix containing Glu173 [Krishnan et al. (2021) Nat. Comm. 12]. By comparing LHCII fluorescence quenching in the presence of wildtype or E173Q PsbS, we aim to understand the role of amphipatic helix movements on establishing functional inter-protein interactions.

74 Pre Recorded Poster

Exploring the pH-sensing mechanism of PsbS by measuring structural changes under different pH conditions.

Anouska van Troost, Willem Marulanda Valencia, Anjali Pandit

Leiden Institute of Chemistry, Leiden University, Leiden, Zuid-Holland, Netherlands

During photosynthesis light energy is converted to chemical energy. However, at excess light, photo-oxidative damage can occur in the plant. To prevent damage the plant dissipates excess energy as heat in a process called non-photochemical quenching (NPQ). In higher plants the pH-sensing protein Photosystem II subunit S (PsbS) protein is a key player in sensing light stress by changes in luminal pH. This change is sensed by two glutamates (E69 and E173) that induce conformational changes upon protonation. Understanding of the molecular mechanism of PsbS will create new possibilities for optimization of biomass production via adjustment of NPQ.

This project aims to look at the conformational changes of spinach and *Arabidopsis* PsbS in response to pH. Hereto recombinant PsbS will be refolded in detergent micelles and model membranes. 13C, 15N MAS NMR measurements will be executed in combination with isotopic labeling of several key residues. As a first step, we will determine the experimental pH range for protonation of pH-responsive residues in spinach PsbS, using NMR spectroscopy for probing glutamate and aspartate protonation states.

75 In Person Poster

Photosynthesis and carbon use strategies for surviving at sub-desert coastal scrub in Tenerife

Laura Díaz-Jiménez, Alicia V. Perera-Castro, Yauci Espinosa-González, Celine I. García-Rodríguez, Beatriz Fernández-Marín, Jaime Puértolas, Águeda M. González-Rodríguez

Department of Botany, Ecology and Plant Physiology, University of La Laguna, La Laguna, Canary Islands, Spain

Tenerife Island (Canary Islands, Spain) is an ideal scenario for studying the diverse mechanism of adaptation in extremophilic plants. Due to the island's exposure to the trade winds on the north slope and the varying elevation of the territory, it is possible for diverse ecosystems to be established with a wide variety of conditions of humidity and temperature. All along the island's coastline, both on the northern and southern slopes, grows the sub-desert coastal scrub with species such as *Euphorbia balsamifera*, *E. lamarckii*, *Salsola divaricata*, *Schizogyne sericea* or *Kleinia neriifolia*, alongside many others. This specific coastal environment is defined by its low annual precipitation in addition to high temperatures and solar irradiance, that force the species to acquire certain adaptative strategies for surviving in these conditions. Although they are cohabiting species, they have adapted in very different ways (e.g., C4 and facultative CAM metabolism, leafless periods, presence of trichomes) that have a direct result in the general metabolism of these plants. During this year, seasonal measurements of the photosynthetic rate and hydric status of the most representative species were taken in two locations of the island (windward and leeward), which are dominated by this ecosystem. With these long-term observations of gas exchange, annual carbon balance and phenology, we have been able to estimate and compute the cost of having one adaptative strategy over another in terms of total carbon uptake. Which one will be the most economic?

76 In Person Poster

Molecular characterization of fucoxanthin-chlorophyll *a/c* proteins in the diatom *Chaetoceros gracilis*: the unique diversification process of the light-harvesting complexes in red-algal lineage.

Minoru Kumazawa[1], Hiroyo Nishide[2], Ryo Nagao[3], Natsuko Inoue-Kashino[4], Ikuo Uchiyama[2], Yasuhiro Kashino[4], Jian-Ren Shen[3], Takeshi Nakano[5], Kentaro Ifuku[1]

[1]Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto, Japan. [2]National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Aichi, Japan. [3]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama, Okayama, Japan. [4]Graduate School of Science, University of Hyogo, Kamigohri, Hyogo, Japan. [5]Graduate School of Biostudies, Kyoto University, Kyoto, Kyoto, Japan

Diatoms belong to a group of photosynthetic Stramenopiles (or Heterokonts), which are secondary symbiotic algae with plastids derived from red algae. The light-harvesting pigment-protein complex (LHC) in diatoms is called fucoxanthin chlorophyll *a/c* binding protein (FCP) based on its photosynthetic pigment composition (e.g., chlorophyll *a/c*, fucoxanthin, diatoxanthin and diadinoxanthin), which effectively absorb light including blue-green region wavelengths in aqueous environments. It has been reported that diatoms have many genes encoding various-types of the FCPs; however, their functional analysis has not been completed yet. In this study, we identify 46 FCP genes in the newly assembled genome and transcriptome of *Chaetoceros gracilis*, a centric diatom: the genome of *C. gracilis* contains ~15,000 prediced genes. Molecular phylogenetic analyses suggest that the FCPs of *C. gracilis* can be classified into five subfamilies: Lhcr, Lhcf, Lhcx, Lhcz, and a novel Lhcq, in addition to a distinct type of Lhcr, CgLhcr9. The FCPs in Lhcr, including CgLhcr9 and some Lhcqs, have orthologous proteins in other diatoms, particularly those found in the PSI–FCPI supercomplex. By contrast, the FCPs in the Lhcf subfamily, some of which were found in the PSII–FCPII structure, seems to be diversified in each diatom species, and the number of Lhcqs differs among diatom species. These findings indicate that the difference in the FCP composition may contribute to species-specific adaptations to light. Further phylogenetic analyses of the FCP and LHC proteins, using genome data and assembled transcriptomes of other diatoms and microalgae in public databases, suggest that the above classification of FCPs is common among various red-lineage algae derived from secondary endosymbiosis of red algae, including Haptophyta. These results provide insights into the loss and gain of FCP/LHC subfamilies during the evolutionary history of the red algal lineage.

[Kumazawa et al. (2022), Physiol. Plant., e13598, 174(1)]

77 Pre Recorded Poster

The molecular pH-response mechanism of the plant light-stress sensor PsbS

Maithili Krishnan[1], Patrick Konold[2], John Kennis[2], Anjali Pandit[1]

[1]Leiden University, Leiden, Netherlands. [2]VU Amsterdam, Amsterdam, Netherlands

Plants need to protect themselves from excess light, which causes photo-oxidative damage and lowers the efficiency of photosynthesis. Photosystem II subunit S (PsbS) is a pH sensor protein that plays a crucial role in plant photoprotection by detecting thylakoid lumen acidification in excess light conditions via two lumen-faced glutamates. However, how PsbS is activated under low-pH conditions is unknown. To reveal the molecular response of PsbS to low pH, we performed an NMR and infrared spectroscopic analysis of PsbS and of a E176Q mutant in which an active glutamate has been replaced. The PsbS response mechanism at low pH involves the concerted action of repositioning of a short amphipathic helix containing E176 facing the lumen and folding of the lumenal loop fragment adjacent to E71 to a 310-helix, providing clear evidence of a conformational pH switch. We propose that this concerted mechanism is a shared motif of proteins of the light-harvesting family that may control thylakoid inter-protein interactions driving photoregulatory responses.

We aim to further explore the pH-response mechanism of wildtype and mutant PsbS by investigating the pH-dependent changes in protonation state and protein fold, and its relationship with functional protein-protein interactions. Hereto we will set up ^{13}C-MAS NMR experiments and study wildtype and mutant PsbS co-reconstituted with LHCs in model membranes.

78 In Person Poster

Preparation of a functional core complex of green sulfur bacterial reaction center

Tomomi Inagaki, Kazuki Terauchi, Chihiro Azai

Graduate School of Life Sciences, Kusatu, Shiga, Japan

The photosynthetic reaction center complex of green sulfur bacteria (Gsb-RC complex) consists of five proteins: PscA, PscB, PscC, PscD and FMO. Two PscA-PscC dyads forms a rigid homodimer, which is a membrane-spanning core complex and will have a perfect two-fold symmetry axis. PscB, PscD and FMO are water-soluble proteins and peripheral subunits. The recently reported cryo-EM structure revealed that each one molecule of PscB, PscD, and trimeric FMO are bound onto the same hydrophilic surface of the core subunit. Therefore, the overall structure of the Gsb-RC complex is asymmetric by binding of the water-soluble proteins. The goal of our study is to judge the impact of the asymmetric binding of the peripheral subunits on electron transfer reactions inside the core complex. In the present study, we explored mild conditions to strip out the peripheral subunits from the Gsb-RC complex.

We investigated effect of two different treatments, increase of NaCl and change of pH, on an isolated Gsb-RC complex of *Chlorobaculum tepidum*. Higher than 1 M of NaCl concentration leaded to dissociation of PscD and most of FMO from the complex, but few of PscB. By raising pH, however, all the water-soluble proteins were detached. All the dissociation events were reversible and a fully functional Gsb-RC complex was recovered by desalting and pH neutralization. We tracked electron transfer reactions in the core complex stripped all the peripheral subunits by transient absorption spectroscopy and found that dissociation of the peripheral subunits affected not only forward electron transfer mediated by the iron-sulfur cluster, FX, but also electron donation from cytochrome cz of PscC to the photo-oxidized primary electron donor, P840+. Our findings suggest that a negative electrostatic surface potential of PscB is responsible for binding of all the peripheral subunits and binding of PscB tunes the electron transfer reactions of the core complex.

79 In Person Poster

QM/MM study of the role of valine 185 in the oxygen-evolving center of photosystem II

Mitsuo Shoji[1], Koichi Miyagawa[1], Kizashi Yamaguchi[2]

[1]University of Tsukuba, Tsukuba, Ibaraki, Japan. [2]Osaka University, Suita, Osaka, Japan

Photosynthetic water oxidation occurs at the Mn cluster of the oxygen-evolving complex in photosystem II. Valine 185 in the D1 protein (D1-V185) located at the outside of the first coordination of the Mn cluster is one of the highly conserved amino acids and is important for the water splitting reaction. In order to elucidate the explicit role of the D1-V185 and insights to the water oxidation reactions, hybrid quantum mechanics/molecular mechanics (QM/MM) simulations were performed for the wild type and threonine mutant (D1-V185T). We found that the hydrogen bond network around T185 is changed upon the D-V185T mutation by the hydrophilic side chain of D1-T185. The present QM/MM results suggest that the hydrophobic covering of the Mn cluster by D1-V185 is one of the key elements for natural photosynthesis to achieve the efficient water-splitting reaction.

80 In Person Poster

Anatomical and physiological determinants of water use efficiency in the C_4 crop sorghum

Yazen Al-Salman[1], Francisco Javier Cano[2], Ling Pan[3], David Jordan[4], Oula Ghannoum[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Sydney, NSW, Australia. [2]Centro de Investigación Forestal, Instituto Nacional de Investigacion Tecnologia Agraria Alimentaria, Madrid, Spain. [3]College of Forestry, Hainan University, Haikou, Hainan, China. [4]Hermitage Research Facility, The University of Queensland, Warwick, QLD, Australia

Rising global temperatures will increase evapotranspiration and compromise leaf intrinsic water use efficiency (*iWUE*). Due to their CO_2 concentrating mechanism (CCM), C_4 crops achieve nearly CO_2-saturated photosynthesis rate (A_n) at a lower stomatal conductance (g_s), increasing their *iWUE*. Hence, avenues other than improving A_n are needed to improve *iWUE*, including reducing g_s without compromising A_n, which requires a greater understanding of what controls g_s in C_4 plants. Building on recent work linking *iWUE* with leaf width (*LW*), we sought to identify leaf anatomical traits that underpin the correlation between *LW* and *iWUE* in ten sorghum lines grown under three different temperatures. We subsequently grew a subset of six lines in growth chambers to investigate the common stomatal traits which control both diurnal variation in *iWUE* and dynamic *iWUE* (measured following step-change in light conditions). Leaf steady-state and dynamic gas exchange, together with surface and internal cross-sectional anatomy, were determined. Narrower leaves had lower stomatal conductance (g_s) and higher *iWUE* across growth temperatures. Narrower leaves had smaller stomatal size (*SS*), surface area of intercellular airspaces, mesophyll thickness and bundle sheath and mesophyll cell size. Structural modelling showed that the developmental association between *LW* and anatomy is underpinned by *SS*. Sorghum lines that maintained high morning and midday *iWUE* exhibited faster stomatal closure but not faster opening, favouring water conservation over maximising photosynthesis. Stomatal kinetics were mainly regulated by aperture response rather than structural traits. In conclusion, the anatomical arrangement of narrow leaves in C_4 sorghum ensures efficient hydraulic supply to the substomatal spaces via reducing outside-xylem hydraulic path, allowing for precise control of the stomatal pore. Stomatal aperture dynamics were also the main determinants of stomatal kinetic responses, and underpinned the association of rapid stomatal closure with increased diurnal *iWUE*.

81 Pre Recorded Poster

Structural and spectroscopic analysis of qH-dissipative antennae in Arabidopsis

Aurélie Crepin, Pierrick Bru, Alizée Malnoë

Umeå Plant Science Centre (UPSC), Umeå, Sweden

Photoprotection in plants is generally divided in an array of “quenching” mechanisms affecting fluorescence emission. The most studied and described of these mechanisms generally represent short- or mid-term acclimation to changes in conditions. However, recently, an increasing number of studies have brought to light various mechanisms of “sustained quenching”, which affect photosynthesis with activation and relaxation timescales of tens of minutes to hours. One such mechanism, termed qH, has been shown to be independent of most well-known quenching pathways, as its activation requires neither lumen acidification, nor xanthophyll pigments, or, as far as is known, phosphorylation. Instead, several effectors have been described, whose exact role in qH remains to be characterized. These include LCNP, a lumenal lipocalin, SOQ1, a membrane multi-domain protein that operates upstream of LCNP and prevents quenching, and ROQH1, a possibly NADPH-dependent dehydrogenase-reductase, involved in qH relaxation.

Recently, quenched LHCII trimers (the main antenna of photosystem II) with active qH have been isolated directly from the model plant *Arabidopsis thaliana*. This very stable, native dissipative state does not depend on a single isoform of Lhcb1-2-3 proteins, composing the LHCII.

Here, we push further the investigation of the changes underlying the formation of this quenched state in plant antenna proteins. Using a combined approach of biochemistry, biophysics and structural biology, we explore the changes brought to LHCII trimers in qH conditions that could be at the origin of the dissipative state.

82 Pre Recorded Poster

How rocks shift the limits of photosynthesis for cyanobacteria in the Namib Desert

Michal Gwizdala[1,2], Pedro H. Lebre[3], Gillian Maggs-Kölling[4], Eugene Marais[4], Don A. Cowan[3], Tjaart P. J. Krüger[1,2]

[1]Department of Physics, University of Pretoria, Pretoria, South Africa. [2]Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. [3]Centre for Microbial Ecology and Genomics, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa. [4]Gobabeb – Namib Research Institute, Walvis Bay, Namibia

Despite its beauty, the Namib Desert in western Namibia is a hostile habitat. Not many photosynthetic organisms thrive in this hyper-arid, extremally hot and exposed to the full solar radiation environment. Cyanobacteria in the Namib Desert are largely restricted to hypolithic habitats – they occupy the ventral surfaces of translucent quartz pebbles in desert pavements. This unique sub-lithic habitat defines the environmental conditions for hypolithic organisms and is reflected in their strategies to cope with this extreme environment.

During our research expeditions to the Namib Desert, we combined fluorometric, spectroscopic, biochemical and metagenomic approaches to investigate the light transmission properties of quartz stones and to assess the photosynthetic and photoacclimatory activity of the underlying hypolithic cyanobacterial biofilms. We aimed to provide a complete characterisation of hypolithic photosynthesis without moving hypoliths out of the desert.

We showed that quartz pebbles greatly reduced the total photon flux to the ventral surface biofilms and filtered out primarily the short wavelength portion of the solar spectrum. Cyanobacteria were the only photosynthetic organisms in hypoliths in the Namib Desert, and chlorophylls d and f were not detected in biofilm pigment extracts. Hypolithic cyanobacterial communities showed some other evidence of adaptation to sub-lithic conditions, such as the prevalence of genes encoding Helical Carotenoid Proteins, which were suggested to be associated with desiccation stress. Under water-saturated conditions, hypolithic communities showed no evidence of light stress, even when the quartz stones were exposed to full midday sunlight. Hypolithic cyanobacteria in the Namib Desert do not use the Far-Red Light Photoacclimation (FaRLiP) or Complementary Chromatic Adaptation to respond to light. This work adds to the understanding of mechanisms behind the unique robustness of photoautotrophic organisms in extreme environments.

Gwizdala et al. (2021) Environ. Microbiol. 23:3867-3880

83 Pre Recorded Oral

Switching photoprotection on and off in a single phycobilisome

Michal Gwizdala[1,2], Joshua Botha[1], Adjélé Wilson[3], Diana Kirilovsky[3], Rienk van Grondelle[4], Tjaart Krüger[1,2]

[1]University of Pretoria, Pretoria, Gauteng, South Africa. [2]Forestry and Agricultural Biotechnology Institute, Pretoria, Gauteng, South Africa. [3]Centre National de la Recherche Scientifique, Gif-sur-Yvette, France. [4]Vrije Universiteit Amsterdam, Amsterdam, Netherlands

A sudden increase in light intensity can be lethal for photosynthetic organisms. Therefore, cyanobacteria, like other oxygenic photoautotrophs, use various strategies to cope with these unexpected fluctuations. In many cyanobacteria, the deadly effects of an increase in light intensity are mitigated by the interaction between the photosynthetic light-harvesting complexes called phycobilisomes (PBs) and the Orange Carotenoid Protein (OCP). OCP senses high light intensities through photoactivation, binds to the core of a PB, and triggers thermal energy dissipation from the PBs. When the OCP-related photoprotective mechanism is on, photosynthetic reaction centres are protected from excessive illumination.

Due to the brightness of their emission, the properties of PBs can be investigated at the level of individual complexes using Single Molecule Spectroscopy (SMS). We showed that energy dissipation from individual PBs can be reversibly switched on and off using only light and OCP. Thanks to SMS, we revealed the presence of quasi-stable intermediate states during the binding and unbinding of OCP to PB, with a spectroscopic signature indicative of transient decoupling of some of the PB rods during docking of OCP [Gwizdala et al. (2018) J. Phys. Chem. Lett. 9:2426-2432]. Real-time control of emission at the level of individual PBs has allowed us to improve our understanding of the molecular foundations of the photoprotective strategies used by cyanobacteria.

84 Pre Recorded Poster

Dynamics of ChlF, plastocyanin, P700, and ferredoxin in fluctuating light

Yuxi Niu[1], Shizue Matsubara[1], Dušan Lazár[2], Silvia D. Schrey[1], Ladislav Nedbal[2]

[1]Institute of Bio- and Geosciences/Plant Sciences, Forschungszentrum Jülich, Jülich, Germany. [2]Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic

Light fluctuations occurring in natural plant canopies can be deconvoluted into elemental harmonic components [Nedbal J. et al. in these Proceedings]. Experimental approach to study response of photosynthetic machinery to harmonically oscillating light was introduced in [Nedbal L. and Březina V. (2002) Biophys. J. 83:2180-2189] and further developed in [Nedbal L. and Lazár D. (2021) Plant Physiol. 187:646-661]. Responses of *Arabidopsis thaliana* to oscillating light with periods between 1s and 8min were reported in [Niu Y. et al. (2022) bioRxiv. doi:10.1101/2022.02.09.479783].

Here, we offer a detailed description of chlorophyll fluorescence (ChlF), plastocyanin, P700, and ferredoxin dynamics measured in light that was oscillating with periods of 30s and 4min. The responses to light oscillations were measured using the Dual-KLAS-NIR spectrometer with *A. thaliana* wild types and the *npq1* mutant lacking the violaxanthin de-epoxidase, the *npq4* mutant lacking the PsbS-protein, and the mutants *crr2-2*, and *pgrl1ab* impaired in the NDH-like and PGRL1-PGR5-dependent pathways of the cyclic electron transport.

The amplitude of the oscillating-light-driven variation of ChlF in wild types was strongly damped by non-photochemical quenching for oscillation with periods of 4min, but less so for fast oscillations with periods of 30s. Regardless of period, P700 oxidation mostly followed the light modulation. Plastocyanin signals were not always synchronized with P700, instead a clear trough occurred when the light approached its maximum for oscillation with periods of 4min. The absence of the PGRL1-PGR5-dependent pathway affected the dynamics of all components, with P700 failing to follow light changes, whereas the absence of the NDH-like dependent pathway resulted in a milder effect to all signals.

Plants do not need to be dark-acclimated for such measurements, which is an advantage for field and greenhouse applications. We therefore propose that the frequency domain measurements presented here provide a new way to characterize dynamics of photosynthesis in light-acclimated plants.

85 Pre Recorded Poster

Strong plasmonic fluorescence enhancement of the main plant light-harvesting complex: experiment and theory

Farooq Kyeyune, Luke Ugwuoke, Joshua Botha, Bertus van Heerden, Tjaart Krüger

University of Pretoria, Pretoria, Gauteng, South Africa

Plasmonic coupling of metallic nanoparticles and adjacent pigments can dramatically increase the brightness of the pigments due to the enhanced local electric field. Here, we demonstrate that the fluorescence brightness of individual LHCII complexes of plants can be enhanced by two orders of magnitude when coupled to a gold nanorod (AuNR) [Kyeyune et al. (2019) Nanoscale 11:15139–15146]. The significant enhancement was achieved by carefully tailoring the longitudinal localized surface plasmon resonance of chemically synthesized AuNRs to match the absorption and emission bands of LHCII. The fluorescence enhancement was accompanied by a shortening of the fluorescence lifetime of up to two orders of magnitude. Using analytical expressions based on classical plexcitonics, we investigated the effect of the local near-field of a gold nanorod on the excitation rate enhancement of a single LHCII placed near the tip of the nanorod [Ugwuoke et al. (2021) Proc. SPIE: 11661E-1–7]. This enabled us to model a single LHCII as a dielectric nanoparticle whose Q_y ground-state transition follows two Lorentzian functions. We show that the LHCII-nanorod distance-dependent resonant coupling of the longitudinal localized surface plasmon resonance of the nanorod to the Q_y band of LHCII leads to strong exciton-plasmon coupling, responsible for the formation of plexcitonic resonances in the hybrid system. The analytical results of our model system are in good agreement with experimental results.

86 Pre Recorded Poster

Is 1+1=2 true for photosynthesis in fluctuating light?

Jakub Nedbal[1], Martin Havelka[2], Dušan Lazár[3], Ladislav Nedbal[3]

[1]16 Salisbury Rd, London E12 6AB, United Kingdom. [2]Gymnázium, Třeboň, Czech Republic. [3]Palacký University, Olomouc, Czech Republic

Plant photosynthesis is an inherently non-linear process. A trivial non-linearity is due to the saturation of photosynthetic reactions by high irradiance. The regulatory non-linearity depends in a complex way on light intensity and on the rate of the light change [Nedbal L. and Lazár D. (2021) Plant Physiol. 187:646-661 37:507-514]. Due to the regulatory non-linearity, a plant's response to a combination of two fluctuating light patterns cannot be directly deduced from responses to the same light patterns that would be applied separately. Similar to other complex systems, the non-linear dynamics of plants may offer attractive opportunities, e.g., potentially suppress, or enhance regulation and, by this, influence growth or stress resilience [Niu Y. et al. (2022) https:// .biorxiv.org/content/10.1101/2022.02.09.479783v2; Prigogine I. and Stengers I. (2018). Order out of chaos: Man's new dialogue with nature. Verso Books.].

A comprehensive mathematical model of photosynthesis was used to predict the dynamic responses of plants to simultaneously or separately applied oscillatory lights. The results of the model were confronted with ChlF yield measurements that were done on a leaf of *Actinidia deliciosa* using a laboratory-built PAM fluorometer. The measuring protocol always contained five 40s-long periods of oscillatory actinic light. This fundamental light pattern was combined with a constant light of varying intensity, or with additional actinic light that was oscillating with periods of 2.5s, 25s, or 50s.

The results revealed strongly non-linear features appearing in response to oscillations of low amplitudes where, according to conventional models, photosynthesis would be expected to be linearly proportional to light. Thus: 1+1≠2 when the light incident on a plant leaf fluctuates. Consequently, the time-domain, Kautsky-type experiments, and the frequency-domain measurements are expected to yield non-identical, partially complementary information: the first on plants in transition between dark- and light-acclimated states, and the latter on dynamics in light-acclimated plants.

87 Pre Recorded Oral

Ethylene inhibits photosynthesis via temporally distinct physiological, morphological, and molecular responses in tomato

Petar Mohorović[1], Marilien Rinia[1], Batist Geldhof[1], Kristof Holsteens[1], Stijn Daems[2], Timmy Reijnders[3], Johan Ceusters[2], Wim Van den Ende[3], Bram Van de Poel[1]

[1]Molecular Plant Hormone Physiology Lab, Division of Crop Biotechnics, Department of Biosystems, KU Leuven, Leuven, Belgium. [2]Department of Biosystems, Bioengineering Technology TC, Campus Geel, KU Leuven, Geel, Belgium. [3]Molecular Biotechnology of Plants and Microorganisms Lab, Department of Biology, KU Leuven, Leuven, Belgium

Ethylene is a volatile plant hormone that regulates many developmental processes such as germination, hypocotyl and root elongation, climacteric fruit ripening, senescence, and responses towards (a)biotic stress. Ethylene can also inhibit photosynthesis and repress vegetative growth, eventually leading to unwanted yield losses in many important crops, including tomato.

To evaluate the temporal effects of ethylene on the photosynthetic performance of tomato plants, we used an automated ethylene gassing system and monitored photosynthesis-associated physiological, biochemical, and molecular responses. Using real-time CO_2 measurements under controlled environmental conditions, we observed that ethylene evoked a dose-dependent inhibition of plant CO_2 assimilation.

One of the earliest responses to ethylene was leaf epinasty (downward bending of leaves) and the accompanying drop in plant canopy cover, which occurred already a few hours after the start of the ethylene gas treatment. Together with a rapid decline in stomatal conductance, followed by a drop in transpiration rates, plant CO_2 assimilation rates plummeted. Concomitantly, our spectrophotometric assays showed that Rubisco activity decreased throughout the course of ethylene treatment, reflecting the observed drop in CO_2 consumption/assimilation rates. These changes were followed by a significant decrease in photosynthetic assimilates, as glucose and fructose levels continued to gradually decline over time. On the other hand, starch content only decreased after 56 hours of treatment, which corresponded with an increase in sucrose levels, indicating that ethylene gradually activates starch breakdown and sugar mobilization, leading to a shift in carbon partitioning in tomato leaves. Eventually, after 3 days of ethylene treatment, chlorophyll fluorescence (quantum efficiency of PSII) and chlorophyll content declined, indicating the onset of senescence.

We are currently analyzing a temporal RNA-seq dataset to get more insight into the effect of ethylene on the transcriptional dynamics of photosynthesis-related genes.

88 In Person Poster

Regulation of non-photochemical quenching in *Chlamydomonas* by orthologs of HY5 and CONSTANS

Gabriella Benko[1], Setsuko Wakao[1,2], Krishna Niyogi[1,2,3]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA. [2]Molecular Biophysics and Integrated Bioimaging, Division Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA, USA

Plants and algae use non-photochemical quenching (NPQ) mechanisms to safely dissipate excess absorbed light energy as heat. The model green alga, *Chlamydomonas reinhardtii*, employs multiple NPQ mechanisms to minimize damage caused by excess light, and the NPQ capacity of the cells is induced by growth in high light (HL). However, the signaling pathway from light perception to expression of NPQ-related proteins is just beginning to be elucidated. Here, we demonstrate that two highly conserved green-lineage transcription factors, ELONGATED HYPOCOTYL5 (HY5) and CONSTANS (CO), are both involved in the induction of NPQ capacity in *Chlamydomonas*. The *Chlamydomonas* mutants, *crhy5* and *crco*, generated by CRISPR-Cas9 editing and insertional mutagenesis, respectively, are differentially affected in the induction of NPQ under HL conditions. While *crco* lacks most NPQ, *crhy5* displays a partial induction of NPQ capacity after six hours of exposure to HL. A double mutant of *crhy5* and *crco* is unable to induce NPQ, similar to *crco*, suggesting that HY5 and CONSTANS may function in the same signaling pathway. The three transcription factor mutants (*crhy5*, *crco*, and *crhy5 crco*) will be used in an RNA-seq time course to further investigate their regulatory roles and direct target genes under HL conditions. These data indicate distinct regulatory functions of these transcription factors in Chlamydomonas versus plants, where HY5 and CONSTANS control photomorphogenesis and flowering time, respectively.

89 Pre Recorded Poster

The effect of D1-E329 mutations on the substrate water exchange in Photosystem II

Abuzer Orkun Aydin[1], Casper de Lichtenberg[2], Petko Chernev[1], Feyian Liang[3], Ann Magnuson[1], Peter Lindblad[1], Johannes Messinger[1]

[1]Molecular Biomimetics, Department of Chemistry, Uppsala University, Uppsala, Sweden. [2]Department of Biology, Copenhagen Biocenter, University of Copenhagen, Copenhagen, Denmark. [3]Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

Biological water oxidation is catalyzed by the $Mn_4CaO_{5/6}$ cluster in Photosystem II. It is well established that molecular water is evolved following the Kok-Cycle through five oxidation states, S0-S4. EPR signals and DFT calculations indicate equilibria between two or more conformations in the different S states, and various water insertion pathways during the S2-S3 transition remain possible, allowing proposals for various pathways for the water splitting. To move forward, one needs to identify the binding sites and modes of the fast (W_f) and slow (W_s) substrate waters required to form O_2. Previous time resolved membrane-inlet mass spectroscopy (TR-MIMS) and ^{17}O-EDNMR investigations favor the central oxygen bridge, O5, as W_s while W2 or the Ca-ligand W3 remain possible options for Wf. Recent in situ XFEL studies on flash-advanced PSII crystals revealed that a new water Ox is inserted between Ca and Mn1 in S2 to S3 transition. Additionally, a high-water mobility is observed in the O1 channel, which leads to a water wheel in close proximity to Ca atom and Y_Z network. Our subject of investigation, D1-E329 resides on a gateway position of this pathway and it was shown to be part of an extended H-bond network around the manganese cluster. To gain further understanding on the role of the O1 channel for substrate water delivery, we investigated substrate water exchange kinetics of *Synechocystis* sp. PCC 6803 D1-E329 mutants using TR-MIMS. Additionally, EPR spectroscopy and fluorescence decay measurements were employed to gain insight about the conformational heterogeneity of the $Mn_4CaO_{5/6}$ cluster and electron transfer kinetics.

90 In Person Poster

What photosynthesis can tell us about post-fire alpine shrub dynamics

Alicia V Perera-Castro[1], Laura Díaz-Jiménez[1], Laura Pulido-Suárez[2], Beatriz Fernández-Marín[1], Jaime Puértolas[1], Milagros León-Barrios[2], Águeda M. González-Rodríguez[1]

[1]Department of Botany, Ecology and Plant Physiology, University of La Laguna, La Laguna, Canary Island, Spain. [2]Department of Biochemistry, Microbiology, Cellular Biology and Genetic, University of La Laguna, La Laguna, Canary Island, Spain

For the last decades, an increase of the frequency and strength of fire events have been observed in Teide National Park (Canary Island, Spain). The effects of fire on the structure and dynamic of this Macaronesian high mountain ecosystem were evaluated during one year after fire. Heat itself killed all seeds except those of the two main Fabaceae species of this ecosystem (*Spartocytisus supranubius* and *Adenocarpus viscosus*), whose germination rate were even enhanced after heat treatment. That explains the *sorpasso* of *S. supranubius* as the most abundant species (59%) in burned soils, a podium that was occupied by a brassicacean specie (*Descurainia bourgaeana*) in non-perturbed patches of the study site (42.4%). Can this initial higher abundance of *S. supranubius* be maintained for long-term? Since nitrogen content, pigments and electron transport rate did not differ among plants that were growing on burned vs. unburned soils, we projected the future scenario of the recovered ecosystem from gas exchange, annual carbon balance and phenology of the studied species. How long will it take for the ecosystem to recover pre-fire structure? Our study is the first example of an attempt of modelling gas exchange data with the aim of answering this question and help the management of the National Park.

91 In Person Poster

Elucidating mechanisms of LHCX- and zeaxanthin-dependent non-photochemical quenching in *Nannochloropsis oceanica*

Thien Crisanto[1,2], Dagmar Lyska[1,2], Marlena Tavernier-Fine[1], Grant Reskey[1], Cailyn Sakurai[1], Setsuko Wakao[1,2], Krishna K. Niyogi[1,2,3]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA. [2]Molecular Biophysics and Integrated Bioimaging, Division Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [3]Howard Hughes Medical Institute, University of California, Berkeley, CA, USA

Photosynthesis is dynamic and requires constant adjustments to changing light intensities. These adjustments are controlled through an interplay of light-harvesting complex (LHC) proteins and pigments that associate with the photosystems, and they include photoprotective processes known as non-photochemical quenching (NPQ). We are investigating NPQ in the photosynthetic stramenopile alga *Nannochloropsis oceanica* CCMP1779 (Eustigmatophyceae). Wild-type cells reach an NPQ of 5 in high light, whereas cells inhibited with an uncoupler or dithiothreitol (DTT) have major reductions in both the fast-acting, energy-dependent quenching (qE) and slowly inducible zeaxanthin-dependent quenching (qZ). Cells inhibited with DTT also show decreases in accumulation of de-epoxidized xanthophyll cycle pigments, indicating that violaxanthin de-epoxidase (VDE) is a key player. We generated knockouts of the genes encoding VDE and three stress-related LHC proteins, LHCX1, 2, and 3. Measurement of NPQ using PAM fluorometry revealed that the vde mutant abolishes qE and qZ, lhcx1 is inhibited in qE, whereas lhcx2 and lhcx3 have no effects in low light and high light-grown cells. To examine the possible role of LHCX1 as a pH sensor, we are generating targeted mutants of putative protonatable residues via a “knock-in” method that uses Cas9-RNP-mediated homologous recombination to insert mutated versions of the *LHCX1* gene into the endogenous locus. Preliminary results show that mutation of lumen-exposed acidic residues does not alter qE capacity, suggesting that LHCX1 may not be involved in sensing thylakoid lumen pH, in contrast to orthologous LHCSR proteins in *Chlamydomonas reinhardtii*.

92 In Person Oral

The role of bryophytes in the understanding and improving photosynthesis

Alicia V Perera-Castro[1], Melinda J. Waterman[2,3], Beatriz Fernández-Marín[1], Águeda M. González-Rodríguez[1], Sharon A. Robinson[2,3], Jaume Flexas[4]

[1]Department of Botany, Ecology and Plant Physiology, University of La Laguna, La Laguna, Canary Island, Spain. [2]Centre for Sustainable Ecosystem Solutions, School of Earth, Atmosphere and Life Sciences, University of Wollongong, Wollongong, NSW, Australia. [3]Securing Antarctica's Environmental Future, University of Wollongong, Wollongong, NSW, Australia. [4]Universitat de les Illes Balears, Department of Biology, INAGEA, Palma de Mallorca, Balearic Islands, Spain

Bryophytes are the group of land plants with the lowest photosynthetic and relative growth rates, at approximately 10% of those reported for tracheophytes. This has been considered to be a consequence of their higher anatomical CO_2 diffusional limitation compared with tracheophytes, resulting from them having the thickest cell wall among land plants and their simple tissue structure limiting the surface for gas exchange. Recent studies have also considered a possible biochemical limitation of photosynthesis as a result of very low investment in Rubisco. The predominance of each factor in limiting photosynthesis is still under debate, since the calculations of percentage limitation by Grassi and Magnani (2005, Plant, Cell & Environ. 28:834-849) are extremely sensitive to the method used to obtain mesophyll conductance in bryophytes. Even a gas exchange independent method for calculating mesophyll conductance (by anatomical modelling) does not break the deadlock between diffusional or biochemical limitation. The mesophyll conductance calculated by anatomical modelling seems to draw two possible scenarios depending on the assumed values of effective porosity assigned to bryophyte cell walls: 1) a predominance of diffusional limitation to photosynthesis due to low porosity, thick cell walls, or, on the contrary, 2) a predominance of biochemical limitation due to the low Rubisco concentration of tissues in a scenario where porosity of cell walls is very high in bryophytes. The second scenario would explain the asymptotic relationship between CO_2 assimilation rate and cell wall thickness when bryophytes are considered. The resolution of this debate is of interest, since the second scenario would open a pathway for improving crop photosynthesis by providing crops with porous bryophyte cell walls.

93 Pre Recorded Poster

Revisiting non-photochemical quenching (NPQ) by a novel wavelength resolved chlorophyll fluorescence detection method

Sanchali Nanda[1], Pushan Bag[1], Alfred Holzwarth[2], Stefan Jansson[1]

[1]Umeå Plant Science Centre, Umeå, Sweden. [2]Vrije Universiteit, Amsterdam, Netherlands

Photosynthetic organisms absorb light energy using carotenoids and chlorophylls. A part of this absorbed light energy drives photosynthesis, the remainder of this absorbed light energy is either dissipated as heat (NPQ) or re-emitted in the form of chlorophyll fluorescence. NPQ can be estimated by chlorophyll fluorescence measurements. The existing methods of chlorophyll fluorescence analysis are based on the assumption that chlorophyll fluorescence mainly originates from PSII, disregarding the contribution from PSI. These methods cannot capture the difference between the contributions from PSII and PSI and thereby the information obtained is limiting. ChloroSpec (manufactured by ChloroSpec B.V. Amsterdam; .chlorospec.com) is a newly developed chlorophyll fluorescence analyzer which systematically addresses this knowledge gap. ChloroSpec takes chlorophyll fluorescence measurements from intact leaves and photosynthetic microorganisms to a higher dimension by adding the so-far neglected wavelength resolution to all the fluorescence parameters. In this study we have used leaves and needles from five different wild-type plant species (Arabidopsis thaliana, Monstera deliciosa, Hybrid Aspen (Populus tremula x Populus tremuloides), Picea abies and Pinus sylvestris) for NPQ characterization using ChloroSpec. Photosynthetic parameters such as dark fluorescence yield (Fo), maximum fluorescence yield (Fm), PSII quantum yield (Fv/Fm, (II)), NPQ kinetics and light response curves all show distinct dependencies on the detection wavelength, but also on the species, excitation wavelength, and growth conditions. The resolved NPQ spectra in the range 675 nm to 800 nm facilitates the possibility of identifying multiple NPQ components differing both spectrally as well as kinetically. Further deconvolution of these extensive wavelength-resolved data sets allows to uncover a tremendous amount of novel information and enhance our understanding of these mechanisms.

94 In Person Poster

Excitation energy transfer from bacteriochlorophyll *c* to bacteriochlorophyll *a* in artificial light-harvesting antennas

Tomas Malina[1], David Bina[2], Jakub Psencik[1]

[1]Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University, Prague, Prague, Czech Republic. [2]Biology Centre, Czech Academy of Science, České Budějovice, South Bohemia, Czech Republic

Aggregates of bacteriochlorophyll (BChl) *c* can be found in chlorosomes, the main light-harvesting antennas of green photosynthetic bacteria. The aggregates do not contain any proteins and self-assemble into large structures (on the order of 100 nm). The lack of proteins makes it possible for the aggregates to self-assemble also in vitro, and to modify their properties on demand. Therefore, they are a promising piece of puzzle in the developing field of artificial light harvesting.

To improve their spectral coverage, we are incorporating other natural pigments into the aggregate structure. Primarily, we are interested in pigments absorbing in the green region of the solar spectrum (like carotenoids), which is otherwise not covered by BChl *c*. In our previous work [Malina et al. (2021) Sci. Rep. 11: 18739], we have found that the excitation energy in chlorosomes and artificial aggregates of BChl *c* remains delocalized for a relatively long time after absorption (tens of ps). This contributes to a higher efficiency of excitation energy transfer not only within the aggregates, but also out of the aggregates.

To address the transfer of energy out of the aggregates, we are incorporating an acceptor of excitation energy into the artificial aggregates. Inspired by natural chlorosomes, we are using BChl *a*. Its S_1 energy is slightly lower than for BChl *c* aggregates, which makes it an ideal candidate to direct the energy flow from BChl *c* to BChl *a*. In this contribution, we will present results of experiments on the efficiency of energy transfer from the artificial BChl *c* aggregates to the incorporated BChl *a*, which reaches up to 95 % for a certain type of artificial aggregates.

95 Pre Recorded Poster

Accuracy of approximate methods for calculating linear spectra of light-harvesting complexes

Johan Nöthling[1,2], Tjaart Krüger[1,2], Tomáš Mančal[3]

[1]University of Pretoria, Pretoria, Gauteng, South Africa. [2]National Institute for Theoretical and Computational Sciences, Stellenbosch, South Africa. [3]Charles University, Prague, Czech Republic

Linear optical spectroscopy is a crucial analytical tool in photosynthesis research, but is hard to simulate exactly—even for small model systems. We give an overview of four linear spectroscopy techniques (absorption, linear dichroism, circular dichroism, and fluorescence) and discuss four approximate methods for calculating linear spectra (the Full Cumulant Expansion, the coherent time-dependent Redfield method, and two methods in which the time-independent Redfield and modified Redfield rates are used). We also present the site-basis expressions for the calculation of linear dichroism and fluorescence anisotropy, which have not been derived previously. In order to determine the accuracy of the approximate methods, we compare approximate spectra of a pigment dimer with exact spectra calculated using the method of Exact Stochastic Path Integral Evaluation—which, to our knowledge, is used for the first time since its introduction. The comparison of approximate and exact spectra is performed for a range of energy gaps and couplings typically found in light-harvesting complexes, and a realistic spectral density is used that includes the first eight intramolecular vibrational modes of chlorophyll *a*. The results of this comparison are used to determine the conditions under which the linear spectra of the plant light-harvesting complex CP29 may be calculated accurately.

96 Pre Recorded Poster

Effects of the LHCA3 or LHCA7 subunit deletion on the structure and function of the Photosystem I Light-Harvesting Complex I in the green alga *Chlamydomonas reinhardtii*

Shin-Ichiro Ozawa[1], Michiyo Takagi[2], André Vidal-Meireles[3], Felix Buchert[3], Michael Hippler[1,3], Yuichiro Takahashi[4]

[1]Institute of Plant Science and Resources, Okayama University, Kurashiki, Japan. [2]Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan. [3]Institute of Plant Biology and Biotechnology, Münster University, Münster, Germany. [4]Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan

Photosystem I (PSI) core complex associates Light-Harvesting complex I (LHCI) to form the PSI-LHCI supercomplex (PSI-LHCI). The PSI-LHCI of the green alga *Chlamydomonas reinhardtii* contains ten LHCI subunits (one LHCA2-9 and two LHCA1s). Four LHCI subunits (LHCA3, LHCA7, LHCA8, and LHCA1 from PSAK to PSAG) bind to the PSI core at PSAF/PsaJ side to form an inner layer, another four LHCI subunits (LHCA5, LHCA6, LHCA4, and LHCA1 from PSAK to PSAG) associate with the inner layer to form an outer layer, and the other two LHCI subunits (LHCA9 and LHCA2) bind PSAG and PsaH/I/L side of PSI core. The green algal LHCI structure provides a larger antenna size to collect more light under lower light environments. Here we characterized the *lhca3* and *lhca7* mutants deficient in LHCA3 and LHCA7, respectively, in *C. reinhardtii* obtained from Chlamydomonas Library Project. Both mutants grow photoautotrophically and accumulate PSI like WT. The *lhca7* cells accumulated LHCA3 protein at a very low level, whereas the *lhca3* cells accumulated LHCA7 protein normally. The in vivo fluorescence emission spectra at 77K revealed that the peak at 712 nm from PSI in wild type was replaced by a peak at 710 nm in the *lhca3* and at 708 nm in the *lhca7*, although a peak at 688 nm from PSII was not affected. The flash-induced optical cross-section analysis indicated lower light energy transfer efficiency in both mutants. We purified PSI-LHCI from the two mutants in which PsaB is fused with His20 tag by affinity chromatography and found that the outer layer of LHCI was significantly dissociated while LHCA2/9 remained associated normally. These results indicate that the LHCA3/7 defect impacts the LHCI structure. We will discuss the efficiency of light energy transfer from LHCIs to PSI core in the absence of LHCA3 and/or LHCA7.

97 In Person Poster

Visualization of thylakoid lumen environment in the dark by Photochemical Reflectance Index (PRI)

Kaori Kohzuma

Department of Biological Science, The University of Tokyo, Tokyo, Tokyo, Japan. Astrobiology Center, National Institutes of Natural Sciences, Mitaka, Tokyo, Japan

The electrochemical potential formed across the thylakoid membrane in the chloroplast is essential to photosynthetic regulation. To visualize the thylakoid membrane environment in real-time in an intact leaf, we focused on the photochemical reflectance index (PRI). PRI is a spectral reflectance parameter calculated from (R_{531nm} - R_{570nm}) / (R_{531n}m + R_{570nm}), which normalizes the change in reflectance at 531nm with 570nm (R is reflectance). It was proposed by Gamon et al. in the 1990s and has contributed as a remote sensing parameter for estimating photosynthetic capacity and carbon sequestration mainly in forests or other wide-area studies because of its light-dependent changes. Since PRI had been suggested to be closely related to the xanthophyll cycle, we expected it to be used as a tool to monitor proton accumulation in the thylakoid lumen. However, we questioned the consistency of ΔA_{505}, which reflects xanthophylls, and ΔA_{535}, which reflects qE (Energy-dependent quenching), in spectral transmittance and PRI in spectral reflectance, and thus we performed a physiological validation using Arabidopsis mutants. The results revealed that the reflectance signal at 531 nm differs from the transmittance signal and specifically detects conversion of xanthophylls rather than qE. The advantage of PRI is that measurements can be made with only a few seconds of shooting light, meaning that the dynamics of xanthophylls can monitor at night. We are currently working on visualizing the thylakoid lumen environment during dark, observing that PRI is induced 3-4 hours after the turned light off from a steady state. In this study, we discuss the possibility of changes in the thylakoid membrane environment in the dark, especially the formation of membrane potentials.

98 Pre Recorded Oral

Balancing photoprotection and energetic robustness to large scale structural dynamics in the PSI-IsiA supercomplex

Dvir Harris[1], Hila Toporik[2], Yuval Mazor[2], Gabriela Schlau-Cohen[1]

[1]MIT, Cambridge, MA, USA. [2]ASU, Tempe, AZ, USA

Photosynthesis is carried out by large antenna-reaction centers super-complexes that mostly reside in flexible cellular membranes. These cellular assemblies maintain near perfect quantum efficiency with regards to light harvesting. Within individual light harvesting entity, chromophores are bound by their respective protein partner to maintain inter pigment distances and orientations within tight tolerances, which is thought to be critical for maintaining highly efficient energy transfer. In contrast, structures of photosynthetic super-complexes revealed large flexibility or variability in the distance and orientations of some individual antennae elements within super-complexes. The scale of this variability and the features that maintain high efficiency energy transfer in flexible membranes remain an open question. Here, we report a high-resolution CryoEM structure of the 2-MDa cyanobacterial (*Synechocystis PCC 6803*) PSI-IsiA super-complex, revealing heterogeneous conformations with large-scale differences in the relative positions of IsiA antenna with respect to PSI reaction center. Single-molecule fluorescence spectroscopy (SMFS) indicated that while an efficient IsiA to PSI energy transfer channel is maintained across all super-complexes, small heterogeneous, lower efficiency states were also present in some complexes, likely due to energetically decoupled IsiA monomers. In addition, IsiA monomers were purified and interrogated with SMFS and spectrally resolved time correlated single photon counting. We identified a quenched, red-emissive state in the IsiA monomer, suggesting that the quenching functionality is accessible even in its monomeric form. Based on the unique IsiA chlorophyll network architecture and theoretical calculations we performed, we unraveled how IsiA is capable of achieving energetic robustness to large scale structural flexibility while balancing it out with photoprotection. This is attained by designated, specific chlorophylls within the network, some of which are unique to IsiA, and the protein assembly dynamicity. Thus, this work provides a comprehensive explanation for IsiA functionality as an outstanding antennae system.

99 In Person Poster

Quantitative assessment of excitation spillover from PSII to PSI in leaf discs at 77 K

Ichiro Terashima[1], Riichi Oguchi[2], Masaru Kono[1]

[1]Department of Biological Sciences, School of Science, The University of Tokyo, Hongo, Tokyo, Japan.
[2]Department of Biology, School of Science, Osaka Metropolitan University, Katano, Osaka, Japan

Spillover of excitation energy from PSII to PSI is believed to be facilitated by the PSI-PSII megacomplex. It is also argued that intensive spillover may not occur in thick grana, because most PSI and PSII are separately located in such thick grana. Fluorescence induction was measured at both 690 nm (PSII) and 760 nm (PSI) in the leaf discs at 77K with a modified PAM 101 to estimate excitation spillover from PSII to PSI. Effects of growth light levels, state transition and the *b*-less mutation on spillover were examined in spinach, cucumber, *Alocasia odora* (a shade tolerant plant), and a Chl *b*-less mutant and a wild type of barley. According to [Kitajima M. and Butler W.L. (1975) BBA 408: 297-305], PSI-fluorescence consists of the intrinsic PSI-fluorescence (FIα) and that originated from the excitations spilt over from PSII (FIβ). The FIα did not show induction, whereas FIβ increased synchronously with FII. By transition from state 2 to 1, F_m of PSII fluorescence (FII_m) increased and FIα decreased by up to 30%, while in the Chl *b*-less mutant, neither FII_m nor FIα changed. For PSI-fluorescence, $FI\beta_m/FI\alpha$ (β_m/α) ratios ranged from 0.3 to 0.6 and were greater in state 1. In contrast to the prevailing view, β_m/α ratios were higher in leaves grown in low light. The *b*-less barley, lacking in LHCII, also showed a considerable β_m/α ratio. If spillover of a similar extent occurs at physiological temperatures, it will be effective for avoidance of PSII photoinhibition especially when the leaves are suddenly exposed to strong light such as sunflecks, as proposed in [Terashima I. et al. (2021) Photosynth. Res. 149: 69-82]. It will be also necessary to incorporate the large spillover term in the fluorescence models.

100 In Person Poster

Directed evolution of a chaperonin complex allows for the production of maize Rubisco in *E. coli*

Timothy Rhodes, Tanya Skinner, Spencer Whitney

The Australian National University, Canberra, ACT, Australia

Expression of plant Rubisco in *E. coli* is an essential step to improve its reaction efficiency using bioengineering techniques such as directed evolution. Only recently was heterologous expression of *Arabidopsis* and tobacco Rubisco successfully demonstrated in *E. coli* [Aigner, ., et al. (2017) Science 358.6368: 1272-1278; Lin, Myat T., et al. (2020) Nature Plants 6.10: 1289-1299]. Despite these landmark breakthroughs, our findings indicate these *E. coli* expression systems are unable to meet the folding and assembly requirements of Rubisco from important monocot crops such as wheat (*Triticum aestivum*) or maize (*Zea mays*). We hypothesized this could be only be achieved by co-expressing monocot Rubisco with its cognate auxiliary folding/assembly chaperones. We tested this hypothesis by developing a modular, Golden Gate compatible cloning system to construct a 2 plasmid expression system, one coding the wheat or maize Rubisco large (RbcL) and small (RbcS) subunits and the second plasmid their cognate CPN60α, CPN60β, CPN20, Raf1, Raf2, RbcX, BSD2 chaperones. While this approach still precluded the production of wheat or maize Rubisco in *E. coli*, subsequent combinatorial chaperone exchange experiments in conjunction with mutagenic screening using Rubisco-dependent *E. coli* directed evolution selection facilitated the identification of a mutation in CPN60α that facilitates the formation of L_8S_8 maize Rubisco, but not wheat Rubisco, in *E. coli*. Presented will be an update of how this new mutant chaperonin complex facilitated the characterization of a faster maize Rubisco mutant.

101 In Person Poster

Tailoring a new *E. coli* expression system for the directed evolution of a golden Rubisco

Tanya Skinner, Spencer Whitney

The Australian National University Research School of Biology, Canberra, ACT, Australia

Progress in Rubisco bioengineering in recent years has successfully improved the carboxylation properties of various bacterial and archaea isoforms of Rubisco by laboratory evolution using *E. coli* selection, a feat not yet reproduced for plant Rubisco. The longstanding barrier to producing functional plant Rubisco in *E. coli* has been recently solved [Aigner et al. (2017) Science 358:1272-1278], providing new opportunities for its directed evolution. Impeding such efforts is the requirement for structural compatibility between the Rubisco large subunits (RbcL) and one or more of its many folding and assembly chaperones. As a result, the use of heterologous chaperones can impair, and sometimes prevent, the biogenesis of plant Rubisco in *E. coli*. This project therefore sought to develop a modular genetic cloning system tailored to expressing canola Rubisco in *E. coli*, an agronomically important crop amenable to both chloroplast and nucleus transformation. Using golden gate cloning a library of genetic parts was used to rapidly assemble a two-plasmid expression system. One plasmid coded the canola Rubisco large and small subunits (RbcL/S) either with or without Rubisco activase (Rca). The second plasmid coded the seven canola chloroplast chaperone proteins required for Rubisco biogenesis: this included the folding chaperonin cage components, Cpn60α, Cpn60β and Cpn20; and the Rubisco specific assembly chaperones, Raf1, Raf2, RbcX and BSD2. Each gene was equipped with independent bacterial T7 promoter and terminator elements – allowing for their regulatable expression in BL21 Star *E. coli* with IPTG. Here I provide an update on analyses undertaken to optimise canola Rubisco expression in *E. coli* and its capacity for use in directed evolution applications to select for mutations that enhance canola Rubisco catalysis for downstream translational testing via editing of the canola nuclear and/or chloroplast genome.

102 In Person Poster

Ability of P700 oxidation in Photosystem I causes varietal difference of chilling tolerance in cucumber

Ko Takeuchi[1], Yufen Che[1], Takeshi Nakano[1], Chikahiro Miyake[2], Kentaro Ifuku[3]

[1]Graduate School of Biostudies, Kyoto University, Kyoto, Japan. [2]Graduate School of Agriculture, Kobe University, Kobe, Hyogo, Japan. [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Low temperature inhibits photosynthesis and negatively affects plant growth. Cucumber (*Cucumis sativus* L.) is a chilling-sensitive plant, and its greenhouse production requires considerable energy during the winter. Therefore, a useful stress marker for selecting chilling-tolerant cucumber cultivars is desirable. In this study, we evaluated chilling-stress damage in different cucumber cultivars by measuring photosynthetic parameters. The majority of cultivars showed decreases in the quantum yield of photosystem (PS) II [Fv/Fm and (II)] and the quantity of active PS I (Pm) after chilling stress. In contrast, (ND)—the ratio of the oxidized state of PSI reaction center chlorophyll P700 ($P700^+$)—differed among cultivars and was perfectly inversely correlated with (NA)—the ratio of the non-photooxidizable P700—. It has been known that $P700^+$ accumulates under stress conditions and protects plants to suppress the generation of reactive oxygen species. In fact, cultivars unable to induce (ND) after chilling stress showed growth retardation with reductions in chlorophyll content and leaf area. For further analysis, two cucumber cultivars, HM and HG, were selected as a tolerant and a sensitive cultivar with respect to the chilling-induced PSI photoinhibition, respectively. After chilling-stress treatment, HM could maintain the P700 oxidation in PSI by the proper photosynthetic control, while HG could not oxidize P700. Loss of photosynthetic control in HG was due to the decrease of the proton motive force (*pmf*) across thylakoid membranes. The above damaging process was not observed in the dark, suggesting the involvement of reactive-oxygen species (ROS). Therefore, the ability of P700 oxidation would determine differences in the initial ROS levels under chilling-stress, which would dictate stress tolerance.

103 In Person Oral

The rate constants of photo-damage and repair of photosystem II differ among woody plants during photoinhibition

Shoko Tsuji[1], Kaori Kohzuma[2], Kouki Hikosaka[3], Kentaro Ifuku[1]

[1]Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto, Japan. [2]Department of Biological Science, The University of Tokyo, Tokyo, Tokyo, Japan. [3]Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan

Light is essential for plant photosynthesis, but when light irradiating leaves is too strong, it causes damage, especially in photosystem II (PSII), leading to reduced photosynthetic activity (photoinhibition). It is known that plants have effective mechanisms to repair the photo-damaged PSII under excessive light energy, and photoinhibition occurs when the rate of photo-damage exceeds that of repair. These two processes can be analyzed separately by infiltration of lincomycin into intact leaves, which inhibits protein synthesis in chloroplasts and hence the PSII repair. Rates of photo-damage and repair have been examined mainly for herbaceous plants, while studies are limited for those in woody plants. In this study, we have investigated the mechanism of photoinhibition using 18 woody species growing under different environments. Their leaves were exposed to strong light, changes of the PSII quantum yields were monitored by measuring chlorophyll fluorescence in the presence and absence of lincomycin, and the rate constant for photo-damage (inactivation) (k_{pi}) and that of the repair (k_{rec}) were calculated. The results suggest that the 18 woody plant species have different properties in the balance between k_{pi} and k_{rec}, indicating the different strategies to cope with excess light energy. We discuss the physiological and ecological aspects of the woody plant species, in relation to their habitat and leaf morphological characteristics.

104 In Person Oral

The CbbQO-type rubisco activases encoded in carboxysome gene clusters can activate carboxysomal form IA rubiscos

Yi-Chin Candace Tsai, Lynette Liew, Zhijun Guo, Di Liu, Oliver Mueller-Cajar

School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

The CO2-fixing enzyme rubisco is responsible for almost all carbon fixation. This process frequently requires rubisco activase (Rca) machinery, which couples ATP hydrolysis to the removal of inhibitory sugar phosphates, including the rubisco substrate ribulose 1,5-bisphosphate (RuBP). Rubisco is sometimes compartmentalized in carboxysomes, bacterial microcompartments that enable a carbon dioxide concentrating mechanism (CCM). Characterized carboxysomal rubiscos, however, are not prone to inhibition, and often no activase machinery is associated with these enzymes. Here, we characterize two carboxysomal rubiscos of the form IAC clade that are associated with CbbQO-type Rcas. These enzymes release RuBP at a much lower rate than the canonical carboxysomal rubisco from Synechococcus PCC6301. We found that CbbQO-type Rcas encoded in carboxysome gene clusters can remove RuBP and the tight-binding transition state analog carboxy-arabinitol 1,5-bisphosphate from cognate rubiscos. The Acidithiobacillus ferrooxidans genome encodes two form IA rubiscos associated with two sets of cbbQ and cbbO genes. We show that the two CbbQO activase systems display specificity for the rubisco enzyme encoded in the same gene cluster, and this property can be switched by substituting the C-terminal three residues of the large subunit. Our findings indicate that the kinetic and inhibitory properties of proteobacterial form IA rubiscos are diverse and predict that Rcas may be necessary for some α-carboxysomal CCMs. These findings will have implications for efforts aiming to introduce biophysical CCMs into plants and other hosts for improvement of carbon fixation of crops.

105 Pre Recorded Poster

Energy transfer reaction upon carotenoids excitation in the photosynthetic reaction center of heliobacteria

Risa Kojima[1], Masatoshi Kida[2], Daisuke Kosumi[3], Hirozo Oh-oka[1,4]

[1]Center for Education in Liberal Arts and Sciences, Osaka University, Toyonaka, Japan. [2]Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan. [3]Institute of Industrial Nanomaterials, Kumamoto University, Kumamoto, Japan. [4]Graduate School of Science, Osaka University, Toyonaka, Japan

Heliobacteria are obligatory anaerobic and anoxygenic photosynthetic bacteria. Their reaction center in heliobacteria (hRC) is classified as type-1 RC, which is the same as those of cyanobacterial and higher plants' photosystem I and green sulfur bacteria. The three-dimensional structure of the hRC from *Heliobacterium modesticaldum* was revealed by the X-ray crystallography [Gisriel et al. (2017) Science 357:1021-1025]. The hRC contains 54 BChl *g*, 4 BChl *g'*, two 8^1-hydroxychlorophyll a_F, and two 4,4'-diaponeurosporene, those are all arranged along with a completely symmetrical *C2* axis. Although the excitation energy transfer mechanisms among BChl *g*s in the hRC has been discussed so far, the involvement of carotenoids (Cars) has not been clarified yet with spectroscopic methods. In the present study, we measured transient absorption spectra after a selective excitation at 490 nm using the highly purified RC in the pico-second time range with femtosecond pomp-probe spectroscopy at 140 K. A global fit analysis was carried out to obtain kinetic components relating to the energy and subsequent electron transfer reactions. It revealed the efficient excitation energy transfer from Cars to the nearby BChl *g*s' pigment pool, $B_{md}787$, with a time constant (τ) of 0.04 ps, and subsequently to $B_{md}812$ with τ = 0.43 ps. The 45 ps component with a maximum negative peak at 814 nm was supposed to be the formation process of the charge-separated state of $P800^+A_0^-$. A positive peak also appeared at 786 nm with the same constant, which was not previously observed in the 810 nm excitation experiment at room temperature [Kojima et al. (2020) J. Photochem. Photobiol. A: Chemistry, DOI: 10.1016/j.jphotochem.2020.112758]. Moreover, the photochromic band sift of Cars observed immediately after the excitation at 820 nm also suggested that the red-BChl *g*s and Cars exist in proximity and would form the π-electron systems over them.

106 In Person Poster

Exploring the sugar sensing mechanisms regulating C_4 photosynthesis and productivity

Lily Chen[1], Yogesh Chaudhari[1], Urs Benning[1], Alexander Watson-Lazowski[1,2], Robert Furbank[3], Oula Ghannoum[1]

[1]Western Sydney University, Sydney, NSW, Australia. [2]John Innes Centre, Norwich, United Kingdom. [3]Australian National University, Canberra, ACT, Australia

How sugars are sensed within the photosynthetic leaves of C_4 grasses is poorly understood. In C_4 species, sugar sensing is complicated by the anatomical and biochemical partitioning of both photosynthetic and carbohydrate metabolism between the bundle sheath and mesophyll cells. C_4 plants have superior photosynthetic performance, thus likely produce more soluble sugars compared to C_3 counterparts. Many important crops are C_4 grasses, for example sorghum, maize, and millets such as Setaria. Further studies into how sugars are sensed in these plants is needed. We have initiated a long-term project to compare sugar sensing pathways in the C_3 model crop *Oryza sativa* and C_4 model millet *Setaria viridis*. Putative sugar sensors explored include hexokinase (HXK), Sucrose nonfermenting 1-Related Kinase (SnRK), Target Of Rapamycin (TOR) and the sugar signalling metabolite trehalose-6-phosphate (T6P). These sensors elicit downstream effects on gene expression and leaf level physiology in response to intracellular sugar levels. Our preliminary studies used a bioinformatics and reverse genetics approach to understand the interplay between photosynthesis and sugar sensing. Previous RNA deep sequencing data (RNAseq) was mined from the source tissue of C_3 and C_4 grasses to see how the expression of key genes encoding sugar sensors change along the leaf gradient and between the leaf mesophyll and bundle sheath cells of C_3 and C_4 grasses. Expression analyses showed some changes in transcript abundance particularly for *HXK* between mesophyll and bundle sheath cells. Additionally, the overexpression of *Setaria virdis HXK6* (*SvHXK6*) and *SvSnRK1a* driven by the *Zea mays* PEPC promoter was examined in Setaria to determine how sugar signalling in the source leaf affect plant photosynthesis and development. The transgenics showed changes in photosynthesis, stomatal conductance and plant growth under standard conditions relative to the null and wild type. These results form a framework to further study sugar signalling in C_4 photosynthesis.

107 Pre Recorded Poster

Energy transfer fluctuation in the photosynthetic reaction center

Toru Kondo[1], Risa Mutoh[2], Shun Arai[1], Genji Kurisu[3], Hirozo Oh-oka[4], Satoru Fujiyoshi[1], Michio Matsushita[1]

[1]Tokyo Institute of Technology, Meguro, Tokyo, Japan. [2]Toho University, Funabashi, Chiba, Japan. [3]Institute for Protein Research, Osaka University, Suita, Osaka, Japan. [4]Osaka University, Toyonaka, Osaka, Japan

Photosynthetic reaction center (RC) is a chromoprotein responsible for the photoelectric conversion in the light reaction of photosynthesis. Pigment molecules embedded in the RC interact with each other to mediate the efficient excitation energy transfer to a central part where the charge separation is induced. The energy transfer mechanism has been discussed based on the precise molecular arrangement given by x-ray crystallography and electron microscopy. Meanwhile, resent studies using single-molecule approaches and molecular dynamics simulation suggest that the protein conformational dynamics can have a significant impact on the light-harvesting function of photosynthetic proteins. We perform the cryogenic single-molecule spectroscopy of heliobacterial RC (hRC), which is a type-I homodimeric RC containing fifty-eight bacteriochlorophylls (Bchls) as well as two chlorophylls *a*s (Chl *a*s) serving as the primary electron accepter. Single hRCs exhibit heterogeneous fluorescence spectra reflecting conformational inhomogeneity. Temporal fluorescence peak shifts are likely associated with fluctuations in the energy transfer pathway. By analyzing the fluorescence excitation spectrum of Bchls and Chl *a*s in a single hRC, we explore how the local conformational dynamics affect the energy transfer process and contribute to the light-harvesting function of the RC.

108 Pre Recorded Poster

Structural uniqueness and diversity of photosystem-light-harvesting supercomplexes

Ryo Nagao[1], Koji Kato[2], Seiji Akimoto[3], Jian-Ren Shen[1]

[1]Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan. [2]JASRI, Hyogo, Japan. [3]Graduate School of Science, Kobe University, Hyogo, Japan

Oxygenic photosynthetic organisms have developed light-harvesting complexes (LHCs) to capture solar energy, which is subsequently transferred to two photosystems (PSI and PSII) to initiate charge-separation reactions. Different from the core components of photosystems, LHCs have a wide variety of subunit compositions as well as different species and binding positions of chlorophylls and carotenoids, leading to the adaptation of individual organisms to a variety of living environments. To examine the variety of LHCs, we have determined eight types of PSI and PSII structures with/without LHCs purified from various photosynthetic organisms using cryo-electron microscopy (cryo-EM) single-particle analysis since 2019. Here we present the structural characteristics of pigment arrangements and subunit compositions of LHCs in these structures. In particular, we show the structures of PSI and PSII in complex with unique LHC, fucoxanthin chlorophyll (Chl) *a*/*c*-binding proteins (FCPs), from the diatom *Chaetoceros gracilis*. These structures exhibit characteristic oligomeric states and pigment arrangements different from the structures of PSII-LHCII and PSI-LHCI from land plants. In addition, the PSI complex from the glaucophyte alga *Cyanophora paradoxa* shows a tetrameric structure, albeit a photosynthetic eukaryote. Moreover, the PSI structure from the primordial cyanobacterium *Gloeobacter violaceus* exhibits a plausible candidate for low-energy Chls in PSI. These structural findings prove the significant uniqueness and diversity of pigment-protein complexes in photosynthetic organisms, most probably due to adaption to the different light environments that each organism occupies.

109 Pre Recorded Poster

Triplet states in the reaction center of Photosystem II

Sinjini Bhattacharjee, Frank Neese, Dimitrios A. Pantazis

Max Planck Institut für Kohlenforschung, Mülheim an der Ruhr, Germany

Photosystem II (PSII) catalyzes the oxidation of water into molecular oxygen and the reduction of plastoquinone (PQ_B) in the light-driven electron transfer chain of photosynthesis. The reaction center (RC) of PSII is the site of primary charge separation upon photo-excitation, forming the $Chl_{D1}^{+}Pheo_{D1}^{-}$ radical pair (RP) [Sirohiwal et al. (2020) J. Am. Chem. Soc. 142:18174-18190]. When the forward electron transfer from $Pheo_{D1}$ to the acceptor plastoquinone (PQ_A) is blocked or delayed, a charge recombination and spin dephasing can lead to formation of triplet states. These triplet states can produce chemically active singlet oxygen which might lead to photodamage and photoinhibition. They also serve as important probes to investigate the electronic structure and chemical environment of the RC chromophores. EPR/ENDOR and other spectroscopic studies suggest that the 'primary donor' triplet is located on an accessory chlorophyll (Chl_{D1} or Chl_{D2}) [Niklas et al. (2022) Photosynth. Res.], however the precise nature or location of this triplet state remains unclear. In this work we characterize all possible triplet states that can be formed at the RC. We employ multiscale modelling approaches based on quantum-mechanics/molecular-mechanics (QM/MM) calculations, combined with range-separated time-dependent density functional theory (TDA-TDDFT) to identify all low-lying triplet excited states at the PSII-RC. In addition, we compute zero-field splitting parameters and hyperfine coupling constants to enable comparisons with experiment. Our results show that the lowest energy triplet is localized on a monomeric chlorophyll, in agreement with experimental interpretations. We further extend our methodology on pigment-pairs to include coupled interactions that may lead to RP charge recombination, and propose a plausible step-by-step pathway that leads to formation of the observable triplet state. This work provides a basis for the atomic level understanding of secondary electron transfer pathways involving photosynthetic pigments and photo-protection mechanisms when the PSII-RC is exposed to excess light.

110 In Person Poster

Carotenoid interconversion in LHCII of *Codium fragile* stimulated by blue-green irradiation

Soichiro Seki[1], Kazuhiro Yoshida[1], Naohiro Oka[2], Yumiko Yamano[3], Yasuhiro Kamei[4], Mitsuru Sugisaki[1], Ritsuko Fujii[1,5]

[1]Graduate School of Science, Osaka Metropolitan University, Osaka, Osaka, Japan. [2]Bio-Innovation Research Center, Tokushima University, Tokushima, Tokushima, Japan. [3]Comprehensive Education and Research Center, Kobe Pharmaceutical University, Kobe, Hyogo, Japan. [4]Spectrography and Bioimaging Facility, National Institute for Basic Biology, Okazaki, Aichi, Japan. [5]Research Center for Artificial Photosynthesis, Osaka Metropolitan University, Osaka, Osaka, Japan

Siphonous algae, a group of marine green algae, inhabit a sea bottom on the coast where the light conditions vary from weak blue-green light to strong white light depending on the sea depth. Also, siphonous algae have invaded the diverse sea environment around the world [Williams SL & Smith JE. (2007) Annu. Rev. Ecol. Evol. Syst. 38: 327–359.]. Therefore, a light-harvesting system requires adaptation to the conditions of alternating extreme irradiation. Siphonous algae have a major light-harvesting complex (LHCII) containing unique carbonyl carotenoids, siphonaxanthin (Sx) [Anderson JM (1983) Biochim. Biophys. Acta 724:370-380.]. Sx has a carbonyl group attached to the conjugated polyene, which induces the intramolecular charge transfer (ICT) characters. In siphonous LHCII, the strong coupling between the ICT-state and the S_1 state may result in the utilization of the green light. In contrast, the photoprotective mechanism of the species has long been unknown because they have no xanthophyll cycle [Christa G. et al. (2017) New Phytol. 214:1132-1144.]. Moreover, Sx contains a hydroxymethyl group adjacent to the conjugated carbonyl group, but the biological significance of this unconjugated hydroxy group has not yet been clarified.

In this study, we cultivated siphonous alga, *Codium fragile* under red, green, and blue light in various irradiances and traced the diurnal changes in the pigment composition accumulated for 7 days. Then, we discovered the accumulation of a novel Sx biosynthetic precursor that lost the hydroxymethyl oxygen (deoxy-Sx). We also found that the deoxy-Sx preferentially binds to the siphonous LHCII instead of Sx [Seki S. et al. (2022) FEBS Lett. https://doi.org/10.1002/1873-3468.14357]. Furthermore, by comparing the optical properties of Sx and deoxy-Sx in several organic solvents, we discovered that deoxy-Sx has a lower ICT character than that of Sx. We will discuss a plausible photoprotective mechanism of the Sx to deoxy-Sx conversion in siphonous LHCII.

111 Pre Recorded Poster

Assessment of photosynthetic activity and singlet oxygen production in Symbiodiniaceae protoplasts in a microfluidic platform

Faiza Bashir[1,2], Imre Vass[1], Milan Szabo[1,3]

[1]Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary. [2]Doctoral School of Biology, University of Szeged, Szeged, Hungary. [3]Climate Change Cluster, University of Technology Sydney, Ultimo, Australia

Symbiodiniaceae is an important dinoflagellate family which lives in endosymbiosis with corals. As coral reefs are currently under threat from climate change, it is of high importance to understand the stress avoidance mechanisms of Symbiodiniaceae. Reactive oxygen species (ROS) are central players in mediating various stress responses; however, the detection of certain ROS for example singlet oxygen is still far from definitive at the single-cell level in Symbiodiniaceae cells due to the hampered uptake of fluorescent sensor dyes through the cell wall. Protoplast technology therefore provides a potential tool for studying oxidative stress. Previous studies have successfully applied cellulose-based protoplast preparation in Symbiodiniaceae; however, the suitability of protoplasts for uptake of ROS-sensing fluorescent dyes remained unclear. We applied a microfluidics-based platform that enabled protoplast isolation from individually trapped Symbiodiniaceae cells, by using a precisely adjusted flow of cell wall digestion enzymes. Trapped single cells exhibited characteristic morphological changes, alterations of cell division and photosynthetic activity during protoplast formation. However, cells regained photosynthetic activity and motility, indicating that protoplasts could be regenerated into normal cells in the microfluidic traps. Physiologically competent protoplasts in microfluidic chambers showed the uptake of the singlet oxygen sensing fluorescent dye, therefore the method allowed for the first time the visualization of the intracellular localization of singlet oxygen in Symbiodiniaceae. The microfluidics-based protoplast technology may open new avenues for studying oxidative stress in parallel with the assessment of photosynthetic activity and morphological changes of Symbiodiniaceae.

112 Pre Recorded Oral

Linking photosynthesis to leaf carbonyl sulfide uptake through stomatal controls

Wu Sun[1], Joe Berry[1], Dan Yakir[2], Ulli Seibt[3]

[1]Department of Global Ecology, Carnegie Institution for Science, Stanford, California, USA. [2]Department of Earth and Planetary Sciences, Weizmann Institute of Science, Rehovot, Israel. [3]Department of Atmospheric and Oceanic Sciences, University of California, Los Angeles, Los Angeles, California, USA

At canopy to continental scales, we have limited ways to quantify photosynthesis, because any flux measurements necessarily entail a substantial respiratory signal. Carbonyl sulfide (COS) has emerged as an atmospheric tracer for photosynthesis in vascular plants. The COS approach to photosynthesis is based on the coupling between leaf COS and CO_2 fluxes through stomatal and mesophyll diffusion. This plant uptake can lead to strong continental drawdown of atmospheric COS concentrations. To extract information about photosynthesis from COS measurements, however, requires knowing the relationship between leaf COS uptake and photosynthesis, which varies with environmental conditions and across species.

We show that variability in the leaf COS : CO_2 relative uptake (LRU) ratio results from the coupling between stomatal conductance and photosynthesis, as explained by the Ball–Berry stomatal conductance model and the Farquhar–von Caemmerer–Berry photosynthesis model [Farquhar et al. (1980) Planta 149:78–90]. We present a framework for interpreting LRU changes with light, humidity, and ambient CO_2 concentration. This framework reproduces observed LRU responses for a collection of C_3 and C_4 species in laboratory incubations and a freshwater marsh plant in the field. The finding that LRU variability embodies stomatal conductance–photosynthesis coupling will aid in accurate quantification of photosynthesis at ecosystem to continental scales from globally distributed COS flux and concentration measurements.

113 Pre Recorded Poster

Isolation and characterization of PSI-IsiA supercomplexes from the cyanobacterium *Anabaena* sp. PCC 7120

Naoki Tsuboshita[1], Takehiro Suzuki[2], Naoshi Dohmae[3], Jian-Ren Shen[4], Ryo Nagao[4]

[1]Graduate School of Natural Science, Okayama University, Okayama 700-8530, Japan. [2]Biomolecular Characterization Unit, RIKEN Center for Sustainable Resource Science, Saitama, Japan. [3]Biomolecular Characterization Unit, RIKEN Center for Sustainable Resource Science, Saitama 351-0198, Japan. [4]Research Institute for Interdisciplinary Science, Okayama University, Okayama 700-8530, Japan

Iron is one of the essential elements for oxygenic photosynthetic organisms. Upon iron deficiency, cyanobacteria produce iron-stress-induced family proteins including IsiA to maintain their photosynthetic ability. IsiA is grouped into light-harvesting pigment proteins binding to a photosystem I (PSI) trimer in either an open or closed ring, and plays an important role in supplying excitation energy to PSI. Most cyanobacteria having the trimeric PSI cores possess a single *isiA* gene, whereas some cyanobacteria have multiple *isiA* genes. The cyanobacterium *Anabaena* sp. PCC 7120 (*Anabaena*) has four *isiA* genes (*isiA1*, *isiA2*, *isiA3*, *isiA5*), and its PSI is a tetramer. Two questions arise as to how IsiAs bind to a PSI tetramer and whether all four *isiA* genes are expressed or not. In this study, we examined the gene expression and association patterns of the four IsiAs with PSI in *Anabaena*. The PSI-IsiA supercomplex was isolated from wild-type cells grown under an iron-deficient condition using anion-exchange chromatography, followed by trehalose density gradient centrifugation. All four IsiAs were found in the PSI-IsiA supercomplex by mass spectrometry. Among them, IsiA1 showed the highest density of the band on the SDS-PAGE gel, and IsiA2 exhibited the largest molecular weight. BN-PAGE analysis showed that the PSI-IsiA supercomplex consists of a PSI monomer with several IsiA subunits. This was verified by negative-staining electron microscopy. Thus, we revealed the expression of the four types of IsiAs and their binding properties to PSI. Based on these results, we discuss why the *Anabaena* IsiAs are not associated with a PSI tetramer.

114 Pre Recorded Poster

Characterization of the wave phenomenon in flash-induced fluorescence relaxation and its importance in microalgae

Priyanka Pradeep Patil[1], Sabit Mohammad Aslam[1,2], Milán Szabó[1,3], Imre Vass[1]

[1]Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary. [2]Doctoral School of Biology, University of Szeged, Szeged, Hungary. [3]Climate Change Cluster, University of Technology Sydney, Ultimo, Australia

Microalgae are considered as important model organisms to investigate the biology of photosynthesis, moreover, they are valuable sources of biomolecules for several biotechnological applications. Photosynthetic electron transport processes provide the energetic balance of the utilization of the energy of light harvesting. It consists of linear electron flow, which ensures the basic functions of photosynthetic productivity and carbon fixation and alternative electron flow, e.g. the cyclic electron flow, which plays a role in fine-tuning of photosynthesis and balancing the ATP/NADPH ratio under stress conditions. In this work, we characterized the cyclic electron transport pathways in microalgae species that are important model organisms (e.g. *Chlamydomonas reinhardtii*) or have a high importance in applied research and industry (*Chlorella sorokiniana*, *Haematococcus pluvialis*, *Dunaliella salina*, *Nannochloropsis limnetica*), by using flash-induced chlorophyll fluorescence relaxation kinetics. Flash-induced fluorescence relaxation is a powerful tool to monitor the reoxidation reactions of the reduced primary quinone acceptor, QA− by QB and the plastoquinone (PQ) pool, as well as the charge recombination reactions between the donor and acceptor side components of Photosystem II (PSII). Our results showed that a wave phenomenon appeared in the fluorescence relaxation profiles of microalgae, however, its characteristics were quite different compared to the wave phenomenon previously identified in cyanobacteria. Furthermore, the wave phenomenon showed species-specific features, as we found that different conditions were required to induce the wave in the different species. Furthermore, the wave phenomenon could be blocked by specific inhibitors of the cyclic electron flow pathways. Therefore, the fluorescence wave phenomenon is an important indicator of the redox reactions of the plastoquinone pool and its interconnected electron transport pathways in microalgae.

115 Pre Recorded Poster

Photosynthetic responses of the coral endosymbiont algae Symbiodiniaceae under acute heat stress, revealed by flash-induced fluorescence relaxation kinetics

Sabit Mohammad Aslam[1,2], Priyanka Pradeep Patil[1], Imre Vass[1], Milán Szabó[1,3]

[1]Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary. [2]Doctoral School of Biology, University of Szeged, Szeged, Hungary. [3]Climate Change Cluster, University of Technology Sydney, Ultimo, Australia

Symbiodiniaceae is a family of unicellular dinoflagellate algae, which live in endosymbiosis with corals. This symbiotic relationship is threatened by global climate change. Global temperature increase has a significant impact on the endosymbiont algae, disturbing the whole symbiosis, which leads to coral bleaching. The heat tolerance is strongly determined by the species (formerly genetic clades) harbored by the coral host, however, the relationship between heat tolerance and the regulation of the photosynthetic electron transport processes are not fully understood. The aim of the current work was to investigate the photosynthetic efficiency and the physiological role of alternative electron transport pathways in different species of Symbiodiniaceae under acute heat stress. Flash-induced chlorophyll fluorescence relaxation is a useful tool to monitor various components of the photosynthetic electron transport chain, the redox reactions of plastoquinone pool and the alternative electron transport processes, which are manifested in a so-called wave phenomenon in cyanobacteria e.g. under microaerobic conditions. However, this phenomenon has not been investigated so far in Symbiodiniaceae. We found that microaerobic treatment alone was not sufficient to induce the wave phenomenon, but it required the decrease of the activity Photosystem II relative to Photosystem I (by acute heat stress) in combination with strongly reduced plastoquinone pool (microaerobic conditions). The characteristics of the fluorescence wave were temperature dependent and strain-specific and possibly related to the transient oxidation and re-reduction of the plastoquinone pool. Furthermore, the wave phenomenon appears to be related to alternate electron flow pathways, which also display strain-specific features, as revealed by accompanying fluorescence measurements such as post-illumination chlorophyll fluorescence rise and fast fluorescence induction kinetics. Therefore, the wave phenomenon in the flash-induced chlorophyll fluorescence relaxation could be used as a potential indicator of heat stress in coral symbiont algae.

116 In Person Oral

Improving the primary carboxylase of C_4 photosynthesis to boost crop production under future climates

Robert Sharwood[1], Rowarne Leith[2], Robert Furbank[2], Susanne von Caemmerer[2], Maria Ermakova[2]

[1]Western Sydney University, Sydney, NSW, Australia. [2]Australian National University, Canberra, ACT, Australia

An escalating challenge facing Australia's grain crop production is sustaining the supply of nutritious food within water limited environments. Extreme climate events and decline in available water are threatening crop yield and quality necessitating new solutions to ensure optimal water use within future food systems. We have sought to identify natural biochemical solutions within C_4 plants that confer improved responses to carbon assimilation and plant productivity under future climates. We have taken a transgenic approach to overexpress the primary carboxylase of C_4 photosynthesis - phosphoenolpyruvate carboxylase (PEPC) in *S. viridis* and a biochemical approach to investigate the *in vitro* biochemistry of PEPC across biochemical subtypes within the Paniceae tribe of C_4 grasses. We successfully expressed recombinant PEPC isoforms within *E. coli* and discovered that the enzyme predominated in its dimeric form. This raised new questions on the regulation of tetramer formation and catalytic competency of the enzyme. In addition, we discovered that the PEPC isoform from the PCK subtype were catalytically superior and contained an amino acid signature that corresponds to decreased malate inhibition which is crucial for increasing flux through the CCM. Therefore, we have identified a number of new avenues to improve carbon in C_4 plants particularly under abiotic stress conditions. An update on the current progress of this research will be presented.

117 In Person Poster

Tracking water oxidation through time-resolved FTIR spectroscopy

Paul Greife[1], Matthias Schönborn[1], Matteo Capone[2], Ricardo Assunção[1], Daniele Narzi[2], Leonardo Guidoni[3], Holger Dau[1]

[1]Freie Universität Berlin, Berlin, Germany. [2]University of L'Aquila, L'Aquila, Italy. [3]University of L'Aquila, L'Aquila, Germany

In the light-driven reaction cycle of Photosystem II (PSII), two 'substrate' water molecules are oxidized resulting in the release of O_2 at the oxygen-evolving complex (OEC), which consists of a Mn_4Ca-oxo cluster and its water-protein environment. Driven by a sequence of light flashes, the OEC cycles through its five S-states, alternating the release of electrons and protons.

Infrared spectroscopy (IR) offers unique insight into these processes, as even slight structural or environmental changes are impactful. Spectrally extensive Fourier Transform IR (FTIR) investigations of the semi-stable S-state intermediates have been performed under near-native and perturbed conditions [Schuth et al. (2017) Biochemistry 56:6240-6256; Debus (2015) Biochim. Biophys. Acta – Bioenergetics 1847:19-34]. Typically, rapid-scan FTIR results in insufficient time resolution for PSII. Therefore, recent IR works have focused on resolving kinetics at specific bands [Takemoto et al. (2019) Biochemistry 58:4276-4283; Mäusle et al. (2020) J. Chem. Phys. 153:215101], sacrificing broader spectral context.

To investigate events associated with the PSII photocycle, we heavily modified the sample compartments of two commercial FTIR spectrometers to push the time resolution to their respective limits. Furthermore, optimizations in sample preparation and data analysis allows for the efficient processing of thousands of excitation sequences.

In a step-scan experiment, more than 230,000 excitation cycles of dark-adapted PSII were recorded and combined to resolve processes from 50 µs to 130 ms over a range of 1800-1300 cm^{-1}, for all S-state transitions. Alongside quantum chemical simulations, analysis of spectral features associated with the evolution of dioxygen (~2.5 ms) during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition identifies a critical transient carboxylate deprotonation preceding the formation of an oxygen radical at the OEC. In another series of experiments, sampling up to 3800 cm^{-1} while pushing rapid-scan time resolution below 10 ms allows for the investigation of PSII systems with slowed kinetics (mutations, water analogues, etc.).

118 In Person Oral

The *Gossypium* genus: an untapped pool of novel photosynthetic traits for cotton?

Demi Sargent[1,2], Warren Conaty[2], David Tissue[1], Spencer Whitney[3], Michael Bange[4], Robert Sharwood[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia. [2]Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation, Narrabri, NSW, Australia. [3]Research School of Biology, Australian National University, Canberra, ACT, Australia. [4]Cotton Seed Distributors, Wee Waa, NSW, Australia

Increasing annual temperatures and more frequent and extreme heatwaves as a result of climate change are predicted to reduce cotton yield potential. 'Future-proofing' cotton cultivars (*Gossypium hirsutum*) to withstand rising temperatures is required to maintain and further improve cotton yields under increasingly challenging future climates. Improving the resilience, performance and efficiency of photosynthesis is a potential target to improve crop yields under suboptimal conditions. Exploiting the natural diversity of photosynthetic processes in crop varieties and wild relatives is a promising approach.

This research aims to elucidate the potential to exploit photosynthetic traits from within the *Gossypium* genus in the development of climate-adapted cotton cultivars. This research interrogated a range of diverse *Gossypium* species for variation in photosynthetic performance under a variety of temperature-manipulation experiments.

Interspecies variation in leaf photosynthetic gas exchange, mesophyll conductance and Rubisco catalytic properties was revealed. The Australian *Gossypium* species exhibited the greatest photosynthetic performance at high temperatures at both the leaf and biochemical level. The Diurnal Canopy Photosynthesis and Stomatal Conductance (DCaPST) simulation platform was used to test the effect of exploiting photosynthetic traits from one Australian *Gossypium* species (A1) on cotton radiation use efficiency (RUE). These simulations showed that cotton RUE and therefore biomass accumulation rate could be increased by up to ~5% at temperatures above 33°C by introducing photosynthetic components from the Australian *Gossypium* species A1.

In conclusion, this research has revealed that the *Gossypium* species are a valuable target for sourcing novel photosynthetic and heat tolerance traits.

119 Pre Recorded Poster

Time-resolved rapid-scan FTIR measurements on photosynthetic oxygen evolution by Photosystem II

Janosch Brandhorst, Paul Greife, Holger Dau

Freie Universität Berlin, Berlin, Berlin, Germany

Photosystem-II (PSII) of plants and cyanobacteria splits water, thereby providing electrons and protons eventually needed for carbohydrate formation from CO_2. Photon absorption triggers charge separation within PSII. An electron hole is transferred to the oxygen evolving complex (OEC) consisting of a Mn4Ca-µ-oxo cluster and its immediate environment. By sequentially absorbing four photons, four oxidizing equivalents are accumulated at the OEC. This process is coupled to a series of electron/proton transfer reactions (S-state cycle). The final redox potential is sufficient to oxidize water and leads to O_2 formation.

Infrared spectroscopy is a method widely used to analyze changes of protein conformations, solvent accessibility of specific protein domains, local electrostatic environments, -bond networks, and protonation states of individual amino acids. In Fourier-transform infrared spectroscopy (FTIR), a large number of spectra can be acquired rapidly by fast interferometer-mirror movement. We observe PSII processes using rapid-scan FTIR with a time resolution of down to 10 ms, covering a large wavenumber range in a single measurement (1000-4000 cm-1). We use a commercial FTIR spectrometer with a modified vacuum sample chamber and a high degree of automated data acquisition. After illuminating PSII samples with saturating laser flashes (5 ns, 532 nm) we can record thousands of excitation sequences of dark-adapted PSII. This allows us to study transient processes in PSII with an excellent signal-to-noise ratio.

The oxygen evolution transition can be slowed down by targeted mutagenesis [e.g. Preston et al. (2013) Biochemistry 52:6824-6833] or by ammonia binding [Assunção et al. (2019) BBA 1860:533-540] to tens of milliseconds. Using rapid-scan FTIR, we aim to observe water splitting and O2 formation in a time-resolved manner to gain insight into the mechanisms of perturbed (slow) PSII. First results on PSII verifying the instrument performance will be presented and a scheme is proposed to break the 10 ms limit.

120 In Person Poster

S-state Transitions of Photosystem II from Spinach and *T. elongatus* – Insight by Time-Resolved Single-Frequency Infrared Spectroscopy

Sarah M. Mäusle[1], Philipp S. Simon[1,2], Rebeca Perez[1,3], Yvonne Zilliges[1], Holger Dau[1]

[1]Freie Universität Berlin, Berlin, Germany. [2]Lawrence Berkeley National Laboratory, Berkeley, California, USA. [3]AZTI - Member of Basque and Technology Alliance, Sukarrieta, Spain

The Photosystem II (PSII) of plants and cyanobacteria catalyses the oxidative splitting of two water molecules, thereby yielding dioxygen, four protons and the four electrons that are transferred to the PSII acceptor side. When driving PSII by laser flashes through its S-state cycle, variations in protein-internal -bond networks and electric fields as well as metal-ion oxidation can be investigated by infrared (IR) difference spectroscopy.

We have developed a time-resolved single-frequency (TRSF) IR experiment with automated samples exchange that facilitates tracking of electron transfer as well as protonation dynamics at high temporal resolution (~50 ns), which previously was applied to determination of activation energies [Mäusle et al. (2020) J. Chem. Phys. 153:215101]. PSII membrane particles from spinach were investigated in the mid-IR regime at several pH values (pH 5.4, 6.2 and 7.0), in H_2O and D_2O. The transients of the oxygen-evolution transition ($S_3 \rightarrow S_4 \rightarrow S_0 + O_2$) revealed a relatively small /D kinetic isotope effect (KIE) of 1.2-1.4 for the rate-determining 2.8 ms electron transfer (ET) step. For the pre-ET kinetics assignable to proton removal from the oxygen-evolving complex, we observed a larger KIE (2.3-2.6). These results show that the reactions previously detected i.a. by photothermal spectroscopy [Klauss et al. (2012) Proc. Natl. Acad. Sci. U.S.A. 109:16035-16040] are 'sensed' by specific carboxylate and amide vibrations.

For the $S_2 \rightarrow S_3$ transition, a KIE-value of about 2 supports the notion of a proton-coupled ET (PCET) step. For the pre-ET phase of ~100 µs, a KIE of 2.3-2.8 and a significant pH-dependency contrasts previous TRSF-IR reports on PSII core particles from *T. elongatus* [Takemoto et al. (2019) Biochemistry 58:4276-4283]). Performing our own experiments on *T. elongatus* PSII confirmed surprising species-specific differences.

Aside from future avenues of the TRSF-IR experiment, the relation between our time-resolved IR results and the mechanism of photosynthetic water oxidation is discussed.

121 In Person Poster

The crucial role of the PTOX and CET pathways in light-dependent reactions in mesophyll chloroplasts of C3 and C4 plants

Aleksandra Urban, Paweł Rogowski, Elżbieta Romanowska

Department of Molecular Plant Physiology, Institute of Environmental Biology, University of Warsaw, Warsaw, Poland

Apart from the linear electron flow (LET), several regulatory and alternative electron transfer pathways exist in chloroplasts, among them the cyclic electron transport around photosystem I (CET) and PTOX enzyme which oxidizes the plastoquinone pool. In CET electrons passed to ferredoxin to the PQ/PQH_2 through the PGR5/PGRL1 complex or NDH complex, ATP is generated but without NADPH production. Regulation of the alternative electron transfer pathways plays a major role in adjusting photosynthesis to changes in light intensity. It remains unclear how C4 plants regulate the flux through these pathways as compared to C3 plants in response to environmental changes and how these pathways adjust ATP content for metabolism. We conducted a comparative study of the CET pathways in maize (C4) and pea (C3) mesophyll chloroplasts by measuring the activity of PSI in the presence of the inhibitors PGR5/PGRL1 complex and PTOX, estimating the content of thylakoid proteins that participate in CET, and determining ATP content and ATP synthase activity, and 77K fluorescence analysis. We found that NDH-CET plays an important role in maize chloroplasts, whereas PGR5/PGRL1 proteins play a dominant role in pea chloroplasts. The obtained results indicate a substantial involvement of PTOX activity in regulating electron flow in response to ATP demand. Based on the study findings, we propose a model of regulation of the linear electron transport (LET) and CET pathways in mesophyll chloroplasts of C4 and C3 plants under low light intensity based on ATP level and PTOX activity.

122 Pre Recorded Poster

Long-term acclimation of the photosynthetic apparatus of an extremophilic red microalga *Cyanidioschyzon merolae* to nickel stress

Francesca Marchetto[1], Sergio Santaeufemia[1], Daniel Solymosi[2], Yagut Allahverdiyeva- Rinne[2], Magdalena Pich[3], Eliza Kurek[3], Ewa Bulska[3], Joanna Kargul[1]

[1]Solar Fuels Laboratory, Center of New Technologies, University of Warsaw, Warsaw, Poland. [2]Molecular Plant Biology Unit, Department of Life Technologies, University of Turku, Turku, Finland. [3]Analytical Expert Center, Center for Biological and Chemical Sciences, University of Warsaw, Warsaw, Poland

The primordial conditions on Earth are similar to the natural ecosystem of an extremophilic red microalga *Cyanidioschyzon merolae*. In fact, this phototroph lives at high temperatures (up to 56°C), low pH (0.02 – 4) and in the presence of various heavy metals.

The aim of this work was to evaluate the physiological changes in *C. merolae* cells during their long-term adaptation to nickel stress. The cells were exposed to 1-6 mM Ni concentrations over a period of 15 days. Examination of the growth curves of Ni-treated cell suspensions demonstrated the capability of *C. merolae* to adapt to high concentrations of Ni. In particular, the growth rate of cells treated with 1 mM Ni showed no significant difference with the untreated control. On the other hand, at 3 mM Ni the cellular growth was partially inhibited as demonstrated by a 2.5-fold decrease of optical density at 750 nm and respectively a 2.12- and 1.66-fold lower total chlorophyll a and carotenoid content compared to the control sample followed by the full recovery of these parameters after 7 days of Ni treatment.

The DUAL-PAM analysis of key photosynthetic parameters demonstrated that *C. merolae* cells exposed to up to 3 mM Ni were photosynthetically viable. Specifically, the maximum quantum efficiency of PSII (Fv/Fm) after 3 days of 3 mM Ni treatment decreased 1.88 fold compared to the control; however, on day 5 this parameter was fully recovered. The non-photochemical quenching (NPQ) mechanism in Ni-treated cells was similar to that in cyanobacteria. The latter parameter was lower in 3 mM treated cells by 1.8-fold compared to the control cells on day 15 of the Ni treatment.

Overall, this work demonstrates the remarkable resilience of the photosynthetic apparatus of *C. merolae* to high Ni concentrations.

123 Pre Recorded Poster

Site-dependent state transition and ultrastructural changes of thylakoid in *Chlamydomonas*

XianJun Zhang[1,2], Yuki Fujita[1], Naoya Kaneda[1], Ryutaro Tokutsu[3], Jun Minagawa[3], Shen Ye[1], Yutaka Shibata[1]

[1]Tohoku University, Sendai, Miyagi, Japan. [2]Division for Interdisciplinary Advanced Research and Education, Tohoku University, Sendai, Miyagi, Japan. [3]National Institute for Basic Biology, Okazaki, Aichi, Japan

Photosynthetic organisms have developed a regulation mechanism called state transitions (ST) to rapidly adjust the excitation balance between the two photosystems by migration of light-harvesting complex II (LHCII). Though many researchers have assumed coupling of the dynamic transformations of the thylakoid membrane to ST, it remains elusive. To clarify the above-mentioned coupling in live Chlamydomonas cells, here we used two advanced microscope techniques, the excitation-spectral microscope (ESM) developed recently by us [Jana&Shibata, (2020) Biophys. J. 118:36-43], and the super-resolution imaging based on structured illumination microscopy (SIM). ESM enables the chlorophyll-b bound to LHCII-related spectral component at each pixel (~250 nm) on fluorescence images [Zhang et al. (2021) Plant Cell Phys. 62:872-882] and SIM provides a satisfactory spatial resolution (~120 nm) to resolve the ultrastructural changes of the thylakoid in the sub-micron scale. Our results provided two new insights into the understanding of the molecular events during ST: (i) the ESM observations revealed ST-dependent spectral changes upon repeated ST inductions. Surprisingly, it clarified less significant ST occurrence in the region surrounding the pyrenoid, which is a subcellular compartment specialized for the carbon-fixation reaction, than the other domains. This suggests the intracellular site-dependent ST activity. (ii) the SIM observations resolved partially irreversible fine thylakoid transformations induced by the ST-inducing illumination. Such thylakoid transformation was also observed in mutant cells lacking LHCII phosphorylation. This result revealed that the fine thylakoid transformation is not induced solely by the LHCII phosphorylation. Although some studies have assumed the necessity of LHCII phosphorylation for the thylakoid-membrane regulation in higher plants, our measurements suggested that LHCII phosphorylation is not necessary for the thylakoid transformation upon ST illumination in Chlamydomonas. The present study clarified the highly flexible nature of the thylakoid ultrastructure which is even susceptible to the ordinary photosynthetic activity [Zhang et al. (2022) BioRxiv].

124 Pre Recorded Poster

Modelling photosynthetic physiology performance of extremophilic red microalgae in response to nickel stress

Sergio Santaeufemia[1], Francesca Marchetto[1], Małgorzata Śliwińska[2], Kamil Sobczak[3], Aleksandra Naziębło[4], Małgorzata Wierzbicka[4], Joanna Kargul[1]

[1]Solar Fuels Laboratory, Center of New Technologies, University of Warsaw, Warsaw, Poland. [2]Tissue Structure and Function Imaging Laboratory, Nencki Institute of Experimental Biology, Warsaw, Poland. [3]Laboratory of Microscopy and Electron Spectroscopy, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland. [4]Laboratory of Ecotoxicology, Institute of Botany, Faculty of Biology, University of Warsaw, Warsaw, Poland

Organisms living in extreme environments have attracted considerable attention in recent years for their ability to survive such harsh conditions. *Cyanidiales* are the only photosynthetic eukaryotes living in extreme conditions associated with volcanic activity, characterised by high temperature, low pH and high concentrations of heavy metals. Unexpectedly, the literature is scarce on the information on the photosynthetic activity in these organisms.

The aim of this study was to dissect the adaptative mechanisms and structural changes of the photosynthetic apparatus of a model red microalga *Cyanidioschyzon merolae* upon long-term exposure of the cells to high Ni concentrations (1-10 mM). Microscopic and elemental analysis of the *C. merolae* cells exposed to various Ni concentrations showed the remarkable survival capability of this phototroph. Confocal microscopy analysis of the cells exposed to 3 mM Ni showed their high survivability (over 80%). The TEM-EDX and SEM-EDX visualization of the cells demonstrated that Ni accumulates mainly on the surface of the cells. The 77K fluorescence analysis showed that *C. merolae* cells exposed to different Ni concentrations exhibit a dynamic adjustment of the functional PSI and PSII light harvesting antenna size. Finally, it is showed that oxygen evolution activity of *C. merolae* cells exposed up to 1 mM Ni was comparable to the untreated control.

Overall, this work shows the high adaptability and dynamic remodelling of the photosynthetic apparatus of *C. merolae* cells upon the exposure to high Ni concentrations. Based on the SEM-EDX and TEM-EDX results, it is postulated that the functional resilience of the *C. merolae* photosynthetic apparatus in the cells exposed to the heavy metal stressor is due to the fast extrusion of Ni cations from the cytoplasm/chloroplast as confirmed by the observed accumulation of this metal on the cell surface and high photosynthetic activity.

125 Pre Recorded Poster

Near-infrared excitation of the oxygen-evolving complex: An electronic structure perspective

Maria Drosou, Dimitrios A. Pantazis

Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany

The oxygen-evolving complex (OEC) of Photosystem II catalyses the light-driven oxidation of water to molecular oxygen by going through a five-state cycle, S_0-S_4, that includes four one-electron oxidation steps of the Mn_4CaO_5 cluster, mediated by a redox-active tyrosine Y_Z. Interesting phenomena arise due to absorption of near-infrared (NIR) light by the OEC. For example, NIR illumination of the low-spin S_2 state induces the transition to forms exhibiting high-spin EPR signals, [Boussac et al. (1996) Biochemistry 35:6984–6989] while in the S_3 state, NIR illumination of an EPR-invisible form facilitates the oxidation of Y_Z by the Mn_4CaO_5 cluster resulting in a modified $(S_2Y_Z^{\bullet})'$ split EPR signal. [Ioannidis et al. (2000) Biochemistry 39:5246–5254]

NIR absorption is attributed to the Mn_4CaO_5 cluster itself, but the nature of electronic excitation remains unknown. Proposed photochemical processes include intervalence charge transfer between two Mn^{IV} and Mn^{III} [Boussac et al. (1996) Biochemistry 35:6984–6989], d-d transition of the trigonal bipyramidal d^3 Mn^{IV} center in the S_3 state, [Retegan et al. (2016) Chem. Sci. 7:72–84] and ^4A–^2E spin-flip excitation of a d^3 Mn^{IV} site. [Morton et al. (2018) BBA - Bioenergetics 1859:88–98] In this work, we investigate the nature of NIR-induced excitations using multireference ab initio methods on computational models of the OEC. The detailed description of the NIR-induced processes has important implications for elucidating the electronic structure of the OEC and for identifying intermediates involved in the S_2 to S_3 transition.

126 Pre Recorded Poster

Quenched LHCII accumulation around PSI in *Chlamydomonas* cells in State 2 revealed by cryo-fluorescence lifetime and spectral microscopy

Yuki Fujita[1], XianJun Zhang[1], Yutaka Shibata[1], Ahmed Ali[1,2], Shen Ye[1],

[1]Tohoku University, Sendai, Miyagi, Japan. [2]Centre Énergie Matériaux Télécommunications, Varennes, Québec, Canada

State transitions (ST) is a short-term regulation mechanism of photosynthetic light-harvesting to maintain the excitation balance between the two photosystems (PSs). The process underlying the ST involves migrations of the light-harvesting complex II (LHCII) and their dynamic connection to and detachment from each of PSs. Clarifying the functional state and intra-chloroplast localizations of LHCII is key to understanding the actual molecular processes during ST. Here, the fluorescence lifetime imaging technique is combined with the cryo-spectral microscope recently developed by us [Fujita et al. (2018) J. Photo. Photo. B 185:111-116] to visualize the light-harvesting activity at various intra-chloroplast local regions. We investigated the local light-harvesting activity in *Chlamydomonas* cells locked to state1 (LHCII associated with PSII) and state2 (a part of LHCII associated with PSI) by detecting the fluorescence decay at 680 nm at every pixel of an image. Simultaneous detection of the local fluorescence spectra at every pixel at 80 K enabled the visualization of intracellular segregation of PSs based on the specific fluorescence bands of PSII (685 nm) and PSI (715 nm). We identified a component showing rapidly decaying fluorescence with a lifetime of ca. 3 ps and emitting at around 676 nm by the observation. The component was assigned to the free LHCII that is isolated from both PSs and in a quenched state. This component was observed only for state2-locked cells and not observed for mutant cells lacking STT7 kinase which is responsible for the LHCII phosphorylation. Simultaneous spectral observations revealed the accumulation of this free LHCII in the PSI-enriched region in state2-locked cells. Further, we found a tendency of the free LHCII to be observed in the surrounding vicinity of the pyrenoid, which plays a potential role in CO_2 fixation. The result implied a connection between the free LHCII formations and the CO_2 fixation mechanism.

127 Pre Recorded Poster

Activation energies of the Photosystem-II reaction cycle from time-resolved infrared spectroscopy in the amide-II region

Mohamad Yahia Dekmak, Sarah M. Mäusle, Holger Dau

Freie Universität Berin, Berlin, Germany

In photosystem II (PSII) light drives the oxidation of P680, a protein-bound chlorophyll unit. $P680^+$ oxidizes the oxygen-evolving complex (OEC), consisting of a Mn_4Ca-oxo cluster and surrounding amino acids. Driven by 4 photons, the OEC accumulates the oxidizing equivalents needed to split 2 water molecules, releasing O_2 as a byproduct. Infrared (IR) spectroscopy is powerful in investigation of these processes. Monitoring kinetics at the amide-II band provides insight into structural or electrostatic changes coupled to electron and proton transfers (ET, PT).

Time-resolved single-frequency IR spectroscopy (TR-SFIR) is a suitable method to monitor absorption changes after laser-flash excitation, with high time-resolution (ca. 50 ns). To facilitate signal averaging in experiments on dark-adapted PSII, our setup employs an x- -movable sample holder for automated sample exchange. A sequence of saturating laser flashes advances the sample through the S-state cycle; the IR source is a quantum cascade laser tunable to single frequencies between 1300-1650 cm^{-1}.

We monitored the flash-induced IR changes of spinach PSII membrane particles at an amide-II band at 1544 cm^{-1} [Noguchi et al. (2003) Biochemistry 42, 6035-6042], allowing us to extract rates for several processes; experiments at 5-30 °C enable estimation of activation energies. The activation energy for a crucial PT step in the $S_2 \rightarrow S_3$ transition agrees with values from the carboxylate-stretching region [Mäusle et al. (2020) J. Chem. Phys. 153, 215101], obtained by constraining the subsequent ET rate to literature values. Steps to solve the significance problem in multi-exponential simulations of single-frequency transients are discussed.

In the $S_3 \rightarrow S_0$ an activation energy of ~260 meV was determined, agreeing with earlier results [Klauss et al. (2012) PNAS 109, 16035-16040]. We conclude that IR absorption changes of the amide-II band reflect various ET and PT kinetics in the PSII photo-cycle. The molecular origin of the informative amide-II absorption changes is discussed.

128 Pre Recorded Poster

Exploring NPQ kinetics and mechanistic correlations in the photoprotective state

Lennart A. I. Ramakers, Jeremy Harbinson, Emilie Wientjes, Herbert van Amerongen

Laboratory of Biophysics, Wageningen University, Wageningen, Netherlands

The sessile nature of plants combined with the variability of light intensity in the environment has led to the evolution of a range of processes to regulate and protect the photosynthetic apparatus within chloroplasts. In PSII the fast acting, major protective mechanisms are collectively referred to as non-photochemical quenching (NPQ), of which the fastest (qE) is activated by the protonation of the PsbS protein. While NPQ has been investigated for a long time, many questions about its molecular mechanisms are still unanswered.

NPQ in wild-type (wt), PsbS-lacking (npq4) and PsbS-over-expressing *Arabidopsis thaliana* was explored, utilising a combination of pulse-amplitude-modulated fluorometry and multivariate analysis, to unpick its various subprocesses. Multivariate analysis of NPQ induction curves over a range of light intensities revealed several phases driven by the thylakoid membrane proton gradient (ΔpH), inducing both PsbS protonation and the formation of zeaxanthin during the xanthophyll cycle (qZ). The first phase seems to proceed *via* a consecutive process containing two distinct quenching states, while the induction of quenching by zeaxanthin occurs on a slower time scale. While PsbS is essential for the fastest phase of the quenching process it is also required for qZ to participate in quenching. Taken together, the data indicates that all these processes work in concert offering a temporary initial high amount of photoprotection upon high-light activation while the slower qZ part allows the overall amount of NPQ to be better fine-tuned to the light conditions. Finally, the overall amount and rate of NPQ were found to be linearly related to the fraction of closed PSII reaction centres, illustrating a discrepancy from a more intuitive sigmoidal relationship. Inhibition of the xanthophyll cycle by dithiothreitol led to the collapse of the linear correlations leaving the plants with a constant degree of NPQ, irrespective of the fraction of closed reaction centres.

129 Pre Recorded Oral

Exciton states on the Type-I reaction center of green sulfur bacterium *Chlorobaculum tepidum* studied by genetic manipulation of bacteriochlorophyll *a* and the structure-based theoretical modeling

Tomohiro Azai[1], Hirozo Oh-oka[2], Hirotaka Kitoh-Nishioka[3], Akihiro Kimura[4], Shigeru Itoh[4]

[1]Col. Life Sci., Ritsumeikan Univ, Kusatsu, Japan. [2]Dept. Biology, Grad Sch. Science, Osaka Univ, Osaka, Japan. [3]Fac Science Engineering. Kindai Univ, Higashi-Osaka, Japan. [4]Dept. Physics, Grad, Sch. Science, Nagoya Univ, Nagoya, Japan

1) In the type-I reaction center of green sulfur bacterium *Chlorobaculum tepidum* (gRC), His-residues at four sites were genetically converted to Leu residues to selectively remove the bacteriochlorphyll *a* (BChl *a*) molecule ligated by each His-residue. Four His residues, which are conserved among the amino acid sequences of the type-I RC proteins of PSI, heliobacteria and green sulfur bacteria, were selected as the targets.

2) Each His-Leu conversion was found to differently modify the absorption spectrum of gRC measured at 77 K.

3) The Hamiltonian of gRC was calculated based on the excitonic coupling strength between the pigments and the site energy shift calculated for each pigment using the Poisson-TrESP and the charge density coupling- (CDC) methods.

4) The calculated gRC model reproduced the whole absorption spectra of gRC well and predicted the distribution of excitation on each pigment in each exciton state.

5) Pre Recorded His-Leu exchanges in silico on the gRC model qualitatively reproduced the experimentally-obtained absorption spectra indicating the specific contribution of each pigment to the exciton states.

6) The constructed gRC model was compared with those of Heliobacterial type-I RC [1,2] PSI, and PSI of Chl *d*-containinig *Acaryouchloris* [3].

[1. Kimura, A., Itoh, S. J. Phys. Chem. B (2018) 122, 11852–11859. 2. Kimura, A., Kitoh-Nishioka, H., Shigeta, Y., Itoh, S. J. Phys. Chem. B (2021) 125, 3727–3738. 3. Kimura, A. et al. J. Phys. Chem. B (2022) 126, 4009-4021]

130 Pre Recorded Oral

New insights into a dimeric PSI structure, a high resolution model of PSI:plastocyanin complex and PSI dependent hydrogen production

Michael Hippler[1,2], Laura Mosebach[1], Thi Thu Hoai Ho[1], Felix Buchert[1], Yuichiro Takahashi[3], Tamar Elman[4], Iftach Yacoby[4], Andreas Naschberger[5], Alexey Amunts[5]

[1]Institute of Plant Biology and Biotechnology, University of Muenster, Muenster, Germany. [2]Institute of Plant Science and Resources, Okayama University, Okayama, Japan. [3]Research Institute for Interdisciplinary Science, Okayama University, Okayama, Japan. [4]School of Plant Sciences and Food Security, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel. [5]Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden

Photosystem I (PSI) enables photo-electron transfer and regulates photosynthesis in the bioenergetic membranes of cyanobacteria and chloroplasts. Being a multi-subunit complex, its macromolecular organization affects the dynamics of photosynthetic membranes. Here we reveal a chloroplast PSI from the green alga *Chlamydomonas reinhardtii* that is organized as a homodimer, comprising 40 protein subunits with 118 transmembrane helices that provide scaffold for 568 pigments [Naschberger et al. (2022) Nat. Plants, accepted]. Our cryo-EM structure identifies that the absence of PsaH and Lhca2 gives rise to a head-to-head relative orientation of the PSI-LHCI monomers in a way that is essentially different from the oligomer formation in cyanobacteria. The light-harvesting protein Lhca9 is the key element for mediating this dimerization. The interface between the monomers is lacking PsaH/Lhca2 and thus partially overlaps with the surface area that would bind one of the LHCII complexes in state transition [Pan et al (2021) Nat. Plants 7:1119-1131]. These findings imply that PsaH/Lhca2 may play a regulatory role in defining the macromolecular organization of PSI -LHCI. Depletion of Lhca2 alters the regulation of photosynthetic electron transfer and hydrogen production in vivo [Ho et al. (2022) Plant Physiol. 189: 329-343], indicating physiological effects related to PSI-LHCI structural dynamics.

131 In Person Poster

Assignment of fluorescence bands of chlorophyll-*f* containing photosystem I by single-molecule spectroscopy

Rin Taniguchi[1], Toshiyuki Shinoda[2], Tatsuya Tomo[2], Shen Ye[1], Yutaka Shibata[1]

[1]Tohoku University, Sendai-shi, Miyagi, Japan. [2]Tokyo University of Science, Shinjuku-ku, Tokyo, Japan

A cyanobacterium containing chlorophyll (Chl)-*f* was discovered in 2011. Chl-*f* has a significantly red-shifted absorbance as compared with Chl-*a*. Photosystem I containing Chl-*f* (FR-PSI) forms a trimer, and each monomer binds ca. 90 Chls, in which seven are replaced with Chl-*f*. The fluorescence spectrum of FR-PSI at 80 K has three characteristic peaks at 745 nm, 755 nm, and 810 nm due to Chl-*f*. Recently, the structure of FR-PSI has been elucidated by the cryo-electron microscopy [Kato et al. (2020) Nature Comn. 11.238:1-10]. While the study determined the binding sites of the seven Chl-*f*s, the mechanism for the large red-shift especially for the 810-nm band has remained unclear. Assignment of the three fluorescence peaks to the seven Chl-*f*s in the structure will provide a hint to solve the puzzle. To do this, we conducted measurements of fluorescence polarization anisotropies of FR-PSI at the single molecule level. We will use the following property: when the trimers take random orientations, the differences in the relative orientations of the transition dipole moments (TDMs) with respect to the threefold axis of symmetry of the trimer result in different polarization anisotropies. For example, Chl-*f* with the TDM parallel/perpendicular to the threefold axis shows a high/low polarization anisotropy of fluorescence. The fluorescence polarization anisotropies of single-FR-PSI trimers were collected by the custom-built cryo confocal microscope with a rotating polarizer set in front of the detector. The distribution of the anisotropy values of each fluorescence band was obtained. We analyzed the obtained data by a clustering algorithm, Gaussian Mixture Models, and successfully separated the peaks into the clusters. However, the most red-shifted peak at 810 nm was not observed in the single-PSI measurement unfortunately. We will discuss a possible assignment of Chl-*f* to the fluorescence bands and possible reason for the missing 810-nm band.

132 Pre Recorded Poster

Enhancing the spectral range of light-harvesting pigment-protein complexes using synthetic chromophores incorporated into lipid membranes

Ashley Hancock[1], Sophie Meredith[1], Minjung Son[2], Muath Nairat[2], Tiejun Wei[3], David Swainsbury[4], Lars Jeuken[1], Kenichi Morigaki[5], Neil Hunter[6], Christopher Duffy[7], Gabriela Schlau-Cohen[2], Peter Adams[1]

[1]University of Leeds, Leeds, West Yorkshire, United Kingdom. [2]Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. [3]Queen's University, Kingston, Ontario, Canada. [4]University of East Anglia, Norwich, Norfolk, United Kingdom. [5]Kobe University, Kobe, Hyōgo, Japan. [6]University of Sheffield, Sheffield, South Yorkshire, United Kingdom. [7]Queen Mary University of London, London, United Kingdom

Light-harvesting (LH) protein complexes found in nature have the role of absorbing solar energy with high efficiency and transferring it to reaction centre complexes where the primary photochemical processes of photosynthesis take place. These LH complexes contain a suite of natural pigments that each absorb light at specific wavelengths, however, the natural combinations of pigments within any one protein do not cover the full range of visible light. In an initial proof-of-principle study, we enhanced the effective absorption range of the plant protein Light-Harvesting Complex II (LHCII) by developing a biohybrid system where LHCII was assembled into lipid-membrane vesicles alongside synthetic lipid-linked Texas Red (TR) chromophores [Hancock et al. (2019) Nanoscale 11: 16284-16292]. TR acted as an energy donor to LHCII via high efficiency Förster resonance energy transfer. More recently, we organised LHCII and TR into lipid nanodiscs to quantify the energy transfer processes occurring at the single-protein level [Hancock et al. (2021) Phys. Chem. Chem. Phys. 23: 19511-19524]. A combination of ultrafast spectroscopy, molecular dynamics simulations and Förster calculations, showed that efficient energy transfer between lipid-bound synthetic chromophores and chlorophyll molecules within the protein can occur at picosecond timescales, comparable to inter-pigment energy transfer rates in natural LH proteins. Finally, we present work that demonstrates the modularity and general application of the approach. Both hydrophilic pigments tethered to a lipid and hydrophobic "free" pigments can be effective excitation energy donors to both plant and bacterial LH protein complexes. The transfer efficiency depends upon the spectral overlap and physicochemical constraints. In summary, these bio-hybrid systems act as useful tools to investigate photophysical interactions between synthetic and natural chromophores and suggest that augmenting natural photosynthetic machinery with synthetic dyes could be an interesting prospect in nanotechnologies, for example, biosensors or biophotovolatics.

133 Pre Recorded Poster

Early-stage oxo-bridge formation of Mn catalysts

Aliya Tychengulova[1], Noemi di Stefano[2], Giuseppe Mattioli[3], Daniele Narzi[2], Leonardo Guidoni[2]

[1]Satbayev University, Almaty, Kazakhstan. [2]Department of Physical and Chemical Science, University of L'Aquila, L'Aquila, Italy. [3]CNR-Istituto di Struttura della Materia, Area della Ricerca di Roma 1, CP 10, Monterotondo Scalo, Italy

Photosynthetic water oxidation is an important source of energy for all life forms which occurs during the oxidation and deprotonation reactions of the oxygen-evolving complex (OEC) in photosystem II (PS II). The Mn_4Ca cluster is also of great interest to the scientific community due to the mechanism of its self-assembly through photooxidation of Mn ions, called photoactivation.

Despite recent progress in describing the mechanism of water splitting catalyzed by PSII, much less is known about the process of self-assembly of the Mn_4Ca cluster under the light, starting from the dissolved Mn2+. In the current situation, a theoretical approach to research can help rationalize the experimental data, shedding light on open questions regarding apo-PSII and the initial steps of photoactivation. The main idea of the work is to study the intermediate states of water-oxidizing catalysts in the process of self-assembly by computational chemistry methods based on the density functional theory. The present work can help to shed light on the structures of intermediate states of transition metal catalysts, as well as the mechanism of the second Mn^{2+} attachment and the formation of oxo-bridges. Moreover, the role of acetate counterions on the self-assembly mechanism and the structure of the Mn catalyst was studied.

134 Pre Recorded Poster

Architecture of the PSI-LHCI-Lhcp supercomplex from the prasinophyte *Ostreococcus tauri*

Asako Ishii[1], Jianyu Shan[2], Xin Sheng[2], Eunchul Kim[1], Akimasa Watanabe[1], Makio Yokono[1], Chiyo Noda[1], Zhenfeng Liu[2], Jun Minagawa[1]

[1]Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki, Japan. [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Prasinophytes, the early-branching green algae, are ubiquitous picophytoplankton in the ocean. *Ostreococcus tauri* is a model prasinophyte species for studying the functional evolution of the light-harvesting systems in photosynthetic eukaryotes. Here, we report a structure and function study on the photosystem I (PSI)-light-harvesting complex (LHC) I supercomplex from *O. tauri*. There are two types of PSI-LHCI supercomplexes in this prasinophyte; one has a larger antenna and is only present under the low light (LL) conditions, while another has a smaller antenna and is more abundant under the high light conditions. We solved a high-resolution cryo-EM structure of the LL-type supercomplex. The revealed structure consists of a PSI core with four Lhca (Lhca1-4) on one side, similar to those in plant PSI-LHCI supercomplex while a Lhca6-Lhca5 heterodimer associate between PsaG and PsaH on the other side, similar to Lhca2-Lhca9 heterodimer in *Chlamydomonas reinhardtii* PSI. Of particular interest is that there are three Lhcp trimers associated with the third side between Lhca6 and PsaK. This side of the PSI core is filled by one or two LHCII trimers in higher plants or green algae only when they are under state 2 conditions. Notably, the PSI-LHCI-Lhcp supercomplex in *O. tauri* does not exhibit far-red peaks in the 77 K fluorescence spectra unlike plants and *C. reinhardtii* [Swingley et al. (2010) Biochim. Biophys. Acta 1797:1458-1464]. We discuss absence of the “red chlorophylls” in the *O.tauri* structure, which were previously proposed in plants and *C. reinhardtii*.

135 Pre Recorded Poster

Photosystem II photoinhibition and the q_I energy dissipation: how many quenching sites exist and do they confer a photoprotective effect?

Wojciech Nawrocki, Maximiliaan Stada, Annamarie Zeekant, Roberta Croce

VU, Amsterdam, Netherlands

Photosystem II (PSII) is a water:plastoquinone photooxidoreductase, the first enzyme in photosynthetic linear electron flow. While light is required for the function of PSII, it also damages it in a process known as photoinhibition. Discovered more than 6 decades ago, photoinhibition results in an irreversible loss of PSII activity, requiring PSII supercomplex disassembly, followed by degradation and resynthesis of at least its reaction centre (RC) protein, D1 [1,2]. Concomitantly with a loss of charge separation capacity in the photoinhibited RC, a gain-of-function process in the form of q_I quenching is induced. Recently, we showed that q_I induced by white light is located within the PSII RC, and proposed that it stems from an oxidative event in the proximity of Chl_{D1} [3]. We extend this study to investigate q_I in terms of its nature- and role, including the spectral dependence of photoinhibition and q_I, the protective effect of q_I energy dissipation, and the limitations in steady-state electron transfer induced by photoinhibition. We discuss the results in the context of the hybrid photoinhibition model [4] including both donor- and acceptor-side impairment of PSII.

[1] E. Tyystjärvi, Photoinhibition of Photosystem II, in: International Review of Cell and Molecular Biology, Elsevier, 2013: pp. 243–303. https://doi.org/10.1016/B978-0-12-405210-9.00007-2.

[2] L. Li, E.-M. Aro, A.H. Millar, Mechanisms of Photodamage and Protein Turnover in Photoinhibition, Trends in Plant Science. 23 (2018) 667–676. https://doi.org/10.1016/j.tplants.2018.05.004.

[3] .J. Nawrocki, X. Liu, B. Raber, C. Hu, C. de Vitry, D.I.G. Bennett, R. Croce, Molecular origins of induction and loss of photoinhibition-related energy dissipation qI, Science Advances. 7 (2021) eabj0055. https://doi.org/10.1126/sciadv.abj0055.

[4] A. Zavafer, A theoretical framework of the hybrid mechanism of photosystem II photodamage, Photosynth Res. 149 (2021) 107–120. https://doi.org/10.1007/s11120-021-00843-1.

136 In Person Oral

Early steps of Photosystem II oxygen-evolving complex assembly are limited by proton release

Brandon Russell, David Vinyard

Louisiana State University, Baton Rouge, LA, USA

The Photosystem II (PSII) oxygen-evolving complex (OEC) is assembled from free Mn^{2+}, Ca^{2+}, and water during biogenesis and repair in a process termed photo-assembly. Building this Mn_4CaO_5 active site requires multiple water deprotonation events to form μ-hydroxo and μ-oxo ligands. Previously, we showed that the rate-limiting step of photo-assembly involves a deprotonation event facilitated by chloride. Using dual mode electron paramagnetic resonance spectroscopy, we observe the binding and oxidation of Mn^{2+} and the accumulation of Mn^{3+} in apo-OEC PSII membranes as a function of chloride concentration and pH. Our results show that chloride facilitated deprotonation events occur in photo-assembly both during the initial Mn^{2+} oxidation event and during the rate-limiting (light independent) step that follows. The ability to efficiently shuttle protons away during both metal cluster assembly and active catalysis is an essential design principle of PSII.

138 Pre Recorded Poster

Investigating the role of *Rubisco activase 3* (*ZmRca3*) in heat stress response in maize

Mae Antonette Mercado[1], Mia Sands[2], Steven Burgess[2], Donald Ort[2], Anthony Studer[1]

[1]Department Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA. [2]Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Rubisco activase (RCA) is a protein that activates Rubisco by removing sugar phosphates. In maize, two Rca genes encode α and β isoforms. The β isoform is encoded by *ZmRca1* (Zm00001eb164390), while the α isoform is encoded by *ZmRca3* (Zm00001eb164380). Previously published RNAseq datasets have shown increased expression levels of *ZmRca3* in maize when exposed to high temperature. Despite the correlation between heat stress and Rca-α expression in C_4 monocot species, its role in heat stress response has not been formally tested. With temperatures expected to rise with climate change, it is important to understand genes and genetic mechanisms that would enable plants to tolerate heat stress. With this in mind, we used transposable elements to identify *ZmRca3* mutants to investigate the role of *ZmRca3* in heat stress response. We used two types of transposable elements to knockdown *ZmRca3*: Dissociation (*Ds*) transposable element (I.S06.1977) and Mutator (*Mu*: mu1022546). Sanger Sequencing results showed that the *Mu* insertion is at the third exon of *ZmRca3*, while the *Ds* insertion mutant *rca3-m2::Ds* is located on the second exon. Prior to investigating the effect of the mutant alleles on heat stress tolerance, we tested five different temperatures in maize to determine the temperature that induces *ZmRca3*. Using qPCR, we found that *ZmRca3* expression increases with exposure to high temperatures, and the presence of *ZmRca3* protein is detected starting at 40°C and further increases at higher temperatures. The initial characterization of the mutant lines shows that homozygous mutant lines disrupt *ZmRca3* expression at 42°C. Gas exchange measurements at 42°C were used to identify variability between the response of the wildtype and mutant lines to heat stress. We will leverage these transposable elements to further understand the effect of heat stress in maize, which will help us develop more resilient crops for the future.

139 Pre Recorded Poster

Red radiation as an inducer of different inhibition mechanisms of photosynthesis in two different types of lettuce (*Lactuca sativa* var. *capitata* L.) – a case study

Krzysztof M. Tokarz[1], Zbigniew Gajewski[1], Stanisław Mazur[1], Edward Kunicki[2], Wojciech Makowski[1], Karolina Miernicka[1], Barbara Tokarz[1]

[1]Department of Botany, Physiology and Plant Protection, University of Agriculture in Krakow, Krakow, Poland. [2]Department of Horticulture, University of Agriculture in Krakow, Krakow, Poland

Plants are exposed to various environmental conditions of which solar radiation is a key factor affecting their growth and development. Frequent and sudden changes in spectral composition and intensity of solar radiation become stressors for plants. Shortwave-length radiation can lead to decrease photosynthesis process by damaging PSII and PSI, reducing quantum yield, disrupting photosynthetic electron transport and photochemical quenching. Whereas longwave-length radiation, in the 700-800 nm range (red light), is the most desirable and effective for photosynthesis.

The aim of the presented study was to describe and compare acclimation strategies of the photosynthetic apparatus in lettuce plants to red light. Plant material comprise of two types of lettuce (*Lactuca sativa* var. *capitata*): butterhead and iceberg. Plants were cultivated in greenhouses covered with transparent glass (control) and glass with red luminophore. After one month of cultivation, gas exchange and chl a fluorescence were measured on plant leaves. Moreover, the content of photosynthetic pigments and selected structural and functional elements of the photosynthetic apparatus were examined (Western Blot).

In both types of lettuce studied, a decrease in the carboxylation efficiency of atmospheric carbon dioxide was observed. In iceberg lettuce, photosynthesis was disrupted on the donor side of PSII. In turn, in butterhead lettuce, limitation on the acceptor side beyond PSI was the cause of the observed disruption.

140 Pre Recorded Poster

Single-molecule analysis of the Cryptophyte light-harvesting subunit PE545

Raymundo Moya[1], Audrey Norris[1], Leah Spangler[2], Gregory Scholes[2], Gabriela Schlau-Cohen[1]

[1]MIT, Cambridge, MA, USA. [2]Princeton University, Princeton, NJ, USA

Cryptophytes are an interesting species of photosynthetic marine algae. Despite containing light-harvesting subunits very similar to the phycobiliproteins found in cyanobacteria, the machinery in cryptophytes is unique. So far, cryptophytes have shown no organization or structure to their phycobiliproteins, unlike the ordered structure found in cyanobacteria. While these light-harvesting subunits of cryptophytes have been investigated at the ensemble level, resulting in a relatively good understanding of how the pigments embedded in the protein-pigment complex couple together and transfer energy, little is known about their single-molecule behavior. Single-molecule investigations are crucial in understanding how the fluctuating protein backbone influences the fluorescence of the pigments. In our work, we have utilized single-molecule fluorescence spectroscopy to identify photophysical substates and how they influence the fluorescence of a specific cryptophyte phycobilin-protein complex, phycoerythrin 545 (PE545). We identified three different conformational states of the subunit and observed rapid switching between them. Furthermore, we determined that the quenched conformation increased in population under high light, suggesting that it may be involved in photoprotectional pathways.

141 Pre Recorded Poster

Single-molecule spectroscopy to probe protein-protein interactions in light harvesting complex II

Madeline Hoffmann[1], Premashis Manna[1], Thomas Davies[2], Matthew Johnson[2], Gabriela Schlau-Cohen[1]

[1]MIT, Cambridge, MA, USA. [2]University of Sheffield, Sheffield, United Kingdom

To cope with fluctuating solar intensity, plants have developed a mechanism known as nonphotochemical quenching (NPQ) to dissipate excess energy as heat. It is well-documented that membrane proteins in the thylakoid regulate NPQ, but a molecular-level understanding of the interactions between said proteins remains elusive. To address this challenge, we reconstitute light harvesting complex II (LHCII) trimers into liposomes at controlled protein-to-lipid ratios, allowing us to study these interactions in an isolated but near-native environment. Time resolved single-molecule fluorescence microscopy reveals that there is increased fluorescence quenching as the protein-to-lipid ratio is increased, indicating significant interactions among trimers. We then model how a range of proposed interaction energies impacts the spatial configuration of the trimers within the liposome, which then enables us to generate simulated lifetime distributions as a function of interaction energy. These distributions are compared to experimental results to extract a coarse-grained estimate for the LHCII lateral inter-trimer interaction energy. To our knowledge, this is the first such estimate for this interaction. We then extended this method to study the system in acidic conditions, as a pH gradient is known to develop across the membrane under excess light conditions. We observed a further reduction in fluorescence lifetime, indicating greater attractive interactions among the trimers. We hypothesize that this may result from reduced repulsive forces due to protonation of amino acid residues within LHCII. In future work, this method will be used to quantitatively investigate the impact of other proteins and biological conditions on LHCII interactions and their role in NPQ.

142 Pre Recorded Poster

P700 and A_{-1} chlorophylls act as one in photosystem I: photoaccumulated FTIR difference spectroscopy of the mutants near the A_{-1} chlorophylls (PsaA-N600M and PsaB-N582M) of photosystem I from *Synechocystis* sp. PCC 6803

Julia Kirpich[1], Michael Nelson[1], Neva Agarwala[1], Wu Xu[2], Gary Hastings[1]

[1]Georgia State University, Atlanta, GA, USA. [2]University of Louisiana at Lafayette, Lafayette, LA, USA

The involvement of the second pair of chlorophyll molecules A_{-1A} and A_{-1B} of photosystem I (PSI) in light harvesting and electron transfer is currently under debate. Therefore, the effect of mutation of asparagine residues that coordinate A_{-1A} and A_{-1B} pigments was studied in PsaB-N582M and PsaA-N600M mutants of PSI from *Synechocystis* sp. PCC 6803 by photoaccumulated FTIR difference spectroscopy at 77 K and 293 K. The comparison of the photoaccumulated FTIR difference spectra of one of the asparagine mutants (PsaA-N600M) to those of wild type and the other mutant (PsaB-N582M) shows significant difference in 1700 cm^{-1} band. This band is commonly assigned to one of the pigments of the chlorophyll pair P700 that absorbs light in PSI and precedes A_{-1} pigments in electron transfer pathway. The observed difference in 1700 cm^{-1} band in the mutant with potentially altered A_{-1} coordination supports the idea that either a) P700 and A_{-1} pigments act as one collective pigment in PSI [Cherepanov et al. (2021) J. Photochem. Photobiol. B, Biol. 217:112154; Cherepanov et al. (2021) Photochem. Photobiol. Sci. 20:1209-1227]; or means that b) the commonly accepted assignment of P700 and A_{-1} pigment bands needs to be reconsidered and the bands for P700 and A_{-1} pigments need to be assigned anew. This study brings up new questions and provides new evidence about the interconnection of P700 and A_{-1} pigments and invokes rethinking of the commonly accepted assignment of infrared bands to specific pigment groups in the characteristic 1800-1500 cm^{-1} region.

143 In Person Oral

The role of Cytochrome b_6f in the control of photosynthesis: a new model

Jennifer Johnson, Joseph Berry

Carnegie Institution for Science, Stanford, CA, USA

From a quantitative perspective, one of the most striking features of the overall photosynthetic process is that the efficiency of light capture decreases as the speed of carbon dioxide fixation increases. Here, we will present experiments examining the role of the cytochrome b_6f complex (Cyt b_6f) in mediating this trade-off. The results provide evidence of two distinct levels of control. The first level of control is purely kinetic: Cyt b_6f catalyzes the rate-limiting step in linear electron flow between Photosystems II and I, which causes upstream electron carriers (and PS II) to accumulate in a reduced state and downstream carriers (and PS I) to accumulate in an oxidized state. In contrast, the second level of control is regulatory: under saturating light intensities, the rate-limiting step at Cyt b_6f is subject to further downregulation based on feedback from carbon metabolism. This form of feedback regulation is termed 'photosynthetic control' of Cyt b_6f, a phrase meant to evoke a chloroplastic analogy to the phenomenon of 'respiratory control' of Cyt bc_1 known in mitochondria. We will discuss how we have translated these insights into a quantitative model that relates the factors limiting electron transport and carbon metabolism, the regulatory processes that coordinate these metabolic domains, and the responses of photosynthesis to light, carbon dioxide, and temperature. Special attention will be given to the innovations that differentiate this model from earlier models of C_3, C_3-C_4, and C_4 photosynthesis, and the new opportunities this creates for interpreting and simulating the environmental responses of photosynthesis across scales.

144 In Person Oral

What did happen to the phycobilisome?

Beverley Green

University of British Columbia, Vancouver, B.C., Canada

The phycobilisome (PBS) is the major photosynthetic light-harvesting antenna in cyanobacteria, red algae, and glaucophyte algae. This large complex structure is made up of phycobiliprotein rods held together by protein linkers, organized to give an efficient energy cascade from phycoerythrin (PE) to phycocyanin (PC) to allophycocyanin (APC) to the reaction centres. In spite of its conserved nature, optimal design and adaptability, the PBS has been lost several times in evolution. Green algae and plants have completely dispensed with it, depending solely on the Chl-binding membrane-intrinsic LHC superfamily. The red algae rely primarily on an elaborate PBS to supply energy to PSII, but employ members of the LHC family to supply PSI. Was PBS loss a matter of chance, enabled by (contingent on) the early evolution of an alternative antenna system, which was expanded in the green lineage? Even within the cyanobacteria, there have been several independent losses of the PBS, accompanied by evolution of a completely different Chl-binding antenna. Eukaryotic phytoplankton are dominated by diatoms, haptophytes, dinoflagellates and cryptophytes, all of which acquired plastids by secondary endosymbiosis from one (or more) red algal ancestor(s). They too, have lost the PBS and massively expanded the LHC family. However, the cryptophytes have retained the PE β-subunit by binding it to small proteins derived from red algal PBS linker proteins. This suggests that the (minor) linkers could even have promoted the demise of the PBS by competing successfully with the red algal PE α-subunit to bind the PE β-subunit, which resulted in the evolution of the unique cryptophyte phycobiliprotein antenna [see Rathbone et al. abstract]. Taking into account recent discoveries from ocean metagenomics, there may have been several mechanisms that led to the loss of the phycobilisome in so many lineages.

145 Pre Recorded Oral

Adaptation to weak light environments in plants - chloroplast photorelocation movements and leaf anatomy changes

Eiji Gotoh

Kyushu University, Fukuoka, Fukuoka, Japan

Plants sense the quality and quantity of light, as well as the direction and intensity of light, and perform optimal photosynthesis through responses at the organelle, cell, and tissue/organ levels. One of the plant light responses is chloroplast photorelocation movements. Chloroplasts move on the light irradiation area under low light and away from the light irradiation area under high light. Analysis of model plant mutants reveals that chloroplast photorelocation movements adjust the light-use efficiency and photosynthesis in leaf to suit the ambient light environment. On the other hand, the physiological meaning of chloroplast photorelocation movements on plants growing in the field has rarely been considered. Therefore, we collected more than 150 plant species from the forest floor all over Japan, and analyzed the presence or absence of chloroplast photorelocation movements and the intracellular arrangement of chloroplasts. The results showed that the pattern of chloroplast photorelocation movements could be classified into several patterns.

Interestingly, about 40% of the plant species growing on the forest floor showed low chloroplast photorelocation movements. Most of their plants grew in very weak-light environments where direct sunlight does not reach the forest floor. Furthermore, plant species with low chloroplast photorelocation movements showed characteristic palisade cell shapes. In this presentation, I will present the latest findings on the change of palisade cell shape adaptation to very weak-light environments.

146 In Person Oral

Enabling a vestigial electron transfer pathway in bacterial photosynthetic reaction centers

Gregory A. Tira[1], James C. Buhrmaster[1], Stephen Hippleheuser[1], Kaitlyn Faries[2], Deborah K. Hanson[1], Dewey Holten[2], Christine Kirmaier[2], Philip Laible[1]

[1]Biosciences Division, Argonne National Laboratory, Lemont, IL, USA. [2]Department of Chemistry, Washington University, St. Louis, MO, USA

Photosynthetic reaction centers (RCs) convert light energy into chemical energy in a series of extremely efficient electron transfer reactions, accomplishing transmembrane charge separation. The structures of RCs reveal two symmetry-related branches of cofactors that are functionally asymmetric; bacterial RCs use the A pathway exclusively. To understand the architectural and energetic factors that underlie the directionality and yields of electron transfer, we coupled extensive rounds of culture passaging and/or random chemical mutagenesis with selective pressure to generate, for the first time, a mutant RC that uses the B pathway preferentially and efficiently. Previous site-specific mutagenesis strategies had not managed to increase the yield of B-branch transmembrane charge separation significantly as we clearly lacked the understanding necessary to use rational design to engineer efficient B-branch electron transfer along with proton uptake. These advances were aided by directed molecular evolution, implementing streamlined mutagenesis and screening steps to sample a larger number of RC variants. The mutations that were discovered in the photosynthetic apparatus and genomes of the *Rhodobacter sphaeroides* expression host system will be highlighted. Insights derived from functional characterization of the revitalized vestigial electron transfer path augment engineering principles needed for successful *de novo* design of biomimetic assemblies capable of both efficient charge separation and charge stabilization. Current experiments are exploring the generality of the altered structure-function relationships in the RCs of the phenotypic revertants in additional RCs from related photosynthetic bacteria. With increased understanding, the capability to deploy these molecules, and their non-biotic mimetics, as molecular switches, nanoscale sensors, and electron gates, will be even closer to realization.

147 In Person Poster

Structural and Spectral Features of Light-Harvesting Complex II Proteoliposomes Mimic Native Thylakoid Membranes

Sam Wilson, Dan-Hong Li Alexander Ruban

Queen Mary University of London, London, United Kingdom

The major photosystem II light-harvesting antenna (LHCII) is the most abundant membrane protein in nature and plays an indispensable role in light harvesting and photoprotection in the plant thylakoid. Here, we show that ‘pseudo-thylakoid characteristics’ can be observed in artificial LHCII membranes. In our proteoliposomal system, at high LHCII densities, the liposomes become stacked, mimicking the in vivo thylakoid grana membranes. Furthermore, an unexpected, unstructured emission peak at ~730 nm (F730) appears, similar in appearance to photosystem I (PSI) emission, but seemingly different in nature. Unlike in PSI, the fluorescence excitation spectrum of the F730 band of LHCII displays an absorption profile identical to that of the bulk chlorophyll a spectrum. This indicates that the F730 fluorescence likely arises from chlorophylls that interact when excited yet exist as single monomers in the ground state. Thus, the F730 state is likely excimeric in origin. These states correlate with the increasing density of LHCII in the membrane and a decrease in its average fluorescence lifetime. The appearance of these low-energy states can also occur in natural plant membrane structures, which has unique consequences for interpretation of the spectroscopic and physiological properties of the photosynthetic membrane.

148 Pre Recorded Oral

Dissolution, diffusion, and assimilation of respired CO_2 in tree stems

Roberto Luis Salomon[1], Kathy Steppe[2]

[1]Universidad Politécnica de Madrid, School of Forestry Engineering, Madrid, Spain. [2]Ghent University, Laboratory of Plant Ecology, Ghent, Belgium

Photosynthetic CO_2 assimilation does not uniquely occur in leaves. Chloroplast-containing cells are present in peripheral tissues of the tree stem, where respiratory metabolism and diffusion barriers make endogenous CO_2 saturating for photosynthesis. Consequently, woody tissue photosynthesis is less responsive to drought stress than leaf photosynthesis because water loss for gas exchange and CO_2 uptake is relatively limited. The relevance of CO_2 re-assimilation in woody tissues is expected to gain importance under drier climates, as it constitutes a continuous C source when leaf assimilation and phloem transport are constrained. Consistent with this hypothesis, woody tissue photosynthesis in poplar trees has been observed to reduce stem CO_2 efflux by half, sustain carbohydrate pools in branches and reduce xylem vulnerability to embolism.

To further investigate the fate of xylem-transported CO_2 at a high spatial and temporal resolution, a ^{13}C aqueous solution was infused at the stem base of three tree species and continuously tracked throughout the plant organs and tissues. Radial CO_2 diffusivity, estimated via cavity ring-down laser spectroscopy, was lower in the conifer species with tracheid anatomy (cedar) than in angiosperm species with ring-porous (oak) and diffuse-porous (maple) anatomy. Similarly, xylem [CO_2] was lower in cedar (< 5 %) than in oak and maple (> 5 %). These two observations suggest disconnected CO_2 pools inside and outside the cambium in conifer relative to angiosperm species, explaining discrepancies commonly observed between taxonomic clades. Second, re-assimilation of the ^{13}C label, up to 9 and 21 % in cedar and oak trees, respectively, was primarily detected in xylem and bark tissues of the stem, with little label reaching foliar tissues. Such non-negligible recycling rates highlight the potential role of woody tissue photosynthesis in tree C budgets under changing climates, while spatial patterns of label loss suggest substantial CO_2 diffusion along the stem-branch transition.

149 Pre Recorded Poster

Long-term adaptation of *Arabidopsis thaliana* to Far-red light

Chen Hu, Wojciech J. Nawrocki, Roberta Croce

Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Vascular plants use carotenoids and chlorophylls a and b to harvest solar energy in the visible region (400-700 nm). Some cyanobacteria have also developed the ability to use far-red (FR) light by redesigning their photosynthetic apparatus and synthesizing red-shifted chlorophylls. Implementing this strategy in plants is considered promising to increase crop yield. To prepare for this, a characterization of the FR light-induced changes in plants is necessary. Here, we explore the behavior of *Arabidopsis thaliana* upon exposure to FR light by following the changes in morphology, physiology, and composition of the photosynthetic complexes. We found that after FR-light treatment, the ratio between the photosystems and their antenna size drastically readjust in an attempt to rebalance the energy input to support electron transfer. Despite a large increase in PSBS accumulation, these adjustments result in strong photoinhibition when FR-adapted plants are exposed to light again. Crucially, FR light-induced changes in the photosynthetic membrane are not the result of senescence, but are a response to the excitation imbalance between the photosystems. This indicates that an increase in the FR absorption by the photosystems should be sufficient for boosting photosynthetic activity in FR light.

150 In Person Oral

Modulating expression levels of mannitol-1-phosphate dehydrogenase (mtlD) gene using different regulatory promoters from *S. elongatus* PCC7942 to stabilize mannitol biosynthesis in cyanobacteria

Prem Pritam, Pramod P. Wangikar

Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

Mannitol is a naturally occurring polyol with a wide number of industrial applications. It is commercially manufactured by the chemical hydrogenation of fructose. Costly purification stage with a low final mannitol yield has prompted active research into the bioproduction of mannitol. Sustainable production by engineered photoautotrophic cyanobacteria proved to have added advantages over heterotrophs . Mannitol production in the cyanobacteria PCC7002 has been established by expressing the mtlD and mlp genes, however gene transformation and gene stability remain key barriers to commercial viability. To address genetic stability, the endogenous compatible solutes synthesis was knocked out in PCC6803, and mannitol production was carried out under salt condition, which enticed PCC6803 to use mannitol as an osmoprotectant and eventually stabilized its production. But impaired growth of the engineered strain and the low productivity still remain a challenge. In this study, we have attempted to explore the potential of the fast-growing strains PCC11801, PCC11802, and PCC7942 for the enhanced and stable photosynthetic conversion of CO_2 to mannitol with the minimal metabolic burden. Two genes were expressed under two different promoters, each of which was chosen based on its regulation in response to abiotic conditions and minimize the toxicity of the intermediate product mannitol-1-phosphate.We adopted this method for modulating expression levels of mtlD gene using different abiotic regulatory promoters from PCC7942 and modified transformation protocol under osmotic stress conditions. Our findings emphasize the significance of using regulatory promoters to control the activity of mtlD gene under different abiotic condition. The highest productivity obtained was 756 mgL-1 in PCC7942 with PcpcB300- mtlD construct under low light and ambient condition after 12 days. With this approach, we provide a valuable hypothesis for stable and eco-friendly commercial mannitol production.

151 In Person Poster

PGRL1 redox states alleviate photoinhibition in Arabidopsis during step increase in light intensity

Amit Kumar Chaturvedi, Orly Dym, Robert Fluhr

Weizmann Institute of Science, Rehovot, Israel

Non-motile plants have evolved regulatory mechanisms to maintain homeostasis for optimal growth. Responses to environmental changes in light are particularly important not only during the diurnal transition from night to day but also to react to light changes caused by passing clouds or by wind. Thioredoxins rapidly orchestrate redox control during environmental change by modifying cysteine residues. Here, we assign a function to regulatory cysteines of PGRL1A, a constituent of the ferredoxin-dependent cyclic electron flow (Fd-CEF) pathway and show their role in the regulation of proton motive force (PMF) and nonphotochemical quenching (NPQ). During step increase of low light intensity (10-60 µE*m-2*s-1), the intermolecular disulfide of the PGRL1A 59-kDa complex is reduced transiently within seconds to the 28 kDa form. In contrast, step increases to higher light intensity (60-600 µE*m-2*s-1) stimulated a stable partially reduced redox state in PGRL1A. Measurements of NPQ, PMF and resultant photosynthetic controls (ND) and (NA) were found to correlate with the redox state of PGRL1A during step increases in light intensity but not in PGRL1mutant plants pgrl1ab or PGRL1A cysteine mutant (PGRL1AC1,2A). Continuous light regimes did not affect mutant growth; however, fluctuating regimes of light intensity showed significant growth reduction in the mutants. Inhibitors of photosynthesis placed control of the PGRL1A redox state as dependent on the penultimate ferredoxin redox state that fuels reducing equivalents to the large set of chloroplasts thioredoxins. Our results showed that redox state changes in PGRL1A are crucial to the optimization of photosynthesis and are regulated by the photosynthetic electron flux.

152 Pre Recorded Oral

How Nature makes O_2: an electronic level mechanism for water oxidation in photosynthesis

Felix Rummel, Patrick O'Malley

The University of Manchester, Manchester, United Kingdom

In this report we combine broken symmetry density functional calculations and electron paramagnetic resonance analysis to obtain the electronic structure of the penultimate S3 state of Nature's water oxidising complex and determine the electronic pathway of O-O bond formation. Analysis of the electronic structure changes along the reaction path shows that two spin crossovers, facilitated by the geometry and magnetism of the water oxidising complex are used to provide a unique low energy pathway. The pathway is facilitated via formation and stabilisation of the [O2]3- ion. This ion is formed between ligated deprotonated substrate waters, O5 and O6, and is stabilised by antiferromagnetic interaction with the Mn ions of the complex. Combining computational, crystallographic and spectroscopic data we show that an equilibrium exists between an O5 oxo and O6 hydroxo form with an S=3 spin state and a deprotonated O6 form containing a two-centre one electron bond in [O5O6]3- which we identify as the form detected using crystallography. This form corresponds to an S=6 spin state which we demonstrate gives rise to a low intensity EPR spectrum compared with the accompanying S=3 state, making its detection via EPR difficult and overshadowed by the S=3 form. Simulations using 70% of the S=6 component give rise to a superior fit to the experimental - band EPR spectral envelope compared with an S=3 only form. The computational, crystallographic and spectroscopic data are shown to coalesce to the same picture of a predominant S=6 species containing the first one-electron oxidation product of two water molecules i.e. [O5O6]3-. Progression of this form to the two-electron oxidised peroxo and three-electron oxidised superoxo forms, leading eventually to the evolution of triplet O2, is proposed to be the pathway Nature adopts to oxidise water.

153 Pre Recorded Poster

Photosynthetic Response of Potato Cultivars to Elevated CO_2

Keshav Dahal, Matthew Milne, Taylor Gervais, Benoit Bizimungu

Agriculture and Agri-Food Canada, Fredericton Research and Development Centre, Fredericton, New Brunswick, Canada

The effects of long-term elevated CO_2 on photosynthetic performance and tuber yield of five potato cultivars (AC Novachip, Atlantic, Kennebec, Russet Burbank, and Shepody) grown at either ambient (400 µmol C mol−1) or elevated (750 µmol C mol−1) CO_2 were studied. Compared to ambient CO_2-grown counterparts, elevated CO_2-grown plants exhibited a 1.06 – 2.2-fold increase in tuber yield, and a 1.1 - 1.4-fold increase in the rates of photosynthesis across the cultivars. Concomitantly, the stomatal conductance and transpiration rates were decreased by 15- 25% and 5-20 %, respectively, and water use efficiency increased by 10-90 % upon growth at elevated CO_2. The CO_2-stimulation of tuber yield varied with cultivars such that the commercial cultivar Russet Burbank and Shepody exhibited a 120% and 58% increase respectively, whereas, AC Novachip, Atlantic and Kennebec exhibited only 6%, 10% and 44% increase in tuber yield respectively at elevated CO_2. This differential stimulation of tuber yield by elevated CO_2 was consistent with variations in CO_2-stimulation of rates of photosynthesis, tuber number and size across the cultivars. Elevated CO_2 increased the tuber number by up to 80% across the cultivars as compared to at ambient CO_2. Although elevated CO_2 increased the tuber size by 6%, 29%, and 89% for AC Novachip, Kennebec, and Russet Burbank respectively, it decreased the size by 12-20% for Shepody and Atlantic. Surprisingly, we did not observe any significant differences in plant growth and morphology in elevated versus ambient CO_2-grown plants. We conclude that the potential for enhancement of photosynthetic performance, water use efficiency and tuber yield of potatoes grown at elevated CO_2 is cultivar dependent.

154 In Person Oral

Manufacturing recombinant proteins in *Chlamydomonas reinhardtii* chloroplast: Modelling and estimating profitability in different scenarios

Eduardo Gorron, John Roles, Ian Ross, Melanie Oey, Ben Hankamer

Centre for Solar Biotechnology, Institute of Molecular Biosciences (IMB), The University of Queensland, Brisbane, QLD, Australia

Chlamydomonas reinhardtii has been proposed as an alternative recombinant protein production system, and several studies has shown the capability of this host to synthesize several recombinant proteins. However, very few studies have analysed the technical, economic and regulatory feasibility for this system. In this study, processes for the manufacturing of five recombinant protein-based products (injectable, topical, diagnostic, GMO aquaculture feed and enzyme for industrial use) employing *C. reinhardtii* chloroplast expression system were modelled. Two different upstream schemes, both encompassing a 1 Ha production area, were compared: a tubular bioreactor PC2 system with artificial illumination, and a Bayes reactor type, single use type of reactor, with natural illumination. The downstream and quality control component of the process was designed in a modular form, that allows for selection of different unit operations according to the desired purity and regulatory considerations. Prices and Internal Rate of Return (IRR) were estimated for different facility areas, specific growth rates and protein yields. The competitiveness of microalgae system for production of recombinant proteins in these different scenarios is discussed.

155 In Person Oral

Light-catalyzed production of fatty acids and their derivatives from CO_2 using cyanobacteria

Shuqin Li, Wim Vermaas

School of Life Sciences, Arizona State University, Tempe, Arizona, USA

Cyanobacteria are excellent organisms for production of excreted biofuels and green chemicals because they are photosynthetic (producing organic compounds from CO_2, water and light) and typically excrete produced compounds much more easily than other phototrophs such as algae do. Excretion of product helps to alleviate feedback inhibition of product formation and enhances the economic feasibility of the process.

We have generated a laurate-producing and -excreting strain of the cyanobacterium *Synechocystis* sp. PCC 6803 that contains a thioesterase from the plant *Umbellularia californica*, releasing the fatty acid laurate when native fatty acid biosynthesis reaches the C12 stage. This strain is efficient in producing laurate, a fatty acid that can be used as a biofuel precursor, from CO_2 that was fixed by photosynthesis. The amount of fatty acid produced, typically in the range of 0.7-1 mM in the medium, represents about 20% of photosynthetically fixed carbon in cells.

However, laurate is readily consumed by many heterotrophic prokaryotes, thus causing challenges for larger-scale applications. Therefore, we added a methylation step to convert laurate to the more stable and water-insoluble methyl laurate. This conversion of laurate to methyl laurate is done by a S-adenosyl methionine (SAM)-dependent enzyme. Main advantages over current biofuel products are methyl laurate's immediate application as biodiesel and its limited solubility in water, thus reducing the availability to heterotrophs in the culture and increasing the ease of harvesting. Morever, lauroyl esters have many additional applications. This approach provides a 'one-stop-shop' cyanobacterial platform that generates liquid transportation fuel from CO_2 and water with sunlight as the energy input.

This work is supported by the US Department of Energy's Office of Energy Efficiency and Renewable Energy (EERE) under the Bioenergy Technology Office (BETO) award number DE-EE0009274.

156 Pre Recorded Oral

Mechanisms of the network and systemic acquired acclimation and the cellular light memory in plants

Stanisław Karpiński

Institute of Biology, Warsaw University of Life Sciences, Warsaw, Mazovia, Poland

Terrestrial plants, unlike animals, have limited mobility and in case of danger cannot escape from stress situation like animals. Therefore, specific acclimation mechanisms have evolved in plants, such as: the Systemic Acquired Acclimation (SAA) [1], the Cellular Light Memory (CLM) [2, 3] or recently discovered the Network Acquired Acclimatization (NAA) [4]. The fundamental quantum-molecular mechanism of non-photochemical quenching (NPQ) in light antennas complexes and photosystems reaction centres in chloroplasts, redox status of the plastoquinone pool (PQ), electrical and Reactive Oxygen Species (ROS) signalling plays a key role in the above mentioned molecular and physiological mechanisms. In a simplified explanation, the absorbed energy is immediately divided into three channels: fluorescence, heat and photochemistry. The absorbed energy division between these channels is regulated by PsbS (22 kD) protein that determine the strength and quality of electrical and ROS retroactive signals from the chloroplast. These signals regulate systemically and networkally NPQ values in other remote photosystems and thus regulate the above-mentioned mechanisms of SAA, CLM and NAA in other cells, tissues, leaves within one plant and in other plants growing besides. In other words, in changing environment plants constantly communicate between leaves and with each other, in a given ecosystem or community of plants, what quantum efficiency of absorbed energy distribution theirs photosystems should achieve in order to adjust nuclear- and organellar-encoded genes expression accordingly thus adjust acclimation responses. Within two trees with 1000 leaves each and with touching leaves, we have several dozen of trillions (10E13) of Pre Recorded communication connections between photosystems. The question is do plants function like a biological quantum computer with a quantum memory of light absorbed in excess?

[[1]Karpiński et al., (1999) Science 284:654-657; [2]Szechyńska-Hebda et al., (2010) Plant Cell 22:2201-2218; [3]Górecka et al., (2019) Plant Cell Environ. 43:649–661; [4]Szechyńska-Hebda et al., (2022) Plant Cell doi.org/10.1093/plcell/koac150].

157 Pre Recorded Poster

Understanding the protective mechanism of small molecules for Photosystem II functionality

Tyler Chapman, Helmut Kirchhoff

Washington State University, Pullman, WA, USA

Glycine betaine (GB), a zwitterionic amino acid derivative naturally produced by a few plant families, has been demonstrated to help stabilize both protein quaternary structure as well as osmotic pressure. GB has also previously been shown to be associated with photosynthesis, specifically with protection of photosystem II (PSII) under abiotic stresses (i.e., high temperature). Our experiments involving the effect of heat stress on the rate of oxygen evolution in isolated Arabidopsis chloroplasts with or without GB support similar conclusions, that GB protects PSII during abiotic stress. We can demonstrate that GB protects only PSII-dependent electron transport against heat stress, but not the electron transport segment from the cytochrome b6f complex to photosystem I (PSI). Experiments examining the protective impact of other naturally produced small molecules (Glycine, sorbitol, and proline) were found to stabilize the rate of oxygen evolution under heat stress to varying degrees, ranging from no impact to a moderate level of protection. However, GB remained the most protective small molecule tested, leading us to investigate further the mechanism by which GB protects PSII. To investigate this unknown mechanism, the protein-ligand interaction modelling program UCSF Chimera was used to characterize interactions between PSII and each small molecule (binding sites, binding energies). The results indicate that small molecules which are more protective often have more interactions with the oxygen evolving complex (OEC) portion of PSII, while molecules which are less protective lack these interactions. Further research aims to elucidate whether certain small molecules may interact with specific amino acid residues, or ions associated with PSII/ions in solution. In addition, results will be presented whether small molecules protect the heat induced dissociation of the light-harvesting complex II (LHCII) from PSII. This data will increase our understanding on the protective mechanism of naturally produced small molecules for photosynthesis under heat stress.

158 Pre Recorded Poster

Heterologous expression of far-red photosystem II subunits in *Synechocystis* sp. PCC 6803

Man QI, Henry Taunt, Peter Nixon

Imperial College London, London, United Kingdom

Certain terrestrial cyanobacteria acclimate to far-red light (FRL) by remodeling their photosynthetic apparatus to bind FRL-absorbing pigments such as chlorophyll d (Chl d) and Chl f that are synthesized in response to FRL. This acclimation response is termed far-red light photoacclimation (FaRLiP) and is under the control of a ~20 gene cluster (FaRLiP cluster) encoding the Chl f synthase (ChlF) and FRL paralogues of core subunits of photosystem I (PSI), photosystem II (PSII) and the phycobilisome (PBS). Heterologous expression of PsbA4/ChlF encoded in the FaRLiP cluster drives Chl f production in non-FaRLiP cyanobacteria but at relatively low levels compared to native systems. This might be due to the lack of the FRL-PSI and FRL-PSII subunits encoded in the FaRLiP cluster that naturally bind Chl f. A recent cryo-EM structure of a native FRL-PSII core complex has revealed the presence of 30 Chl a, 1 Chl d and 4 Chl f per PSII monomer. The Chl d occupies the ChlD1 position in the PsbA3/PsbD3 reaction center complex and the four Chl f are found in the antenna (three in PsbB2 and one in PsbC2). Here, we replaced individual endogenous PSII subunits in *Synechocystis sp.* PCC 6803 (Syn6803) with their FRL paralogues (PsbA3, PsbB2, PsbC2 and PsbD3) encoded by *Chroococcidiopsis thermalis* PCC 7203 to test whether these FRL-PSII subunits accumulate in the absence of Chl f and Chl d and whether they are incorporated into hybrid PSII complexes. Growth tests showed that none of these FRL-PSII mutants grew photoautotrophically. However, a combination of His-tag pull-downs and immunoblotting revealed that PsbA3, PsbC2 and PsbD3 could accumulate within the membrane and were able to form complexes with the endogenous Syn6803 PSII subunits. In contrast, levels of PsbB2 were undetectable. The same strategy has also been used to test the accumulation of Chl d-binding PSII subunits from the *Acaryochloris marina* in Syn6803.

159 Pre Recorded Poster

Regulation of photoprotective qH by LCNP and SOQ1

Jingfang Hao, Alexander Johansson, Johan Svensson Fall, Jianli Duan, Alizée Malnoë

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå, Sweden

Non-photochemical quenching (NPQ) plays an important role in phototrophs for decreasing photo-oxidative damage. The antenna quenching component qH is a sustained form of NPQ that is photoprotective, taking hours or longer to relax. The plastid lipocalin (LCNP) is located in the thylakoid lumen and is required for qH occurrence in cold and high light conditions. The thylakoid membrane-anchored protein SUPPRESSOR OF QUENCHING1 (SOQ1) suppresses qH by inhibiting LCNP via its lumenal domains including thioredoxin (Trx)-like, NHL, and C-terminal fragment (CTD). However, the mechanisms by which SOQ1 inhibits LCNP function, as well as by which LCNP induces qH are unknown. In our work, we investigated the oligomerization of LCNP *in vitro* and *in vivo*. *In vitro*, LCNP forms tetramer and dimer with three intramolecular disulfide bridges. *In vivo*, LCNP mainly exists as a dimer and monomer respectively in Arabidopsis wild type and *soq1* mutant in non-stress conditions. The monomeric form of LCNP is increased in wild type in cold and high light conditions. Our results suggest that monomeric LCNP is the active form for qH occurrence. Several lines of evidence suggest that SOQ1 regulates LCNP function in a redox manner. Our results determined that the SOQ1 lumenal domains display methionine sulfoxide reductase activity and can reduce the methionine sulfoxide (MetO) in LCNP to methionine, implying that the slower mobility of LCNP on SDS-PAGE in the *soq1* mutant is due to methionine oxidation. Furthermore, the slower mobility of LCNP with multi methionine mutated to alanine in *soq1* is no longer observed, supporting that modification of LCNP occurs on methionine in the *soq1* mutant. Altogether, our work indicates that when SOQ1 is nonfunctional, LCNP exists as LCNP-MetO form that would lead to its monomerization, thereby activating LCNP for qH.

160 Pre Recorded Poster

Predicting plant Rubisco kinetics from RbcL sequence data using machine learning

Wasim A Iqbal[1], Alexei Lisitsa[2], Maxim V Kapralov[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is responsible for the conversion of atmospheric CO_2 to organic carbon during photosynthesis. Screening the natural diversity of Rubisco kinetics from plants is the main strategy used to find better Rubiscos for crop engineering efforts. Here, we demonstrate the use of Gaussian processes (GPs), a family of Bayesian models, coupled with protein encoding schemes for predicting Rubisco kinetics from Rubisco large subunit (RbcL) sequence data. GPs trained on published experimentally obtained Rubisco kinetic datasets were applied to over 9,000 sequences encoding RbcL and predicted kinetic parameters close to experimental values. Notably, our predicted Rubisco kinetic values were in agreement with known trends, e.g. higher carboxylase turnover rates (Kcat) for Rubiscos from C_4 or crassulacean acid metabolism (CAM) species compared to ones found in C_3 species. This is the first study demonstrating machine learning approaches as a tool for screening Rubisco kinetics, and this approach could be applied for other enzymes.

161 Pre Recorded Poster

Mg^{2+} deficiency impacts the thylakoid membrane bioenergetics in the cyanobacterium *Synechocystis* sp. PCC 6803

Anne-Christin Pohland[1], Gábor Bernát[2], Stefan Geimer[3], Dirk Schneider[1]

[1]Johannes Gutenberg University, Mainz, Germany. [2]Balaton Limnological Research Institute, Tihany, Hungary. [3]University of Bayreuth, Bayreuth, Germany

Magnesium (Mg^{2+}), the most abundant divalent cation in living cells, serves as a cofactor in many enzymatic reactions and is required for various intracellular processes. In photoautotrophic organisms, Mg^{2+} serves as the central ion of the light-harvesting pigment chlorophyll (Chl), and thus, is of particular importance in chloroplasts and cyanobacteria. Additionally, Mg^{2+} serves as a counterion to balance the pH gradient that is built up across the thylakoid membrane (TM) due to the photosynthetic light reaction. Yet, albeit Mg^{2+} is so crucial, it still is unclear how Mg^{2+} limitation affects the cyanobacterial physiology.

When the cyanobacterium *Synechocystis* sp. PCC 6803 was grown in BG11 medium with reduced Mg^{2+} content, the Chl *a* amount significantly decreased accompanied by a reduced photosystem I (PS I) content when compared to the control. While the amount of photosystem II (PS II) was not severely altered, its effective quantum yield was reduced. In contrast, the effective quantum yield of PS I increased, in agreement with a reduced donor-side limitation and faster P_{700} re-reduction when cells were grown under Mg^{2+}-limiting conditions. EM micrographs revealed an increased s-layer surrounding individual cells when cells were growth under Mg^{2+} limitation.

Using the fluorescent dye acridine orange, we monitored the light-induced generation of a ΔpH across the *Synechocystis* TMs. Following a fast decrease of the fluorescence signal upon switching the light on, due to acidification of the thylakoid lumen, we observed a fluorescence increase, due to alkalization of the cytoplasm. Yet, altered fluorescence signals were observed when *Synechocystis* cells were grown in media with low Mg^{2+} concentrations suggesting a significant role of Mg^{2+} in the formation of a ΔpH across the cyanobacterial TM, most likely due to an impaired ability to counterbalance the pH gradient that is built up across the TM due to the photosynthetic light reaction.

162 In Person Oral

Ultrafast excitation energy transfer of engineered biohybrid light-harvesting 2 complex (LH2) with extrinsic chromophores

Takehisa Dewa[1], Masaya Kito[1], Tetsuya Yamamoto[2], Kazuki Hinago[2], Masaharu Kondo[1], Yutaka Nagasawa[2]

[1]Nagoya Institute of Technology, Nagoya, Aichi, Japan. [2]Ritsumeikan University, Kusatsu, Shiga, Japan

We have recently reported an extension of the light-harvesting ability of light-harvesting 2 complex (LH2) from a purple photosynthetic bacterium by attachment of artificial chromophores (biohybrid LH2), which was driven by a mechanism of ultrafast excitation energy transfer (EET) [Yoneda et al. (2015) J. Am. Chem. Soc. 137:13121–13129; Yoneda et al. (2020) J. Phys. Chem. C 124:8605–8615]. Femtosecond transient absorption (TA) spectroscopy exhibited multi-exponential EET dynamics, which may come from the variation of the locations of the attached extrinsic chromophores. In this study, we addressed constructing faster EET systems to clarify the mechanism of EET by using engineered LH2 in which cysteine (Cys) residues were introduced to either Cys residue nearby B850 (LH2α polypeptides) or B800 (LH2β polypeptides). For the former system, a fluorophore ATTO647N was attached to the Cys residue apart from the B850-coordinated His by five amino acid residues in the α polypeptide. The femtosecond TA spectroscopy showed accelerated EET from ATTO647N to B850 at the time constants at 320 fs and 2.0 ps. When ATTO647N was attached to the vicinity of B800, the time constant of EET from ATTO647N to B800 was 7.1 ps, slower than the former system. These results suggest that the B850 assembly is a remarkable energy acceptor compared to B800, despite the less spectral overlap with the donor ATTO647N. This remarkable ability of B850 as an energy acceptor may come from the higher-lying exciton bands as an ultrafast channel, through which the excitation energy of the donor molecule can be effectively transferred.

163 Pre Recorded Oral

Lemnaceae: floating plants able to combine high levels of zeaxanthin and photosynthesis

Barbara Demmig-Adams, William Adams

University of Colorado, Boulder, CO, USA

The opportunity to study mangroves Olle Börkman brought back from a sabbatical in Australia led to BDA's career-long pursuit of carotenoid roles in plants and as human micronutrients. Without the chance to study non-photochemical dissipation of excess absorbed light in these plants with their remarkable capacity for this process, BDA would not have run into the association between this process and the carotenoid zeaxanthin. Selected examples of continuing questions in this field will be highlighted. Our own group's recent focus has been on aquatic floating plants (Lemnaceae) and their unique features surrounding zeaxanthin accumulation. *Lemna* combines exceptionally high zeaxanthin levels with high maximal photosynthesis rates and very fast growth. This contrasts with the trends seen in land plants where similarly high zeaxanthin levels (and very high levels of non-photochemical dissipation of excitation energy) are seen only in slow-growing species with low maximal photosynthesis rates (and low levels of photochemical dissipation of excitation energy). Moreover, a modest growth light intensity is sufficient to induce *Lemna*'s maximal capacity for photosynthesis and growth rate as well as extraordinarily high contents of high-quality protein and essential human micronutrients (e.g., lutein, β-carotene, vitamin E). This low-light-grown *Lemna* can be transferred to high light for a few hours in a pre-harvest finishing procedure to induce pronounced zeaxanthin accumulation while avoiding the heat damage land plants often experience upon such transfer. We review how the aquatic environment favors features that make Lemnaceae a nutritious crop for sustainable cultivation in small spaces (urban greenhouses or spaceflight environments) with low resource input and remarkable climate resilience. We summarize the roles of zeaxanthin and lutein in human vision as well as opposing systemic inflammation, enhancing cognitive functioning, and combatting chronic and infectious diseases (including a role of zeaxanthin in COVID-19 protection).

164 In Person Oral

Basking in the sun: how mosses photosynthesise and survive on the coldest continent on Earth

Sharon Robinson[1,2], Alicia Perera Castro[3,2], Krystal Randal1[1,2], Melinda Waterman[1,2]

[1]Securing Antarctica's Environmental Future, Wollongong, NSW, Australia. [2]Centre for Sustainable Ecosystem Solutions, University of Wollongong, Wollongong, NSW, Australia. [3]Universitat de les Illes Balears, Palma, Mallorca, Spain

Antarctica is the coldest, highest, driest, windiest continent on Earth. The region has experienced major changes in temperature, wind speed and stratospheric ozone levels over the last 50 years. Whilst West Antarctica and the peninsula have shown rapid warming and consequent ecosystem change, at first East Antarctica appeared to be little impacted by climate warming and thus biological changes were predicted to be relatively slow. Recently, however, Antarctica has experienced extreme climatic events in the form of summer heatwaves in 2019/20 [Robinson et al. (2020) Glob. Change Biol. 26:3178-3180] and an autumn heatwave in March 2022.

Terrestrial plants are limited to the peninsula region and mosses are the dominant plants for most of the frozen continent. Mosses are restricted to sparse ice-free areas and grow where melt water is available through the summer. Soils are generally poor and nutrient sources often come from marine sources, including ancient penguin colonies. Even in the summer, Antarctic mosses and lichens battle sub-zero temperatures, extreme winds and reduced water availability; all influencing their ability to survive and grow. However, in summer, Antarctic moss canopy temperatures can be well above air temperature. At midday, canopy temperatures can exceed 15°C, depending on moss turf water content [Perera Castro et al. (2020) Front. Plant Sci. 11:1178; see abstract 92]. How do these tiny plants protect themselves in such harsh conditions?

This talk will address how mosses photosynthesise on the cold continent, how they protect themselves from excess light and ultraviolet (UV) radiation [Waterman et al. (2017) Journal of Natural Products 80:2224-2231; Waterman et al. (2018) Biological Research 51:49] and what a changing climate might mean for their survival and health.

165 Pre Recorded Poster

Light harvesting protein complement of Photosystem I and II in diatoms grown under different light intensities

Claudio Calvaruso, Konstantinos Stefanidis, Claudia Büchel

Institute of Molecular Biosciences, Goethe University Frankfurt, Frankfurt, Germany

Despite the ecological relevance of diatoms, many aspects of their photosynthetic machinery are still unclarified. Diatoms differ from the green-lineage of oxygenic organisms by their pigmentation and their light-harvesting complex (Lhc) proteins, also called fucoxanthin-chlorophyll proteins (FCP). Three main groups of proteins constitute the FCPs: Lhcf, Lhcr and Lhcx, the latter involved in photoprotection. Different subpopulations of photosystem (PS) I and PSII supercomplexes were isolated from the diatom *Thalassiosira pseudonana* and their subunits identified. The analysis of the antenna composition identified Lhc(s) specific for PSI (Lhcr1, 3, 4, 7, 10-14 and Lhcf10) and for PSII (Lhcf 1-7, 11 and Lhcr2), respectively. From the Lhcx group, only Lhcx6_1 was closely associated with both PS [Calvaruso et al. (2020) Plant Physiol. 183:67-79]. Three-dimensional sub-volume averaging from cryo tomography of thylakoid membranes revealed that the PSII supercomplex of *T. pseudonana* incorporates monomeric FCPs and a trimeric form of light-harvesting antenna, which differs from the tetrameric antenna observed previously in another diatom, *Chaetoceros gracilis*. The organization of the PSI supercomplex is conserved in both diatom species [Arhad et al. (2021) Plant Physiol. 186:2124-2136].

High light acclimation changed the molecular features of the PS and their ratio in thylakoids. In PSII supercomplexeds, no obvious changes in polypeptide composition were observed, whereas for PSI changes in one specific group of FCP proteins were detected. The amount of xanthophyll cycle pigments and their de-epoxidation ratio was increased in PSI under HL. In PSII, however, no additional xanthophyll cycle pigments occurred, but the de-epoxidation ratio was increased as well. This comparison suggests how mechanisms of photoprotection might take place within and in the proximity of the PS, which gives new insights into the capacity of diatoms to adapt to different conditions and in different environments.

166 In Person Poster

Unlocking novel biocatalytic potential of Photosystem II by directed evolution

Tom Dongmin Kim, Tanai Cardona

Imperial College London, London, United Kingdom

Photosynthesis is the very foundation of life on earth, and the reaction starts with the light-driven oxidation of water by photosystem II (PSII). enormous effort has been given to understand this multiprotein complex, in terms of its structure, assembly, mechanism and more recently, its evolutionary history [Oliver et al. (2021) Biochim Biophys Acta Bioenerg. 1862(6):148400]. While PSII is generally highly conserved across photosynthetic organisms, the reaction centre protein D1 in PSII shows incredible sequence diversity compared with the main characterised version that is essential for photosynthesis [Cardona T. (2016) Front Plant Sci. 7:257]. We hypothesize that this diversity of D1 reflects an inbuilt mechanism of adaptability and versatility to a changing environment, which makes the system evolvable. Based on this hypothesis, the current project aims to develop a directed evolution pipeline for engineering PSII to utilise its unexplored potential.

Using a D1-deficient *Synechocystis* sp. PCC 6803 mutant strain as a model vehicle, an *in vitro* D1 library diversification system based on error-prone PCR is established. To increase the throughput of the variant library, a camouflage strategy using one of the *Synechocystis* methyltransferases is employed to evade the restriction-modification system and the resulting library size reaches up to 10^5 variants per µg DNA. With these variant libraries, screening endeavour is in progress including substrate screening for novel photobiocatalysis and growth performance under atypical light conditions. A modular *in vivo* directed evolution system equipped with a Cas9-directed, multi-target hypermutation module is also in development to expand the coverage on the evolutionary landscape of PSII. This approach will provide knowledge and molecular rationales on how to control and manipulate the structure and function of PSII, for any desired purpose, and even to achieve novel functions that go beyond water oxidation, but that might be of interest for biotechnological applications.

167 Pre Recorded Oral

Pending astrobiology questions for photosynthesis research

Nancy Kiang

NASA Goddard Institute for Space Studies, New York, NY, USA

With the latest and upcoming space telescopes, the prospect of detecting life on planets outside our solar system – exoplanets – is an impending reality. Photosynthesis on Earth produces signs of life that can be seen from remote spacecraft, such as the Galileo mission in 1990 and the Cassini fly-by of Saturn in 2013. These signs of life include oxygen in the Earth's atmosphere and the spectral reflectance of land vegetation. While other gaseous and surface signs of life have been proposed, those produced by oxygenic photosynthesis remain the most robust and most rigorously vetted for false positives and negatives for life and remain prime targets in the search for life elsewhere by NASA and other space agencies. However, there remain fundamental unanswered questions about how photosynthesis on Earth can inform our interpretation of observations of exoplanets for the presence – or not—of this major life process. These include how the oxygen-evolving complex (OEC) originated; how might light-harvesting pigments express differently under the light of another star; how might the trap wavelength of the photosynthetic reaction center differ as well as evolve; whether anoxygenic photosynthesis has the potential to dominate at the planetary scale; and other fundamental questions about the photosynthetic apparatus and light use efficiency. I present a review of these astrobiology questions for photosynthesis research and describe proposed frameworks for interpreting exoplanet observations given limited data and theoretical understanding. This review is intended to inspire researchers on diverse aspects of photosynthesis, including but not limited to its origin, evolution, energetics, light harvesting adaptation and regulation, modelling, and alternative configurations in synthetic biology and artificial photosynthesis.

168 In Person Poster

High spin structures of S_2 state manganese cluster

Hiroyuki Mino[1], Shinya Kosaki[2]

[1]Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan. [2]School of Science, Nagoya University, Nagoya, Aichi, Japan

The photosynthetic manganese cluster has five different redox states denoted as the S_n (n = 0–4), and Sn advances to S_{n+1} by one-electron oxidation, and S_4 relaxes to S_0. The structure of the manganese cluster consists of a Mn_3CaO_4 cube and additional manganese and oxygen. EPR experiments have reported some isomers in S_2 states depending on the species. The *g*~2 multiline and the *g*~4 (4.1) signals were detected in plant PSII, however, the *g*~4 EPR signal was not detected in cyanobacterial PSII under physiological conditions. The equilibrium is ascribed to some structural modifications to the manganese cluster caused by the charge effect of the extrinsic proteins [Taguchi et al. (2020) J Phys Chem. B 124:5531-5537].

Another S_2 isomer is detected in the extrinsic protein depleted PSII as a *g*~5 (4.5-4.9) EPR signal with hyperfine structures, depending on the concentrations of divalent cations. The signal should not be easily categorized as the same *g*~4 high spin states. The *g* ~5 signal is similar to the EPR signal previously reported in cyanobacterial PSII at high pH or on the way from the S_3 state, which have no hyperfine structure. The temperature dependence of the signal formation on S_1 to S_2 states is complementary to S_2 to S_3 states [Mino (2022) Photosyn. Res. *in press*].

There are two possibilities for the origin of the *g*~5 spin state, one is the isolated ground spin *S*=5/2, which is closer to rhombic symmetry, or the isolated ground spin *S*=7/2, which is closer to axial symmetry. We will discuss the spin state with some recent results.

169 Pre Recorded Oral

Revisiting the ELIP hypothesis for sustained thermal dissipation in overwintering yew leaves

Ryouichi Tanaka[1], Ye Zihao[1], Mina Sawada[1], Makio Yokono[2], Makiko Iwasa[1], Ryo Moriyama[1], Toshihiko Hara[1], Ayumi Tanaka[1], Mitsutoshi Kitao[3], Junko Kishimoto[1], Atsushi Takabayashi[1]

[1]Hokkaido University, Sapporo, Hokkaido, Japan. [2]National Institute for Basic Biology, Okazaki, Aichi, Japan. [3]Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, Hokkaido, Japan

The photosynthetic antennae of most plants have a thermal dissipation mechanism in which PsbS controls thermal dissipation in response to rapid changes in incident lights. The photosynthetic machinery of evergreen conifers is supposed to have an extra mechanism that keeps thermal dissipation high during wintertime, which is termed "sustained" non-photochemical quenching (sustained NPQ). Several factors, which are not mutually exclusive, have been proposed to take part in the sustained NPQ mechanism which include 1) zeaxanthin accumulation, 2) early light-induced protein (ELIP), 3) the phosphorylation levels of light-harvesting-complex (LHC) proteins, 4) spillover from photosystem II (PSII) to PSI. To gain insights into the sustained NPQ mechanism, we investigated the correlated changes in the photosynthetic responses in yew (*Taxus cuspidata*) leaves and its transcriptomes in Sapporo, Japan. The quantum yield of PSII (Φ2) was measured every week in 2019 and 2021 at the sun and shade side of a yew tree. The results show that Φ2 is correlated with ambient temperatures between December and March, while it was correlated with light intensity in other months, indicating that sustained NPQ is induced in December. The analysis of gene expression profiles a substantial number of genes are upregulated in winter. In particular, the total number of the ELIP transcripts dominated the winter transcriptomes: they account for about 15% of total transcripts in winter. ELIP protein accumulates nearly the same level as LHC in winter. On the contrary, the proportion of spillover estimated by the measurement of delayed chlorophyll fluorescence at 77K did not change in summer and winter, indicating that spillover is not a major factor for sustained NPQ in yew. The results indicate that ELIP plays an important role in over-wintering yew leaves.

170 In Person Oral

Leaf structure-function relationship provides insights into photosynthetic capacity and induced responses under stress in walnut

Mina Momayyezi[1], Andrew McElrone[1,2]

[1]University of California, Davis, Davis, California, USA. [2]USDA-ARS, Davis, California, USA

Leaves are complex bioreactors balancing CO_2 and water exchange and light absorption to maximize photosynthesis. Inherent variation and stress-induced changes in leaf structure and function can provide insight into photosynthetic performance. Walnuts are important crops for California agriculture, and we have begun exploring *Juglans* germplasm for traits of agronomic value related to photosynthetic capacity. Anatomical and biochemical bases of photosynthesis and dehydration response for 11 *J. regia* accessions, collected from various habitats were explored as future potential scions. Accessions with highest photosynthesis at ambient and saturating CO_2 have thicker leaves with more porosity and gas-phase diffusion (g_{IAS}), leaf nitrogen with less leaf mass, native to lower latitudes with more frost-free days, greater precipitation seasonality, and lower temperature seasonality. Drought impairs photosynthesis in all accessions, while low-latitude accessions remain photosynthesis higher even under stress, suggesting their structural and biochemical potentials for future breeding. When walnut species *J. regia* and *J. microcarpa* with contrasting leaf anatomy were compared using a combination of light microscopy, X-ray microCT, gas exchange, and chlorophyll distribution, they exhibited differences in mesophyll CO_2 conductance (g_m) and light absorption under non-stressed and drought conditions. *J. regia* with thicker palisade mesophyll, higher fluorescence in the palisade, greater low-mesophyll porosity, and greater g_{IAS}, has higher stomatal and mesophyll (g_m) conductances and carboxylation capacity. While *J. microcarpa* with more and highly-packed mesophyll cells and bundle sheath extensions (BSEs) show higher fluorescence in spongy and in proximity to the BSEs. Drought reduces mesophyll cell volume in both species, leading to an increase in porosity and g_{IAS}, but significant declines in biochemical activity under drought overrule changes in g_{IAS} and decrease g_m. Drought-induced changes in gas exchange and light absorption are linked to leaf anatomy, suggesting leaf structure and biochemistry that enhances photosynthesis can be used to select genotypes to improve productivity under stress.

171 Pre Recorded Oral

Conifer photorespiration involves a substantial non-enzymatic decarboxylation reaction due to an imbalance between catalase and glycolate oxidase activities in the peroxisomes

Shin-Ichi Miyazawa[1], Takafumi Miyama[1], Ko Tahara[1], Tokuko Ujino-Ihara[1], Hiroyuki Tobita[1], Yuji Suzuki[2], Mitsuru Nishiguchi[1]

[1]Forestry and Forest Products Research Institute (FFPRI), Tsukuba, Ibaraki, Japan. [2]Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan

Previous studies indicated that chloroplastic glutamine synthetase, an essential enzyme of the photorespiratory pathways, was absent from the leaves of conifers. This finding intrigued us to reconsider the current belief that the photorespiratory pathway is identical between conifer gymnosperms and angiosperm C_3 species. We compared the concentrations of the canonical photorespiratory metabolites of the leaves between 13 conifer and 14 angiosperm tree species grown in a field site. The results revealed that the mean glycerate content per unit chlorophyll concentration of the conifer leaves was only one-tenth that of the angiosperm tree leaves. In the canonical photorespiratory pathway, glycerate is produced from serine via an intermediate, hydroxypyruvate, in peroxisomes. To elucidate the lower levels of glycerate detected in conifer leaves, we performed experiments of ^{13}C-labeled serine feeding to the detached shoots of a conifer (*Cryptomeria japonica*) via the transpiration stream and compared the labeling patterns of photorespiratory metabolites with those of an angiosperm tree (*Populus nigra*). Glycerate was mainly labeled in *P. nigra* leaves while glycolate was more labeled than glycerate in *C. japonica* leaves. These results suggested that H_2O_2-mediated non-enzymatic decarboxylation (NED) converting hydroxypyruvate into glycolate occurred in *C. japonica*. The photorespiratory pathway involves an H_2O_2-scavenging enzyme, catalase (CAT), and an H_2O_2-generating enzyme, glycolate oxidase (GLO); both are peroxisomal enzymes in angiosperms. In contrast, database analyses of the peroxisomal targeting signal motifs and analyses of the peroxisomal fractions isolated from *C. japonica* leaves demonstrated that conifer peroxisome was not a major localization of leaf CAT activity. These results suggest that NED substantially occurs during photorespiration in conifer leaves because of an imbalance between CAT and GLO activities in the peroxisomes.

172 Pre Recorded Oral

First contact to gravity – earliest land plants bryophytes adapt to increase in gravity via enhancements of photosynthesis and expression of AP2/ERF transcription factors

Yuko T. Hanba[1], Kaori Takemura[1], Sakihito Kitajima[1], Maki Yokoi[2], Yuki Yamashita[3], Akihisa Shinozawa[4], Ayuko Maeda[1], Hiroyuki Kamachi[5], Atsushi Kume[6], Ichirou Karahara[5], Yoichi Sakata[4], Tomomichi Fujita[3]

[1]Kyoto Institute of Technology, Kyoto, Japan. [2]Hokkaido University, Sapporo, Japan. [3]Hokkaido University, Hokkaido, Japan. [4]Tokyo University of Agriculture, Tokyo, Japan. [5]University of Toyama, Toyama, Japan. [6]Kyushu University, Fukuoka, Japan

Bryophytes, which are one of the earliest land plants that colonized terrestrial environments from aquatic ancestors, have faced the significant increases in gravitational force for the first time over the evolutionary history of land plants. Changes in gravity should have induced significant changes in physiology, anatomy and growth of bryophytes, but there has yet to be any direct link demonstrated between gravity, photosynthesis, and gene expression. Here we show that photosynthesis of a model bryophyte, *Physcomitrium patens*, is strongly enhanced by the increases in gravity (hypergravity, 6 xg and 10 xg), in which acceleration in CO_2 diffusion of P. patens is a key trait. This increase in CO2 diffusion is probably governed by the increase in surface area of chloroplasts facing to the air, which is due to both an increase in plant numbers and an increase in chloroplast sizes. To identify a key gene to this process, we performed RNA-seq analysis and found that AP2/ERF transcription factors were predominantly up-regulated at 10 xg. Hence, we mimicked gene expression of 10 ×g-growing P. patens by artificially overexpressing one of the AP2/ERF transcription factors to test any link of this gene to the anatomical and photosynthetic alterations against hypergravity. In the overexpressed plants, chloroplast size and plant numbers were increased, which lead to increased CO2 diffusion and thus enhanced photosynthesis rate. Thus, our results strongly suggest that the AP2/ERF transcription factors are one of the key genes, which bridge hypergravity response to enhancement of photosynthesis and growth. Together with the fact that gene number of AP2/ERF transcription factors in the genome increase rapidly in bryophytes when compared to the sister group of land plants, multiplication of AP2/ERF transcription factors and establishment of the novel gene network may play a crucial role to adapt towards terrestrial environments during land plant evolution.

173 In Person Poster

Energy migration and quenching models in LHCII

Callum Gray, Chris Duffy

Queen Mary University of London, London, United Kingdom

Non-photochemical quenching (NPQ) is a crucial process by which higher plants defend themselves from photodamage. However, the precise mechanism by which NPQ proceeds within a photosynthetic complex is still not fully understood.

There are well-established theoretical models and detailed experimental data available for the study of single light-harvesting complexes, as well as less invasive measurements on the level of the membrane, which by necessity probe longer length and time scales. However, more coarse-grained theoretical models of photosynthetic membranes have generally included approximations such as idealising the geometry of the system, working at very low excitation density or assuming a continuum model of protein aggregates, none of which truly represent the analogous experimental measurements.

We address this problem by performing Monte Carlo simulations of energy migration and quenching within LHCII aggregates, while also reproducing a TPSPC experiment and fitting the obtained simulation data in the same way. We find that multi-exciton effects such as annihilation are present even at relatively low excitation energies and that simply modelling energy migration within aggregates, even in the absence of any recognised quenching model, has a discernible effect on the excited state lifetime. Finally, we test several qualitatively distinct models of quenching to investigate whether the various proposed mechanisms for NPQ could be differentiated based on experimental data.

174 Pre Recorded Poster

The Ycf48 accessory factor occupies the site of the oxygen-evolving manganese cluster during photosystem II biogenesis

Ziyu Zhao[1], Irene Vercellino[2], Jana Knoppová[3], Roman Sobotka[3,4], James Murray[1], Leonid Sazanov[2], Josef Komenda[3,4], Peter Nixon[1]

[1]Imperial College London, London, United Kingdom. [2]Institute of Science and Technology Austria, Klosterneuburg, Austria. [3]Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic. [4]University of South Bohemia, České Budějovice, Czech Republic

The efficient assembly and repair of the oxygen-evolving photosystem two (PSII) complex needed for robust oxygenic photosynthesis relies on a suite of accessory factors. The highly conserved Ycf48 assembly factor binds to the newly synthesized D1 reaction center polypeptide and promotes the initial steps of PSII assembly, but its binding site is unclear. Here we have used cryo-electron microscopy to determine the structure of a cyanobacterial PSII D1/D2 reaction center assembly complex with Ycf48 attached. Ycf48, a 7-bladed beta propeller, binds to the amino-acid residues of D1 that ultimately ligate the Mn_4CaO_5 cluster that catalyzes water oxidation, thereby preventing the premature binding of Mn^{2+} and Ca^{2+} ions and protecting the site from damage. Interactions with D2 help explain how Ycf48 promotes assembly of the D1/D2 complex. Overall, our work provides new insights into the structural changes that occur to create the binding site for the Mn_4CaO_5 cluster.

175 Pre Recorded Poster

Diurnal regulation of the activities of FBPase and NADP-GAPDH enzymes in some local C4 species of the Chenopodiaceae

Shahniyar Bayramov, Taliyya Orujova, Ulduza Gurbanova, Novruz Guliyev

Institute of Molecular Biology and Biotechnology ANAS, Baku, Azerbaijan

Among dicotyledon families, Chenopodiaceae has the most C4 species and the greatest diversity in structural forms of C4. Diurnal dynamics of changes in the activity of NADP-glyceraldehyde-3-phosphate-dehydrogenase (NADP-GAPDH) and fructose-1,6-bisphosphatase (FBPase) enzymes belonging to the Calvin cycle have been studied in some local C4 species of the Chenopodiaceae family grown under natural climatic conditions. Dynamics of changes in the activity of the mentioned enzymes in *Neoae mucranata*, *Suaeda altissima*, *Salsola dendroides*, and *Atriplex tatarica* species of different photosynthetic subgroups of the Chenopodiaceae family were studied. The experiments were performed during the active developmental stages of vegetation in plants grown under natural conditions. The results showed that the initial activity of the enzyme FBPase was higher in *S. altissima* than in other species. The initial activity of this enzyme was 13.3 and 1.7-fold higher in the afternoon hours compared to the morning and evening hours, respectively. The maximum activity of the enzyme FBPase in *S. altissima* increased 6-fold in the morning and evening hours and 2.5-fold in the afternoon hours compared to the initial activity. The activity of the enzyme NADP-GAPDH in the leaves of the studied plants was significantly higher in the afternoon than in the morning hours. The activities of NADP-GAPDH and FBPase were redox-regulated by light. The results show that the increase in light intensity in the afternoon hours is accompanied by increasing activities of both enzymes, which suggests that the activities of these enzymes are redox regulated by light.

176 Pre Recorded Poster

Photosynthetic machinery, carbon metabolism, and antioxidant status of wheat genotypes under drought stress followed by-re-watering

Durna Aliyeva, Ulduza Gurbanova, Aynura Pashayeva, Ismayil Zulfuqarov, Irada Huseynova

Institute of Molecular Biology and Biotechnologies, Azerbaijan National Academy of Sciences, Baku, Azerbaijan

During water deficiency, vascular plants shorten their vegetation period. We try to illuminate the role of the morpho-physiological and biochemical parameters and changes of the photosynthetic machinery in response to drought followed by re-watering in wheat plants. Consequently, some drought-stress related traits of two bread wheat genotypes (drought-tolerant Gobustan and drought-sensitive Tale-38) have been studied. The hydrogen peroxide content increases under drought in both genotypes. Recovering hydrogen peroxide content occurs faster in the Gobustan genotype after re-watering. We have demonstrated that the isozymes of benzidine peroxidase change dynamically under drought. Similar dynamic changes were observed in the content of the two carbon-fixing enzymes, aspartate aminotransferase, and alanine aminotransferase. In both genotypes, the electron transport rate and photochemical efficiency of photosystem II show similar responses to drought with subsequent re-watering. Nevertheless, the amount of the photosynthetic pigments changes considerably resulting in structural changes of thylakoid membranes. In the drought-tolerant Gobustan genotype, the structure of thylakoid membrane nearly totally recovered after re-watering. Amongst the traits used in our research, the fluctuations in dry biomass, the levels of the phenolic compounds, benzidine peroxidase enzyme, the amount of the photosynthetic pigments, and the proteome of thylakoid membranes respond to the drought with subsequent re-watering more dynamically. Remarkably, these traits show a high recovery rate in the drought-tolerant Gobustan genotype. Hence, we conclude that the drought-tolerant genotype exhibits higher adaptive potential to stress and more dynamic recovery than the drought-sensitive genotype.

177 Pre Recorded Oral

Architecture of the chloroplast PSI-NDH supercomplex in *Hordeum vulgare*

Liangliang Shen[1,2], Kailu Tang[3], Wenda Wang[1], Chen Wang[3], Hangjun Wu[4], Zhiyuan Mao[1,2], Shaoya An[3,4], Shenghai Chang[4], Tingyun Kuang[1], Jian-Ren Shen[1,5], Guangye Han[1], Xing Zhang[3,4,6]

[1]Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing, China. [2]University of Chinese Academy of Science, Beijing, China. [3]Department of Biophysics, and Department of Pathology of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China. [4]Center of Cryo-Electron Microscopy, Zhejiang University School of Medicine, Hangzhou, China. [5]Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan. [6]Liangzhu Laboratory, Zhejiang University Medical Center, Hangzhou, China

Two types of photosynthetic electron transport pathways, linear and cyclic electron transport (LET and CET), operate in the thylakoid membranes of oxygenic photosynthetic organisms. The PSI CET is essential for balancing the changing demands of ATP/NADPH under various physiological conditions and for the regulation of the photosynthetic machineries in response to the varying environmental conditions. In angiosperms, two distinct ferredoxin-dependent CET routes are known to operate. One is a PROTON GRADIENT REGULATION 5 (PGR5) and PGR5-like Photosynthetic Phenotype 1 (PGRL1)-dependent, antimycin A-sensitive pathway. The other one is a chloroplast NADH dehydrogenase-like complex (NDH)-dependent, antimycin A-insensitive pathway. Both pathways contribute to the formation of ATP and can cooperate under various different conditions to regulate the ATP/NADPH ratio.

Plant chloroplast NDH complex is a large thylakoid membrane complex consisting of at least 29 subunits and plays an important role in mediating PSI CET. NDH has been shown to form supercomplexes with the PSI core via light-harvesting complex I (LHCI) subunits to fulfill its function in CET. However, the detailed structure of the PSI-NDH supercomplex remains elusive. Here we report cryo-electron microscopy structure of a PSI-NDH supercomplex from barley (*Hordeum vulgare*) at an overall resolution of 4.4 Å and local resolutions of 3.40 Å and 3.88 Å for two PSI-LHCIs and 3.70 Å for the NDH complex, respectively [Shen et al.(2022) Nature 601:649-654]. The result demonstrates that PSI-NDH is composed of two copies of PSI-LHCI and one NDH complex. Two monomeric LHCI proteins, Lhca5 and Lhca6, mediate the binding of two PSI complexes to NDH. Ten plant chloroplast specific NDH subunits are observed and their interactions with other subunits in NDH are elucidated. Taken together, this study provides a structural basis for elucidating the mechanism of PSI-NDH-mediated CET in angiosperms.

178 Pre Recorded Poster

Effect of soil nitrogen availability on photosynthetic cold acclimation of *Arabidopsis thaliana* plants

Armida Gjindali, Giles Johnson

University of Manchester, Manchester, United Kingdom

Upon transfer of plants to low non-freezing temperature, excitation pressure is imposed on Photosystem II (PSII) due to slowing of metabolism, leading to a decrease of photosynthetic carbon assimilation. Exposure of fully expanded leaves of *Arabidopsis thaliana* plants to sustained low temperature results in changes in gene expression, protein content and leaf metabolism and an establishment of a new steady state with higher photosynthetic rate compared to that prior to cold exposure. This process is called dynamic photosynthetic acclimation and is completed in a time scale of days. We hypothesize that nitrogen, as one of the essential elements of amino acids, affects the ability of plants to acclimate to sustained cold conditions.

In this study Arabidopsis thaliana plants, Columbia 0 ecotype were grown in 20 ^{o}C day/18 ^{o}C night in sand and were watered three time per week with Hoagland's solution modified to three total nitrogen concentrations: 2.5mM, 5mM and 15mM. After 8 weeks, plants were transferred in 5 ^{o}C for a week before photosynthetic acclimation being evaluated. Limiting N availability to 2.5mM N resulted in hindering of the acclimation process. Only plants grown in 15mM N were able to acclimate and reach higher maximum photosynthetic rate after cold exposure. Plants grown in 5mM N concentrations where unable to acclimate to cold but did not exhibit signs of extreme stress with maximum photosynthetic rate remaining in the same level before and after cold exposure.

179 Pre Recorded Oral

Chlorophyll-*f*-producing cyanobacterial communities in selected karst caves of Northern Italy

Francesco Bellamoli[1], Leonardo Latella[2], Marco Cantonati[3], Matteo Calgaro[1], Camilla Laiti[3], Stefano Cazzaniga[1], Nicola Vitulo[1], Matteo Ballottari[1]

[1]Department of Biotechnology, University of Verona, Verona, Italy. [2]Department of Zoology, Museo di Storia Naturale of Verona, Verona, Italy. [3]Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy

Caves are seldom considered an environment where phototrophic organisms can grow, but they can be found in most parts reached by direct or indirect sunlight. Among phototrophs, cyanobacteria have the ability to adapt to harsh environmental conditions, such as dimly illuminated cave sections with low temperatures. Depending on light conditions, through a remodeling of the photosynthetic apparatus (FaRLiP) and by synthesizing alternative chlorophylls (chl *d* and/or *f*), cyanobacteria can also utilize far-red light to drive photosynthetic processes. Although far-red absorbing chlorophylls have been described in several cyanobacterial species, their occurrence and distribution, in terrestrial and especially in cave habitats, is still scarcely documented.

In this study, we examined aerophytic epilithic cyanobacterial populations and associated prokaryotic communities in three karst caves of Northern Italy. In particular, for each sampling site we analyzed pigment content by HPLC, thus identifying chlorophyll-*f*-containing samples, and performed metabarcoding analyses on 16S rRNA V3-V4 region to determine the composition of prokaryotic communities.

Subsequent analyses highlighted complex communities with strong taxonomic differences in very small distances on a spatial scale, with light availability as the main driver. We determined possible cyanobacterial taxa associated with chlorophyll *f* production, and, moreover, we identified non-cyanobacterial taxa strongly associated with the presence of chlorophyll *f* that could be considered putative biomarkers indicating ecological niches for chlorophyll *f*-producing cyanobacteria.

180 Pre Recorded Poster

Photosystem II monomeric antenna CP26 has a key role in regulating Non-Photochemical Quenching in *Chlamydomonas reinhardtii*

Stefano Cazzaniga[1], Matteo Pivato[1], Federico Perozeni[1], Minjae Kim[2], Samim Sardar[3], Cosimo D'Andrea[3], EonSeon Jin[2], Matteo Ballottari[1]

[1]Dipartimento di Biotecnologie, Università di Verona, Verona, Italy. [2]Department of Life Science, Hanyang University, Seoul, Korea, Republic of. [3]Istituto Italiano di Tecnologia, Center for Nano Science and Technology, Milano, Italy

Photosystem II (PSII) must balance between efficient light harvesting and photoprotection from excess light energy absorption. Among photoprotective mechanisms, the thermal dissipation of excitation energy by non-photochemical quenching (NPQ) is the fastest and the more prominent. Dissecting the specific contribute of different antennae is fundamental to understand microalgae response to different lights. PSII antennas are arranged into monomeric LHC and trimeric LHCII. For their position as linker between the core complex and the external LHCII, the monomeric subunits have a pivotal role in both maintaining an efficient light harvesting and activating photoprotection to avoid reactive oxygen species generation. The role of monomeric antenna CP26 was investigated in *Chlamydomonas reinhardtii* using genome editing to obtain a knock-out mutant (named k6). Absence of CP26 partially reduced photosynthetic efficiency: excitation energy transfer, PSII efficiency and oxygen evolution were decreased in the mutant and, at moderate light intensities, k6 grew slower than parental strain. But the main effect of CP26 was on NPQ that was strongly reduced. NPQ was detected with different technique (pulsed fluorescence, 77K-fluorescence emission quenching and acid-induced quenching) all confirming its impairing in k6. The NPQ phenotype was rescued by complementing k6 with the genomic sequence of CP26 confirming the specific role of the protein. This complementing allowed also to study the correlation between amount of CP26 and NPQ; ~50% of CP26 (with respect to wild-type) was able to restore a wild-type level of NPQ while below 50% there was a linear correlation between CP26 content and quenching. These data shows that CP26 has a different role with respect to the other monomeric subunit, CP29, that was strongly involved in light harvesting and only minimally affected NPQ. Thus, the genetic engineering of these two proteins could be a promising target to regulate microalgae photosynthetic efficiency at different light regimes.

181 Pre Recorded Poster

Time-resolved FTIR difference spectroscopy for the study of Photosystem I with non-native naphthoquinones incorporated into the A_1 binding site

Neva Agarwala, Hiroki Makita, Gary Hastings

Georgia State University, Atlanta, GA, USA

Microsecond time–resolved Fourier transform infrared difference spectroscopy (TR FTIR DS) has been used to study photosystem I (PSI) with four high-potential, 1,4–naphthoquinones (NQs) incorporated into the A_1 binding site are 2–chloro–NQ (2ClNQ), 2–bromo–NQ (2BrNQ), 2,3–dichloro–NQ (Cl_2NQ), and 2,3–dibromo–NQ (Br_2NQ). “Foreign minus native” double difference spectra (DDS) were constructed by subtracting TR FTIR DS for native PSI from corresponding spectra of different NQs.

To help assess and assign bands in the DS and DDS, density functional theory based vibrational frequency calculations for different quinones in solvent, or in the presence of a single asymmetric –bond to either a water molecule or a peptide backbone NH group, were undertaken. Calculated and experimental spectra agree best for the peptide backbone asymmetrically –bonded system. We also show that the monosubstituted NQs can occupy the binding site into different orientations, with C_3 hydrogen atom being both ortho and meta to the -bonded C_4=O group.

By comparing multiple sets of DDS, bands of PhQ^- are identified at 1520, 1494, 1476, 1456 and 1414 cm^{-1}. The 1494 and 1414 cm^{-1} bands are well–known to be due to C_1 ̈ O and C_4 ̈ O stretching vibrations, respectively. The band at 1476 cm^{-1} is assigned to C– bending modes of PhQ^- whereas the bands at 1520 and 1456 cm^{-1} are due to a C_2 ̈ C_3 stretching and a C– bending vibration, respectively. Similar bands are identified for the four halogenated NQs.

The frequency separation between the C_1 ̈ O and C_4 ̈ O modes for PhQ^-/$DMNQ^-$/$2MNQ^-$/$2ClNQ^-$/$2BrNQ^-$/$Cl2NQ^-$/$Br2NQ^-$ is 80/69/75/83/83/~60/59 cm^{-1}, respectively and ~30 cm^{-1} for the neutral state NQs, indicating stronger hydrogen bonding to the C_4=O group in the anion state compared to neutral state.

182 In Person Oral

Insights into the evolution of C_4 photosynthesis from endemic Australian taxa

Martha Ludwig[1], Nicole Dakin[1], Roxana Khoshravesh[2], Florian Busch[3], Tammy Sage[4], Rowan Sage[4]

[1]University of Western Australia, Crawley, WA, Australia. [2]Lancaster University, Lancaster, United Kingdom. [3]University of Birmingham, Birmingham, United Kingdom. [4]University of Toronto, Toronto, Ont, Canada

The evolution of plants using C_4 photosynthesis occurred through modifications of the anatomy, biochemistry, and gene expression patterns of species employing the ancestral C_3 photosynthetic pathway. The grass genus *Neurachne* and most members of *Tecticornia*, a group of halophytic succulent eudicots, are endemic to Australia. Previous studies on these taxa showed both contain individual C_3 and C_4 species, with *Neurachne* also containing a C_3-C_4 photosynthetic intermediate species. Using a phylogenetically informed approach, we have characterised the CO_2 fixation pathway employed by targeted species in these two groups, which has resulted in novel insights into the evolution of C_4 photosynthesis. From ultrastructural, immunolabelling, gene expression, and gas exchange analyses we found the C_3-C_4 intermediate *N. minor* carries out C_2 photosynthesis, which employs a CO_2 photorespiratory pump, while *N. annularis* and *N. lanigera* are proto-Kranz and C_2-like species, respectively. Consequently, these species allow resolution of the earliest steps along the C_3 to C_4 evolutionary continuum in this monocot genus. Corresponding studies on the photosynthetic articles of targeted *Tecticornia* species showed *T. auriculata* demonstrates several characteristics indicative of a C_3-C_4 photosynthetic intermediate species, which is the first evidence of photosynthetic intermediacy in this genus. Several other *Tecticornia* species show C_4-associated anatomical traits that may represent evolutionary enablers of C_4 photosynthesis in this genus.

183 Pre Recorded Oral

X-ray absorption spectroscopy of the water splitting reaction

Isabel Bogacz[1], Margaret Doyle[1], Medhanjali Dasgupta[1], Hiroki Makita[1], Philipp Simon[1], Thomas Fransson[2], Clemens Weninger[3], Roberto Alonso Mori[4], Uwe Bergmann[5], Jan Kern[1], Vittal Yachandra[1], Junko Yano[1]

[1]Lawrence Berkeley National Lab, Berkeley, CA, USA. [2]KTH Royal Institute of Technology, Stockholm, Sweden. [3]MAX IV Laboratory, Lund, Sweden. [4]SLAC National Accelerator, Menlo Park, CA, USA. [5]University of Wisconsin: Madison, Madison, WI, USA

Water oxidation in photosynthesis occurs through a four-electron redox reaction that is catalyzed by the Mn_4CaO_5 cluster located in the oxygen-evolving complex (OEC) of Photosystem II. During the Kok cycle, the OEC advances through four stable intermediate states (Si, i = 0 to 3) and one transient (S4) where changes in the redox states of the metal cluster occur. Upon the accumulation of four oxidizing equivalents in the Mn4CaO5 cluster, two waters are oxidized to molecular oxygen. Electron paramagnetic resonance (EPR) shows isomorphism in the S2 state with the presence of both a high spin (5/2, g=4.9/4.1 in PS II from cyanobacteria/higher plants) (HS) and low spin (1/2, g=2) (LS) forms. Previous computational studies proposed a correlation of spin state with significant changes in the structure of the Mn4CaO5cluster. In the current study, we examined the pH-induced HS-S2 state of T. Elongatus with Mn-K edge extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge spectroscopy (XANES). Resulting XANES data shows that pH has the largest impact on edge position, as all pH 8.6 data (S1 and S2, low and high spin) was shifted to higher energy relative to pH 6.5 data. EXAFS data and fits did not indicate significant structural changes. Therefore, it indicates a smaller structural difference between spin isomers, such as that expected from different protonation states of oxo/hydroxo and/or water ligands. We will examine possible HS/LS structures of the S2 states, based on the current EXAFS studies using synchrotron radiation at cryogenic temperature, as well as the room temperature crystal structures of the S2 state, and preliminary results of time-resolved room temperature XAS obtained using an X-ray free electron laser. The S2-state structure from XFEL studies, characterized by the g-2 LS EPR signal, shows an open-structure for the Mn4CaO5 cluster.

184 In Person Poster

Electronic requirements for low barrier O-O bond formation in Natural and Artificial Photosynthesis

Yulia Pushkar

Purdue University, West Lafayette, IN, USA

Using the energy of the light, the Photosystem II (PS II) produces oxygen by splitting water into electrons, protons and O2.The catalytic center stores several oxidative equivalents at ~ +1.23 V. This can proceed via highly oxidized metal-oxo species such as MnIV=O, FeV=O or RuV=O. In these species large spin density is localized on the oxygen making it effectively a radical. Metal-oxo species can undergo two main reactions: i) react with water with the formation of Metal-OOH peroxide or ii) by combining two oxo-species with the formation of the bridging Metal-O-O-Metal peroxide.

Using time-resolved X-ray emission spectroscopy, a method uniquely sensitive to the electronic structure of the Mn4Ca, we measured the step of the O-O bond formation in PSII (S3 to S0 transition). Spectroscopic changes are consistent with reduction of the Mn in the cluster (compared to the S3 state) detected at ~50 µs and ~500 µs ahead of the reduction of the TyrZ·+. These results indicate the multi-step nature of the O-O bond formation and O2 release by Photosystem II; O-O bond formation likely occurs prior to the final electron transfer step. This model resolves the kinetic limitations associated with O-O bond formation and suggests an evolutionary adaptation to avoid releasing of harmful peroxide species. Our atomistic model of the S3 state incorporates a MnIV=O fragment of radicaloid character and exhibits antiferromagnetic alignment with opposite spin orientations between two Mn centers and associated oxygens, allowing for a low barrier to O-O bond formation. [1-3] In D2O the lifetime of the early transient state is extended to 500 µsec allowing further spectroscopic and structural analysis.

1. Davis et al. (2015) arXiv:1506.08862
2. Davis et al. (2018) Phys Rev. X 8: 041014
3. Pushkar et al. (2018) J. Phys. Chem. Lett. 9:3524-3531

185 Pre Recorded Poster

Structural features and green light harvesting of fucoxanthin chlorophyll *a/c*-binding proteins in diatoms

Yue Feng[1,2], Songhao Zhao[1], Cuicui Zhou[1], Zhenhua Li[1,2], Tingyun Kuang[1], Wenda Wang[1], Jian-Ren Shen[1,3]

[1]Institute of Botany, Chinese Academy of Sciences, Beijing, China. [2]University of Chinese Academy of Sciences, Beijing, China. [3]Okayama University, Okayama, Japan

Diatoms rely on fucoxanthin chlorophyll a/c-binding proteins (FCPs) to harvest light energy for oxygen-evolving photosynthesis and contribute about 20% of the global primary productivity each year. Compared with other light-harvesting complexes (LHCI/LHCII) of green photosynthetic organisms, FCP antennae have more diversities in protein subunits and pigments, as well as flexibilities and structures to cope with fluctuating light conditions, enabling diatoms to harvest more green light under dim light environment and to protect the photosynthetic center from potential photoinhibition. In recent years, we solved the structure of a main FCP antenna in a dimeric state from a pennate diatom Phaeadactylum tricornutum [Wang . et al. (2019) Science 3363: 598], as well as a tetrameric FCP complex associated with the PSⅡ core in a centric diatom Chaetoceros gracilis [Pi X. et al. (2019) Science 365: 463]. New cryo-electron microscopic analyses indicate that the tetrameric FCP could form a pentamer with an additional FCP monomer, and the dimeric FCP could directly or indirectly bind to PSII core mediated by a LHCX subunit. Structure, absorption spectra and pigment analysis suggest that the dimeric FCP has an organization different from that has been solved in the isolated state, and may have an enhanced green light harvesting capability than that of the tetrameric FCP. This reveals a C2S2M2 type PSⅡ-FCPⅡ containing FCP dimers and LHCX, which has novel protein and pigment organizations that are important for elucidating the efficient light-harvesting, energy transfer and dissipation mechanisms in diatoms.

186 Pre Recorded Poster

Engineering astaxanthin accumulation reduces photoinhibition and increases biomass productivity under high light in *Chlamydomonas reinhardtii*

Stefano Cazzaniga[1], Federico Perozeni[1], Thomas Baier[2], Matteo Ballottari[1]

[1]University of Verona, Verona, Italy. [2]Bielefeld University, Bielefeld, Italy

Astaxanthin is a high valuable ketocarotenoid with strong antioxidative activity and is natively accumulating upon environmental stress exposure in selected microorganisms as green microalgae. While light is required for photosynthesis, fueling carbon fixation processes, application of high irradiance causes photoinhibition and limits biomass productivity. Here, we demonstrate that engineered astaxanthin accumulation in the green alga *Chlamydomonas reinhardtii* conferred high light tolerance, reduced photoinhibition and improved biomass productivity at high irradiances, likely due to strong antioxidant properties of constitutively accumulating astaxanthin. In competitive co-cultivation experiments, astaxanthin rich *Chlamydomonas reinhardtii* outcompeted its corresponding parental background strain and even the fast-growing green alga *Chlorella vulgaris*. Metabolic engineering inducing astaxanthin and ketocarotenoids accumulation caused improved high light tolerance and increased biomass productivity in the model species for microalgae *Chlamydomonas reinhardtii.* Thus, engineering microalgal pigment composition represents a powerful strategy to improve biomass productivities in customized photobioreactors set ups. Moreover, engineered astaxanthin accumulation in selected strains could be proposed as a novel strategy to outperform growth of other competing microalgal strains.

187 Pre Recorded Poster

The structure of the eukaryotic bilin reductase *Gt*PEBB reveals a flipped binding mode of dihydrobiliverdin

Johannes A Sommerkamp[1], Nicole Frankenberg-Dinkel[2], Eckhard Hofmann[1]

[1]Ruhr University Bochum, Bochum, Germany. [2]Technical University Kaiserslautern, Kaiserslautern, Germany

Cyanobacteria are a large group of photoautotrophic bacteria which efficiently harvest light energy with their antenna complexes, the phycobilisomes. One major chromophore, covalently linked to phycobilisomes is the open-chain tetrapyrrole molecule phycoerythrobilin (PEB). The biosynthesis of bilins starts with the cleavage of heme by heme oxygenases to yield biliverdin IXa (BV). BV serves then as the substrate for ferredoxin-dependent bilin reductases (FDBRs), a radical enzyme family which is independent from any metal or organic co-factors. In cyanobacteria and cryptomonads, BV is reduced by 15,16-DHBV (15,16-dihydrobiliverdin):ferredoxin oxidoreductase (PebA) to the intermediate 15,16-DHBV. A second consecutive two-electron reduction by PEB:ferredoxin oxidoreductase (PebB) generates the final chromophore PEB. Phycoerythrobilin synthase (PebS) encoded by cyanophages, combines the activities of both PebA and PebB in one single enzyme.

Several crystal structures of PebA and PebS were published by our laboratories in the last years but structural evidence for PebB was missing. Here we present the crystal structure (1.9 Angström) of PebB from the cryptomonad *Guillardia theta* which reveals insight into structural differences and provides the basis for further studies on the regio-specific reaction pathways [Sommerkamp et al. (2019) JBC 294:13889-901].

188 Pre Recorded Oral

The anatomy of change: leaf structure in four dimensions

Danny Tholen, Guillaume Théroux-Rancourt

University of Natural Resources and Life Sciences, Vienna, Wien, Austria

Leaves capture light and carbon dioxide for use in the process of photosynthesis. The rate of photosynthesis is not only affected by the biochemical properties of the cells in which this process takes place, but also by the anatomy of the leaf. It is important to recognize that leaf anatomy is not necessarily invariant over time. For example, it is well-known that the leaf anatomy is much affected by growth light intensities. But how do observed differences in cell shape, cell size, mesophyll thickness and porosity contribute to photosynthetic function, and how much do these different traits contribute? We will present a new framework that allows us to better analyze the relative importance of changes or differences in anatomical characteristics. Even on short timescales (minutes), environmental factors such as light, CO_2 levels and water availability may induce rapid changes in some anatomical properties. Organelle movement, opening and closing of stomata, and dehydration of the mesophyll during wilting are examples of rapid changes in the leaf structure. We will review the effects of these anatomical changes on leaf gas-exchange. Classical microscopy using fixed tissue samples is often ill-suited to quantify such fast changes in leaf structure. We will introduce a novel method that allows to concurrently monitor gas-exchange and leaf anatomy in three dimensions over time.

189 Pre Recorded Poster

Toward an effective use of microalgae by disentangle LHCSR role on non photochemical quenching (NPQ) in *Chlamydomonas reinhardtii*

Federico Perozeni[1], Stefano Cazzaniga[1], Julianne Troiano[2], Luca Zuliani[1], Giorgia Beghini[1], Gabriela Shlau-Cohen[2], Matteo Ballottari[1]

[1]University of Verona, Verona, Italy. [2]Massachusetts Institute of Technology, Cambridge, USA

Photosynthetic organisms evolved a photoprotective mechanism inducing thermal dissipation of excessive light energy, called Non-photochemical quenching (NPQ). The proper balance between photoprotection and light conversion for carbon fixation has been proposed as domestication target both in plants and in algae. In the model organism for green algae, *Chlamydomonas reinhardtii*, pigment binding proteins LHCSR(s), were reported as the main actors in NPQ. In this work the mechanism of action of LHCSR was studied using a combination of *in vivo* and *in vitro* approach. Despite high sequence identity of the two isoforms of LHCSR present in *C. reinhardtii*, LHCHSR3 showed a stronger quencher activity compared to the LHCSR1 due to a different occupancy of L2 carotenoid binding site. Two distinct quenching processes, individually controlled by pH and zeaxanthin, were identified within LHCSR3. Either pH- or zeaxanthin-dependent quenching are able to protect against reactive oxygen species, and thus the two quenching processes may together provide different induction and recovery kinetics for photoprotection in a changing environment. These results allow to better understand the NPQ mechanism in *Chlamydomonas reinhardtii* opening the possibility to the generation of mutants with modified NPQ induction fully exploiting the microalgae potential for a growing world.

190 Pre Recorded Oral

Structures of the cyanobacterial phycobilisome in the light-harvesting and photoprotected states

Maria Agustina Dominguez Martin[1,2,3], Paul Sauer[4,5], Henning Kirst[2,3], Markus Sutter[1,2,3], David Bina[6,7], Basil Greber[3,4,8], Eva Nogales[3,9,5,10], Tomáš Polívka[6], Cheryl Kerfeld[1,2,3,11]

[1]MSU-DOE Plant Research Laboratory, East Lansing, Michigan, USA. [2]EGSB Division, LBNL, Berkeley, California, USA. [3]MBIB LBNL, Berkeley, California, USA. [4]QB3, Berkeley, California, USA. [5]HHMI, Berkeley, California, USA. [6]Faculty of Science, Ceske Budejovice, Czech Republic. [7]Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic. [8]Division of Structural Biology, London, United Kingdom. [9]QB3, Berkeley, california, USA. [10]Departament of Molecular and Cellular Biology, Berkeley, California, USA. [11]Departament of Biochemistry and Molecular Biology, East Lansing, Michigan, USA

Phycobilisomes (PBS) are the elaborated light-harvesting antennas in cyanobacteria. To balance the harvesting of light energy against the risks of photodamage, many cyanobacteria have evolved a photoprotective mechanism that relies on the interaction between a photoreceptor, the Orange Carotenoid Protein (OCP), and the PBS. Here we present four cryo-electron microscopy structures, with and without OCP, of the 6.2 MDa PBS from the model organism *Synechocystis* PCC 6803 at overall resolution 2.1-3.5 Å. The structures revealed the existence of three different conformational states of the antenna, two previously unknown, for the unquenched PBS. We found that two of the rods can switch conformation within the complex, suggestive of a potentially new type of regulation. We also discovered a novel linker protein, named ApcG, that binds to the membrane facing side of the PBS. In addition, the structure of the PBS-OCP complex shows four 34 kDa OCPs organized as two dimers quench the PBS. The complex also reveals for the first time, the structure of the active form of the OCP, revealing an ~60 Å displacement of its regulatory C-terminal domain. Finally, we elucidate energy transfer pathways based on structural and spectroscopic properties. These results provide detailed insights into the cyanobacterial light-harvesting and place a foundation for future bioengineering applications.

191 Pre Recorded Poster

Engineering of the fast-growing *Synechococcus* PCC 11901 for the synthesis of high added-value carotenoids.

Nico Betterle, Elia Battagini, Francesco Bellamoli, Matteo Ballottari

University of Verona, Verona, Italy

The industrial-scale production of high added value molecules in microalgae is limited by the growth performance of the latter in industrial systems. *Synechococcus* PCC 11901 (hereafter called Synechococcus), a prokaryotic microalgal strain, has been recently isolated [Włodarczyk et al. (2020) Commun Biol 3, 215]. This microalga showed exceptional properties suitable for large-scale cultivation. In fact, this microalga, using light and CO_2, is a) capable of accumulating biomass in large quantities, b) has a rapid duplication rate, c) grows in soils with a high salt concentration and d) is genetically manipulable. This work focused on the engineering of Synechococcus to produce astaxanthin, a high added value ketocarotenoid not produced in this microalgal strain. Specifically, heterologous β-ketolase from *Chlamydomonas reinhardtii* [Perozeni et al. (2020) Plant Biotechnol J. 18: 2053-67] and β-hydroxylase from *Brevundimonas* sp. SD-212 [Casazza et al. (2019) Physiol. Plant 166 (403-412)] genes were constitutively expressed. Transformed cells (hereafter called BC) efficiently accumulated astaxanthin (~85% of total carotenoids) during photo-autotrophic growth. Moreover, preliminary experiments showed that BC cells grew faster than WT cells in the presence of high-light and continuous bubbling with CO_2, possibly because of the photoprotective activity exerted by astaxanthin. In the stationary phase, WT and BC cells showed a comparable biomass accumulation, reaching ~4g/L dcw upon only 4 days of growth in a photobioreactor. BC cells accumulated ~0.82g of astaxanthin per Kg of biomass produced. Of interest was the evidence that the synthesis of astaxanthin caused a rearrangement of the composition of thylakoidal complexes. In fact, sucrose gradient fractionation showed that BC cells accumulated less trimeric Photosystem I and monomeric Photosystem II and more monomeric Photosystem I than WT cells. In conclusion, the engineered Synechococcus succeeded in accumulating astaxanthin, without impairing cell growth rate, making this fast-growing cyanobacterium an ideal platform for the industrial photoautotrophic synthesis of astaxanthin.

192 Pre Recorded Oral

Structural insights into photosystem II assembly

Marc M. Nowaczyk

Ruhr-University, Bochum, Germany

Membrane protein complexes play a central role in cyanobacterial bioenergetics. We have solved several structures of cyanobacterial membrane protein complexes by cryo-electron microscopy and complemented the work by spectroscopic analysis or molecular dynamics simulation [Schuller et al. (2019) Science 363:257-260, Schuller et al. (2020) Nat. Commun. 11:494]. Photosystem II (PSII) is one of the best studied photosynthetic multi-subunit membrane protein complexes so far. It catalyzes a very unique reaction: the light-driven oxidation of water. Many details of PSII structure and function have already been elucidated. However, the molecular details of its biogenesis, particularly the role auf auxiliary proteins, which assist the assembly of the various cofactors and subunits, remain unclear. Using cryo-electron microscopy, we solved the structure of a partially functional PSII assembly intermediate from the thermophilic cyanobacterium *Thermosynechococcus vestitus* BP-1 at 2.94 Å resolution [Zabret et al. (2021) Nat. Plants 7:524-538]. It contains three assembly factors (Psb27, Psb28, Psb34) and provides detailed insights into their molecular function. Binding of Psb28 induces large conformational changes at the PSII acceptor side, which distort the binding pocket of the mobile quinone (Q_B) and replace the bicarbonate ligand of non-heme iron with glutamate, a structural motif found in reaction centers of non-oxygenic photosynthetic bacteria. These results reveal novel mechanisms that protect PSII from damage during biogenesis until water splitting is activated. Our structure further demonstrates how the PSII active site is prepared for the incorporation of the Mn_4CaO_5 cluster, which performs the unique water splitting reaction.

193 In Person Oral

The nature of the chemical bond of the oxyl/oxo intermediate for water oxidation in oxygen evolving complex of photosystem II

Kizashi Yamaguchi[1,2], Koichi Miyagawa[3], Mitsuo Shoji[4], Hiroshi Isobe[5], Takashi Kawakami[1]

[1]Osaka University, Toyonaka, Osaka, Japan. [2]Riken Kobe, Kobe, Hyogo, Japan. [3]Tuskuba University, Tukuba, Ibaragi, Japan. [4]Tsukuba University, Tsukuba, Ibaragi, Japan. [5]Okayama University, Okayama, Okayama, Japan

The oxyl-radical character of the high-valent (HV) Mn(V)=O bond has been investigated in relation to radical reactions on both experimental and theoretical grounds ((Yamaguchi et al. (1986), Appl. Quant. Chem. 155-184). The HOMO-LUMO energy gap for the HV Mn=O bond is often small, indicating the so-called triplet instability which entails the HOMO-LUMO mixing to afford the broken-symmetry (BS) orbitals localized mainly on the Mn- and O-sites, respectively. The BS orbital on the O-site is responsible for radical reactions. Recently, the oxyl-radical character of the HV Mn=O bonds has attracted great interest in relation to the O-O formation for water oxidation in both native and artificial systems. Full geometry optimizations at the UB3LYP-D3/def2-TZVP level of theory have been performed for elucidation of the geometrical structures of $CaMn_4O_{(5)}$-hydroxide ($O_{(6)}^{-}$) (1) and $CaMn_4O_{(5)}$-Oxyl($O_{(6)}$) (2) intermediates for water oxidation in oxygen evolving complex (OEC) of photosystem II (PSII), The optimized Ca-$O_{(6)}$-Mn_1 geometry of 2 has elucidated the strong coordination of the $O_{(6)}$ site to Ca ion, indicating its Lewis acid effect for stabilization of the high-valent (HV) Mn-oxo (Mn=O) bond. The natural orbital (UNO) analysis of the UB3LYP-D3 solution has been performed to elucidate the occupation numbers of the bonding and anti-bonding UNOs of the spin-polarized (SP) Mn=O bond, which are used for computation of the oxyl-radical character of the HV SP Mn=O bond of 2. The oxyl-radical character of HV Mn=O bond of 2 was reduced to be lower than 15% in a sharp contrast to the large oxyl-radical (>50 %) character of the HV Mn=O bond without the coordination to the Ca ion.

194 In Person Poster

Relative stability and electronic states in the S_1 state of the $CaMn_4O_5$ cluster of the OEC of the PSII by DFT and DLPNO-CC calculations

Koichi Miyagawa[1], Mitsuo Shoji[1], Takashi Kawakami[2], Hiroshi Isobe[3], Kizashi Yamaguchi[2], Yasuteru Shigeta[1]

[1]University of Tsukuba, Tsukuba, Ibaraki, Japan. [2]Osaka University, Toyonaka, Osaka, Japan. [3]Okayama University, Okayama, Okayama, Japan

The natural water oxidation reaction ($2\ H_2O \rightarrow 4\ ^{+} + 4\ e^{-} + O_2$) is catalyzed at the $CaMn_4O_5$ cluster in the oxygen evolving complex (OEC) of photosystem II (PSII). During the reaction, the OEC throughs the five intermediate states (S_0 ~ S_4), referred as the Kok cycle. In 2011, the high-resolution X-ray diffraction structure of the S_1 state of the Kok cycle was elucidated by Umena et al. that $CaMn_4O_5$ cluster has distorted cubane-like structure and four-water molecule was coordinated. However, the XRD structure could not elucidate the protonation state of $CaMn_4O_5$ cluster and coordinated water molecules and hydrogen bond network. DFT calculation helps to interpret the electric structure of the reaction center. However, in order to elucidate the detailed reaction pathway and its energy change, the results are different on the DFT parameters' change. Therefore, post-DFT calculation methods such as coupled cluster method are required. In this research, the relative stability and the spin structure were investigated by DFT, Domain-based Local Pair Natural Orbital (DLPNO)-CCSD(T), and the exact diagonalization of spin Hamiltonian matrix for the right (R)-opened (opened cubane) structures and left (L)-opened (closed cubane) structures of CaMn4O5 cluster with possible protonated condition in the S_1 state of the Kok cycle for the OEC of PSII. The multiple intermediates in the S_1 state revealed by DLPNO-CCSD(T) are compatible with the experimental results for the S_1 state of the $CaMn_4O_5$ cluster in OEC of PSII.

195 Pre Recorded Oral

Tracking Photosynthetic Metabolites Across Bacterial Micro-Compartments using Computational Microscopy

Daipayan Sarkar, Josh Vermaas

Plant Research Laboratory, Michigan State University, East Lansing, MI, USA

The carboxysome is an organelle found in photosynthetic bacteria that locally concentrates carbon dioxide (CO_2) to improve the efficiency for RuBisCO, the key enzyme in photosynthetic carbon fixation. The carboxysome shell is composed of proteins, which encapsulate RuBisCO and its partner protein, carbonic anhydrase. Carbonic anhydrase converts soluble bicarbonate into CO_2, increasing the local CO_2 concentration for RuBisCO. In addition to CO_2, other metabolites such as oxygen (O_2), bicarbonate (HCO^{3-}), adenosine triphosphate (ATP), 3-phosphoglyceric acid (3-PGA) and ribulose-1,5-bisphosphate (RuBP) permeate through the carboxysome shell. Leveraging new GPU-resident molecular simulation engines, we determine the carboxysome permeability for RuBisCO reactants and products through unbiased simulation. We find that the carboxysome itself is not selectively permeable to bicarbonate over carbon dioxide, as originally hypothesized. Instead, the carboxysome shell proteins form a general barrier to maintain the carbon dioxide gradient generated by carbonic anhydrase activity within the carboxysome. Furthermore, based on the observations we made from the thermodynamics (free energy) and kinetics (permeability) from a multimillion atom scale model of the carboxysome shell, separate molecular simulations using replica-exchange umbrella sampling are performed to compare and characterize the permeability, diffusivity, and free-energy profiles for different pore motifs in the hexameric protein of the carboxysome shell originating in different species. The results from all-atom simulations presented here are critical towards the engineering of a smarter synthetic bacterial micro-compartments, to improve photosynthetic efficiency with wide range of applications in sustainable, metabolic, and biomedical engineering.

196 Pre Recorded Poster

The mechanistic bases of genetic variations photosynthetic responses and to environmental stress: Identifying linkages to lipid composition.

Donghee Hoh[1], Christoph Benning[1,2,3], David M. Kramer[1,2]

[1]MSU-DOE Plant Research Laboratory, East Lansing, MI, USA. [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA. [3]Department of Plant Biology, Michigan State University, East Lansing, MI, USA

This work tests hypothetical models for how genetic variations affect climate-resilience of photosynthesis. We used detailed, high-throughput and multi-dimensional phenotyping of a panel of recombinant inbred lines (RILs) from Cowpea (Vigna unguiculata (L.) Walp) parents found to be sensitive or tolerant to chilling stress.

We observed significant differences in a range of photosynthetic responses to chilling stress and found co-localized quantitative trait loci (QTL) intervals among the photosynthetic parameters. We then analyzed this data to assess the interactions among parameters that can reflect the operation of distinct photosynthetic regulatory modes. The data suggest that sensitivity to chilling conferred by specific genomic loci is associated with 1) more reduced Q_A (accumulation of electrons in photosystem II acceptor side) 2) increased cyclic electron flow (CEF) 3) Increased thylakoid proton motive force (*pmf*), leading to accelerated recombination in photosystem II, resulting in the production of highly reactive singlet oxygen. Therefore, we propose that these genetic variations optimize photosynthesis in the tolerant lines under low temperatures, preventing recombination reactions within Photosystem II that can lead to deleterious $^1O_2$ production.

Further, we tested for co-linkages between lipid compositions and chilling responses of photosynthesis. Strikingly, we observed co-linkages between QTL intervals for photosynthetic light reactions and specific fatty acids, the thylakoid-specific localized fatty acid phosphatidylglycerol $16{:}1^{\Delta 3trans}$ (PG 16:1t), suggesting that lipid content directly or indirectly affects the temperature dependencies of the redox state of Q_A, *pmf*, CEF and production of reactive oxygen species. This work offers an effective approach to identifying not only the genetic components, but also the underlying mechanisms of improved performance conferred by natural variations.

197 Pre Recorded Oral

Selective antenna modification in a fast-growing cyanobacterium to improve efficiency and carbon-neutral productivity under outdoor growth conditions

Annesha Sengupta, Himadri Pakrasi

Washington University in St. Louis, St. Louis, Missouri, USA

Phycobilisomes (PBSs) are macromolecular light-harvesting antenna protein-pigment complexes present in cyanobacteria to enhance the capture of light energy. PBSs have an allophycocyanin core and attached to the core are phycocyanin discs stacked via the linker proteins to form rods. The PBS adjusts the length of the rods to optimize light harvest. Studies investigating the impact of antenna modification on strain performance have led to inconclusive results [Krist et al. (2014) Biochim. Biophys. Acta 1837:1653-1664]. During the current study, we systematically truncated the structure of PBS in a fast-growing, high-light thriving strain *Synechococcus elongatus* UTEX 2973 having a bi-cylindrical core with 4 types of linker proteins. Remarkably, one such mutant strain, 2973Δ*rrl* (2973Δ*cpcC1C2DB2A2*), exhibited a growth advantage of up to *36%* as compared to the WT under outdoor growth conditions. In contrast, deletion of the rod-core linker protein (CpcG) was detrimental to growth. We conclude that a carefully selected truncated rod structure is required for optimal growth under high light conditions. Additionally, the Δ*rrl* strain exhibited a five-fold higher EYFP level. Our study indicates the importance of an optimized antenna for improved growth and productivity.

198 Pre Recorded Oral

Photosynthetic "light potentials" of field plants under fluctuating environmental conditions

Atsuko Kanazawa, Abhijnan Chattopadhyay, Sebastian Kuhlgert, Nicholas Fisher, Donghee Hoh, David Kramer

Michigan State University, East Lansing, Michigan, USA

Using the PhotosynQ open-science cloud-based platform, we explored the environmental dependencies of range of photosynthetic parameters, and developed an unsupervised statistical approach to identify patterns consistent with proposed mechanisms, and test under which environmental conditions and species they occurred. There were several surprising findings, where the results in the field were distinct from those seen under typical laboratory conditions: Light reactions: 1) We found cases where increased light induced acidification of the thylakoid lumen that resulted in very rapid NPQ (in the form of qE), resulting in net oxidation of Q_A and P_{700}, i.e., increased NPQ controlled overall electron flow. 2) At lower leaf temperatures, high light-induced lumen acidification occurred, resulting in strong photosynthetic control (PCON) at the cytochrome b_6f complex, but did not trigger strong increases in NPQ, net reduction of Q_A and oxidation of P_{700}. Further experiments in the lab showed that this effect is likely caused by "freezing out" of the qE response at the level of the antenna complexes. 3) We observed limitations at the acceptor side of PSI, only at very short times (a few seconds) after increased light, suggesting that rapid induction of PCON and/or NPQ effectively prevents this effect. We have expanded these studies to test for limitations at the levels of stomatal conductance and assimilatory reactions by assessing the CO_2-dependence of light potentials. Under different conditions, we observe limitations attributable to either the activation states of assimilatory enzymes or stomatal conductance. Surprisingly, strong CO_2-related limitations are seen in both C3 and C4 plants. We will discuss the implications of these effects for understanding climate responses of photosynthesis and prospects for crop improvement.

199 Pre Recorded Oral

Charge separated states of PSII are stabilized by “cryptic” states that prevent recombination reactions

Jingcheng Huang, David Kramer

Michigan State University, East Lansing, Michigan, USA

Charge recombination between Q_A and Y_Z of Photosystem II (PS II) is known to produce singlet oxygen that leads to the damage of photosynthetic apparatus. This process is mostly prevented under steady-state photosynthesis by a set of different regulatory or protective mechanisms; however, these safety valves may not catch up the pace of the rapid changing environment. Our current work investigates how the build-up of transthylakoid membrane potential accelerates the charge recombination. This adversarial scenario could occur under fluctuation light when the dissipation of the ion flux cannot match the rapid photon flux change. We constructed an electroluminescence device that stimulates the charge recombination by applying external electric field to the swollen chloroplasts (spherical thylakoid membrane vesicle), and measures the light emitted from the re-excited chlorophylls which are repopulated from charge recombination. We found that charge recombination from the $S_2Q_A^-$ state is strongly suppressed by formation of a stabilized state with a time constant of about 50 ms. This phase is too slow for known electron/proton transfer events, and does not coincide with lifetime of the global membrane potential. We thus propose the stabilization involves a slow, “cryptic” process. Our working hypotheses is that stabilization is caused by the conformational changes that solvate the charge density on Q_A in the surrounding dielectric environments, or the spin-state transition in S_2 of oxygen evolving complex. If confirmed, this state could act as an unrecognized photoprotective process, and lead us to modify the energy diagram for PSII states. This state may also be related to the long-standing question of why maximum fluorescence (F_M) cannot be reached by single turnover saturation flash.

200 In Person Poster

Iron rescues glucose-mediated photosynthesis repression during lipid accumulation in the green alga *Chromochloris zofingiensis*

Tim L. Jeffers[1], Samuel Purvine[2], Carrie D. Nicora[2], Ryan McCombs[1], Adrien Stroumza[1], Ken Whang[1], Alice Dohnalkova[2], Mary Lipton[1], Krishna K. Niyogi[1], Melissa S. Roth[1]

[1]UC Berkeley, Berkeley, CA, USA. [2]Pacific Northwest National Laboratories, Richland, WA, USA

It is predicted that perhaps most of genes involved in photosynthesis have yet to be experimentally characterized. To develop a model system that reveals novel players in photosynthesis, we exploit the ability of the green alga *Chromochloris zofingiensis* to switch off and on photosynthesis during environmental changes. Here, we reveal that *C. zofingiensis* switches off photosynthesis due to insufficient iron (-Fe) interacting with exogenous glucose (+Glc) and hexokinase (HXK1). Analytical chemistry, photosynthetic measurements, and transmission electron microscopy show that while -Fe+Glc treated cells accumulated triacylglycerols (TAGs) and lost photosynthesis and thylakoid membranes, replete iron and glucose (+Fe+Glc) cultures maintained photosynthesis and thylakoids while still accumulating TAGs. We quantified by mass-spectrometry the proteomes of 12 conditions combining +/-Fe and +/-Glc in WT and two *hxk1* mutant strains, and specifically calculated proteins distinctly regulated in the heterotrophic state (WT-Fe+Glc), where the photosynthetic electron transport chain was largely downregulated. Furthermore, we found hundreds of uncharacterized proteins depleted in heterotrophy with vascular plant orthologs, including those that are upregulated during *Arabidopsis thaliana* thylakoid biogenesis, providing evidence for novel proteins that are universally associated with photosynthetic development. Finally, the role of iron in trophic transitions was revealed by iron transporters and iron containing enzymes that showed respiratory complexes are prioritized over photosynthetic complexes, and ferredoxin-desaturase pathways for TAG accumulation are prioritized over thylakoid lipid synthesis. This systems work provides insight into the roles nutrient deficiencies and sugar signaling have in the regulatory priorities of photosynthetic organisms and reveals novel genes for photosynthesis, metal reallocation, and biofuel production across algae and plants.

201 In Person Poster

Partitioning of excitation energy between Photosystem II and Photosystem I is affected by mutations targeting the His-252 residue of the D1 subunit of Photosystem II

Jack A. Forsman[1], Toby J. Brown[2], Kevin J. Sheridan[2], Tina C. Summerfield[2], Julian J. Eaton-Rye[2]

[1]University of Umeå, Umeå, Sweden. [2]University of Otago, Dunedin, New Zealand

In Photosystem II (PS II), the histidine-252 residue of the D1 subunit is important to the function of PS II [Lupínková et al. (2002) BBA-Bioenergetics 1554:192-201]. Mutants were generated in *Synechocystis* sp. PCC 6803 to change the D1:His-252 residue to an alanine, a glutamine and a tyrosine, creating the H252A, H252Q and H252Y strains, respectively. PS II activity was impaired in these mutants, with the H252A and H252Q, evolving oxygen at 34% and 19% of the control rate, respectively, while the H252Y strain was unable to evolve oxygen. As the oxygen evolution rates decreased in the mutant strains, the variable fluorescence and PS II-specific low temperature fluorescence emissions rose, in an inversely proportional manner. However, western blots of solubilized thylakoid proteins from these mutants indicated that the levels of PS II and Photosystem I (PS I) were approximately the same in the control and mutant strains. We therefore hypothesized that the changes in the variable fluorescence, and low-temperature emissions, were not due to changes in photosystem stoichiometry but rather due to changes in the distribution of excitation energy between the two photosystems. Interestingly, when fluorescence induction assays were performed on isolated thylakoid membranes, the control strain and the mutant strains displayed similar levels of variable fluorescence. Complimentary results were also seen when low-temperature fluorescence emission spectroscopy was performed on isolated thylakoid membranes and whole cells. This suggests that the mechanism responsible for the allocation of excitation energy to PS II or PS I is disrupted during isolation of the thylakoid membranes. As the majority of the phycobilisomes are washed away during isolation of the thylakoids, it seems likely that the increase of excitation energy being passed to PS II in the mutant cells arises from a state transition in response to reduced PS II activity.

202 Pre Recorded Poster

Investigating the structural changes along the Kok cycle around the water oxidation complex of Photosystem II using time-resolved X-ray free-electron laser (XFEL) crystallography

Asmit Bhowmick[1], Rana Hussein[2], Mohamed Ibrahim[2], Phiipp Simon[1], Ruchira Chatterjee[1], Jan Kern[1], Vittal Yachandra[1], Junko Yano[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [2]Humboldt University, Berlin, Germany

The water oxidation complex in Photosystem II is the catalyst for the transformation of two water molecules to molecular oxygen, releasing four electrons and four protons in the process. Starting from the ground state, the complex gets progressively oxidized upon absorption of a photon and moves along the Kok cycle (S_0-S_4 intermediate states). In the last state, the complex releases its stored oxidative power to catalyze the reaction and return to the ground state. Here we present our results on time-resolved structural investigation done at room temperature on Photosystem II of the various stable intermediates along the Kok cycle as well as timepoint in-between these intermediates. The data shows the sequence of events leading to the insertion of a new substrate water in the S_2-->S_3 transition. The water insertion is accompanied by distance changes within the cluster as well as side-chain coordination with the complex. In addition, we observed critical structural changes in the different water and proton channels that are coordinated with the substrate water insertion and tell us about their functional role. We also discuss preliminary results on the final transition that leads to formation of molecular oxygen (i.e S_3-->S_0 transition)

203 Pre Recorded Poster

HltA: a universally conserved cyanobacterial protein phosphatase involved in light tolerance

Patricia Walker

Washington University in St Louis, St Louis, MO, USA

Cyanobacteria have evolved several types of environmental signal sensors and transducers, including two-component systems, serine/threonine protein kinases/phosphatases, and sigma factors of RNA polymerase [Los, D.A., et al. (2010) Sensors (Basel). 10(3): p. 2386-415] which help respond to and overcome environmental stresses. The high light tolerant cyanobacterium Synechococcus elongatus UTEX 2973 is the fastest-growing cyanobacterial strain known and optimally grows under extreme high light intensities of 1500-2500 µmol photons m^{-2} s^{-1} [Ungerer, J., et al. (2018) MBio, 2018. 9(1)]. Although this most advantageous growth under high light conditions is not well understood, especially considering that Synechococcus elongatus do not encode an Orange Carotenoid Protein to perform non-photochemical quenching. We leveraged the few genetic differences between Synechococcus 2973 and the model strain Synechococcus elongatus PCC 7942 to identify new factors with key roles in high light tolerance. We identified an uncharacterized protein that we have termed HltA for High light tolerance protein A. We found hltA to be highly conserved throughout cyanobacteria with few homologs outside of this lineage, indicative of the potentially important role of *hltA* to the photosynthetic lifestyle of these oxygenic phototrophs. Using bioinformatic tools, we determined that HltA is a PP2C-type protein phosphatase belonging to the bacteria-specific Group II family and is closely related to the environmental stress response phosphatase from Bacillus subtilis, RsbU. The single N-terminal regulatory GAF domain may function in light sensing and activation of phosphatase activity. Mutations in the hltA gene resulted in severe defects to high light growth but did not result in any growth defects under nutrient and salt stress [Walker, P.L., and .B. Pakrasi. (2022) Microbiology Spectrum, 0(0): p. e01008-22]. These results provide evidence that *hltA* is important for the light tolerance of Synechococcus 2973 and mechanisms of light response throughout cyanobacteria.

204 Pre Recorded Poster

Highly-resolved P_{700}^{+}/P_{700} spectra from isolated PSI of several cyanobacterial strains

Michael Nelson, Gary Hastings

Georgia State University, Atlanta, GA, USA

Synechocystis sp. PCC6803 (S.6803WT), *Synechocystis* sp. PCC7002 (S.7002WT), and the S.6803 menB-mutant are discussed with measurements made on isolated, trimeric Photosystem I particles. Photoaccumulated ("light minus dark") P_{700}^{+} spectra were collected at $2cm^{-1}$ and $1cm^{-1}$ resolutions from $7000cm^{-1}$ to $915cm^{-1}$ at both 293K and 77K. Splitting of key spectral features are observed. The large negative band (previously described as the ground state P_B 13^1 keto carbonyl)[α] centered around $1700cm^{-1}$, is shown to be composed of at least two distinct components. These components are not evident in $4cm^{-1}$ resolution spectra from the same strains. Lorentzian Fourier self-deconvolution is applied to further elucidate the underlying band structure. Isotopic labeling of S.6803WT is also employed to this end.

[α] [Breton J. et al. (1999) Biochemistry 38: 11585–11592]

205 Pre Recorded Oral

Synthetic control of non-photochemical quenching in a marine diatom

Graham Peers[1], Maxwell Ware[1], Tessema Kassaw[1], Martin Lohr[2], Yu Bai[1]

[1]Colorado State University, Fort Collins, Colorado, USA. [2]Johannes Gutenberg-Universität Mainz, Mainz, Germany

Inducible non-photochemical quenching (NPQ) allows cells to balance efficient photosynthesis with photoprotection while growing in variable light environments. Diatoms are ochrophyte algae that use a xanthophyll cycle involving the pigments diatoxanthin/diadinoxanthin along with LHCX protein for rapidly reversible NPQ. We have now identified the proteins involved in toggling the xanthophyll cycle pigments. In this talk we will focus on the ZEP3 protein and its role in reverting the photoprotective state back to light harvesting. Concurrent with this work we have developed a synthetic gene circuit that permits tunable and reversible gene expression in the diatom *Phaeodactylum tricornutum*. Placing the zep3 gene under the control of this promoter allows us set the level of reversible NPQ, regardless of light environment. We will discuss the control of NPQ and its effects on biomass production and fitness using experimental data and whole genome models of metabolism which integrate realistic light harvesting parameters.

206 Pre Recorded Oral

Allosteric regulation of PHOSPHOENOLPYRUVATE CARBOXYLASE: a key enzyme driving C4 photosynthesis

Ryan Wessendorf, Asaph Cousins

Washington State University, Pullman, Washington, USA

With an exponentially growing human population, further understanding the evolution of C4 photosynthesis may provide insights into bioengineering enhanced photosynthesis in biofuel and food crops. Phosphoenolpyruvate (PEP) carboxylase (PEPC) is found in all terrestrial plants and carboxylates PEP with bicarbonate (HCO3-) to form the four-carbon acid oxaloacetate. This reaction is essential for supplying intermediates to the tricarboxylic acid cycle (TCA) cycle, which balances the carbon and nitrogen pools within plants. In C4 plants, gene duplication facilitated PEPC's neofunctionalization from its anaplerotic function to operate as the first committed and non-reversable step of the carbon-concentrating-mechanism (CCM) that defines C4 photosynthesis. The C4 PEPC enzyme is a key rate limiting enzyme of the CCM and evolved a high sensitivity to its allosteric activator Glucose-6-Phosphate (G6-P), compared to ancestral non-photosynthetic PEPC isoform. Large amounts of the C4 PEPC in leaves requires it to be tightly regulated to keep it from disrupting primary metabolism. Previous research identified a plant-specific-loop, amino acids 349-369, that hydrogen bonds and stabilizes the interaction with G6-P. However, the mechanism controlling PEPC's sensitivity G6-P is not well understood. To investigate the sensitivity of PEPC to G6-P we have used a PEPC-less E. coli cell line to overexpress and purify PEPC isozymes from the two C4 grasses *Oropetium thomaeum* and *Paspalum vaginatium*. These PEPC isozymes display different levels of sensitivity to G6-P and we used single amino acid and domain swaps to generate chimeric PEPCs to test how the enzymes structure influences its G6-P regulation. This research provides valuable information that identifies one mechanism that controls PEPC's sensitivity to G6-P in *P. vaginatium* but not *O. thomaeum*. These data provide new insights into the complicated mechanisms controlling the allosteric regulation of PEPC by G6-P. Support was provided by Office of Basic Energy Sciences, DOE (grant no. DE-SC0001685).

207 Pre Recorded Poster

Theoretical calculations by hybrid-DFT and DMRG-CASCI methods for effective exchange integrals in binuclear manganese complex model for OEC PSII.

Takashi Kawakami[1,2], Masato Torii[1], Koichi Miyagawa[3], Hiroshi Isobe[4], Mitsuo Shoji[3], Shusuke Yamanaka[1], Mitsutaka Okumura[1], Takahito Nakajima[2], Kizashi Yamaguchi[5]

[1]Osaka Univ., Toyonaka, Osaka, Japan. [2]RIKEN, Kobe, Hyogo, Japan. [3]Tsukuba Univ., Tsukuba, Ibaraki, Japan. [4]Okayama Univ., Okayama, Okayama, Japan. [5]Osaka Univ., Ibaraki, Osaka, Japan

Mn-O2-Mn units in binuclear manganese complex is essential structure to study oxygen evolving complex (OEC) in photosystem (PSII). Such magnetic interaction between two spin sources can effectively describe electronic and magnetic properties. As well known, hybrid-DFT methods as high performance MO methods are usually employed and successfully describe the nature. In addition, DMRG-CASCI methods are expected to overcome limitation of hybrid-DFT methods with non-dynamical correlation correction.

208 Pre Recorded Poster

Structural dynamics around the Mn-cluster of Photosystem II in light of decades of time-resolved studies

Philipp Simon[1], Asmit Bhowmick[1], Rana Hussein[2], Mohamed Ibrahim[3], Johannes Messinger[4], Jan Kern[1], Vittal Yachandra[1], Junko Yano[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, USA. [2]Humboldt-Universität zu Berlin, Berlin, Germany. [3]Universität zu Lübeck, Lübeck, Germany. [4]Uppsala University, Uppsala, Sweden

Decades of time-resolved studies on Photosystem II using various experimental techniques lead to a good understanding of its photocycle including the oxidation of the Mn_4CaO_5 cluster by the redox-active Tyr-His unit Y_Z, and deprotonation events compensating the accumulated charges. However specific sites for the latter as well as the nature of the substrate waters and O-O bond formation mechanism are still lacking. In this contribution I will try to summarize the current understanding – without claiming completeness – and highlight discrepancies and unknowns.

In our recent works, we obtained radiation damage free structural intermediates of the oxygen evolving complex at room temperature using X-ray free electron lasers. Those intermediates provided valuable insights into the nature of sites involved in electron and proton transfer events and substrate water binding. In the S_2 to S_3 transition in particular, we observed the insertion of a new water Ox after opening of the Mn-cluster and a reorientation of D1-E65 serving as a proposed proton gate. However, interpreting all the observed changes was and is difficult.

I will attempt to bring the two pieces – structural and transient dynamics – together to obtain a better understanding of the photocycle of PSII and the nature of water oxidation.

209 Pre Recorded Oral

Predicting the oxidation states of Mn ions in the oxygen-evolving complex of Photosystem II using supervised and unsupervised machine learning

Muhamed Amin

University of Groningen, Groningen, Netherlands. CFEL, Hamburg, Germany. CCNY, New York, NY, USA

Serial Femtosecond Crystallography at the X-ray Free Electron Laser (XFEL) sources enabled the imaging of the catalytic intermediates of the oxygen evolution reaction of Photosystem II (PSII). However, due to the incoherent transition of the S-states, the resolved structures are a convolution from different catalytic states. Here, we train Decision Tree Classifier and K-mean clustering models on Mn compounds obtained from the Cambridge Crystallographic Database to predict the S-state of the X-ray, XFEL, and CryoEm structures by predicting the Mn's oxidation states in the oxygen evolving complex (OEC). The model agrees mostly with the XFEL structures in the dark S1 state. However, significant discrepancies are observed for the excited XFEL states (S2, S3, and S0) and the dark states of the X-ray and CryoEm structures. Furthermore, there is a mismatch between the predicted S-states within the two monomers of the same dimer, mainly in the excited states. We validated our model against other metalloenzymes, the valence bond model and the Mn spin densities calculated using Density Functional Theory (DFT) for two of the mismatched predictions of PSII. The model suggests designing a more optimized sample delivery and illumination systems are crucial to precisely resolve the geometry of the advanced S-states to overcome the noncoherent S-state transition. In addition, significant radiation damage is observed in X-ray and CryoEM structures, particularly at the dangler Mn center (Mn4). Our model represents a valuable tool for investigating the electronic structure of the catalytic metal cluster of PSII to understand the water splitting mechanism.

210 Pre Recorded Poster

Investigating the mechanism of substrate binding in the solar water oxidation reaction of Photosystem II

K V Lakshmi[1], Vidmantas Kalendra[1], Krystle Reiss[2], Gourab Banerjee[2], Ipsita Ghosh[2], Ke Yang[2], Victor Batista[2], Gary Brudvig[2]

[1]Rensselaer Polytechnic Institute, Troy, NY, USA. [2]Yale University, New Haven, CT, USA

The photosynthetic solar water-splitting protein, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in Nature by using light energy to drive a catalyst capable of oxidizing water. The water oxidation reaction is catalyzed by the Mn_4Ca-oxo cluster in the oxygen-evolving complex (OEC) which cycles through five light-driven S-state intermediates (S_0-S_4) as it accumulates charge equivalents to split water. However, a detailed mechanism of the reaction remains elusive as it requires knowledge of the binding of substrate water in the higher S-state intermediates of the OEC. In particular, the binding of substrate in the S_2 to S_3 state transition of the OEC that leads to O-O bond formation is poorly understood because of the inability of conventional methods to probe water molecules. We are using state-of-the-art two-dimensional (2D) hyperfine sublevel correlation spectroscopy methods to determine the binding of water in the S state intermediates of the OEC. In this presentation, we will describe ongoing studies that employ small molecule analogs and site-directed mutagenesis of PSII to elucidate the mechanism of the delivery and binding of substrate at the Mn_4Ca-oxo cluster in the S_2 and S_3 states. These studies have important implications on the mechanistic models for water oxidation in PSII.

†This study is supported by the US Department of Energy (DE-FG02-07ER15903 (KVL), DE-SC0001423 (VSB) and DE-FG02-05ER1564 (GWB)).

211 Pre Recorded Oral

Transgenerational effects of elevated CO_2 on rice photosynthesis and grain yield

Chunhua Lv[1,2,3], Zhenghua Hu[4], Jian Wei[1], Yin Wang[1]

[1]Institute of Ecology, College of Urban and Environmental Sciences and Key Laboratory for Earth Surface Processes of Ministry of Education, Beijing, China. [2]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China. [3]University of Chinese Academy of Sciences, Beijing, China. [4]School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing, China

The responses of crops to rising carbon dioxide concentration ([CO_2]) are often validated using single-generation short-term experiments. However, the transgenerational effects of elevated [CO_2] on rice growth have received little attention. Here, we set up ambient [CO_2] (a[CO_2]) and elevated [CO_2] (e[CO_2], a[CO_2]+200 μmol mol^{-1}) treatments using open-top chamber (OTC). Rice was cultivated in different [CO_2] treatments over five growing seasons in 2016-2020. Beginning in 2017, rice seeds harvested in the previous year under a[CO_2] and e[CO_2] conditions were planted in their respective growing environments. In 2021, seedlings derived from a[CO_2] maternal treatment (a[CO_2]$_m$) and e[CO_2] maternal treatment (e[CO_2]$_m$) were planted with both a[CO_2] offspring (a[CO_2]$_o$) and e[CO_2] offspring (e[CO_2]$_o$) conditions to investigate the transgenerational effects of e[CO_2]. Leaf gas exchange and grain yield under different conditions were determined in 2021. The results showed that light-saturated net photosynthesis (A_{sat}) and stomatal conductance of offspring from e[CO_2]$_m$ were significantly lower at the heading and grain-filling stages under e[CO_2]$_o$ compared with a[CO_2]$_m$, and the corresponding stomatal density was also significantly lower. Moreover, Asat was positively correlated with stomatal density. These results suggest that transgenerational effects induce a decrease in stomatal density and thus cause a lower benefit of A_{sat} from e[CO_2]$_o$. These findings contribute new insights into predicting crop growth and yield in the future.

212 Pre Recorded Oral

Overexpression a single gene inducing great yield in rice by simultaneously enhancing nutrient uptake and photosynthesis

Yin Wang[1,2], Maoxing Zhang[3,2,4], Xi Chen[4], Feiyun Xu[5,4], Ming Ding[2,4], Wenxiu Ye[6,2], Yosuke Toda[2], Yuki Hayashi[2], Houqing Zeng[7], Feng Yan[8], Toshinori Kinoshita[2], Yiyong Zhu[4]

[1]Peking University, Beijing, China. [2]Nagoya University, Nagoya, Japan. [3]Foshan University, Foshan, China. [4]Nanjing Agricultural University, Nanjing, China. [5]Fujian Agriculture and Forestry University, Fuzhou, China. [6]Institute of Advanced Agricultural Sciences, Peking University, Weifang, China. [7]Hangzhou Normal University, Hangzhou, China. [8]Justus Liebig University, Giessen, Germany

Rice (*Oryza sativa*), the staple food of more than 3 billion people, is one of the most important crops in the world. Improving the production of rice is not only for tackling the world food crisis, but also for enhancing the carbon sink in farmland ecosystem. In this study, we show that a single rice gene, *Oryza sativa* plasma membrane (PM) $^{+}$-ATPase 1 (*OSA1*), simultaneously regulates nitrogen and carbon uptake. As a result, under laboratory hydroponic conditions, compared with the wild-type (WT) rice, the rate of $^{15}NH_4^+$ absorption was significantly higher in *OSA1*-oxs and lower in *osa1* mutants. Since it was reported that the PM $^{+}$-ATPase was a key enzyme in light-induced stomatal opening process, stomatal conductance in *OSA1*-oxs, under saturated white light conditions, was almost double that in the WT. Because the stomatal conductance is closely related to leaf photosynthetic rate by limiting the CO_2 uptake, we found that the photosynthetic rates in *OSA1*-oxs was 26–28% higher than in the WT. Interestingly, not only the photosynthesis–irradiance response curve but also the A-Ci curve were higher in *OSA1*-oxs, indicating a greater photosynthetic capacity. Comprehensive gene expression profiles in the leaves and roots of WT, *OSA1*-ox and *osa1* mutant rice plants indicated that *OSA1* regulated the expression of genes involved in C and N metabolism. Finally, we conducted field trials at three different locations, and found that *OSA1*-oxs rice plants causes a 33% increase in grain yield and a 46% increase in N use efficiency overall. Even the same grain yield was attained using only half the amount of N fertilizer when the WT was replaced with *OSA1*-oxs. These findings indicate that the manipulation of PM $^{+}$-ATPase could cooperatively improve nitrogen and carbon utilization, potentially providing a vital tool for food security and sustainable agriculture.

213 Pre Recorded Poster

Examining the dynamics of protective non-photochemical quenching in sunflower species with contrasting transpiration rates

Charles Chen, Halina Liu

Azusa Pacific University, Azusa, CA, USA

When plants absorb more light energy than they can use in photosynthesis, they face the threat of damage to their reaction centers from the formation of reactive oxygen species. To minimize this photodamage, plants utilize photoprotective mechanisms such as heat dissipation via non-photochemical quenching (NPQ) and chlorophyll fluorescence. Plant species that are native to arid, sunny regions have developed adaptations to conserve water, but this results in a restriction of CO_2 intake, thus limiting photosynthesis and raising the risk of photodamage. It is expected that the plant species whose photosynthetic capacity is low due to water-saving adaptations will have higher NPQ, but is it sufficient to protect themselves from photodamage? We examined the photosynthetic capacity and photoprotective properties of two sunflower species with contrasting transpiration rates: *Helianthus annuus* (common sunflower, high water use) and *Venegasia carpesioides* (canyon sunflower, low water use). Photosynthetic carbon assimilation rate, stomatal conductance, and chlorophyll fluorescence were measured at a range of light intensities, and fluorescence data was used to estimate the protective non-photochemical quenching (pNPQ) and photochemical quenching in the dark (qPd). The recovery of qPd after extended exposure to high light was also examined. *Helianthus* had higher photosynthetic capacity and lower NPQ than *Venegasia*, but qPd for both species fell below the threshold for full photoprotection at light intensities above 200 µE.

214 Pre Recorded Oral

Bicarbonate-controlled reduction of oxygen by the Q_A semiquinone in Photosystem II

Andrea Fantuzzi[1], Friederike Allgöwer[2], Holly Baker[1], Gemma McGuire[1], Wee Kii Teh[1], Ana P. Gamiz-Hernandez[2,3], Ville R. I. Kaila[2,3], A. William Rutherford[1]

[1]Department of Life Sciences, Imperial College London, London, United Kingdom. [2]The Arrhenius Laboratories for Natural Sciences, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden. [3]Department of Chemistry, Technische Universität Mänchen, Garching, Germany

The electron acceptor side of Photosystem II (PSII) contains a non-heme ferrous iron that is flanked by two quinones, Q_A and Q_B. The non-heme iron is coordinated by four histidine residues and a bicarbonate ion that acts as a bidentate ligand. The bicarbonate is thought to play a role in Q_B protonation. It has been shown that when Q_A is reduced, bicarbonate binds less strongly. Bicarbonate dissociation leads to an upshift in the reduction potential of Q_A leading to a decreased probability of forming singlet oxygen [Brinkert et al., Proc. Natl. Acad. Sci. U.S.A. 113, 12144–12149 (2016)]. It has been suggested, but not demonstrated, that long lived $Q_A^{\bullet-}$ may donate electrons directly to O_2, forming superoxide, $O_2^{\bullet-}$. Here, using chlorophyll fluorescence in plant PSII membranes, we show that, at physiological O_2 concentrations, O_2 does oxidize $Q_A^{\bullet-}$ with a $t_{1/2}$ of 10 s. Superoxide is formed stoichiometrically, and the reaction kinetics are controlled by the accessibility of O_2 to a binding site near $Q_A^{\bullet-}$, Kd_{app} = 70 ± 20 µM. Unexpectedly, $Q_A^{\bullet-}$ could only reduce O_2 when bicarbonate was absent from its binding site on the nonheme iron (Fe^{2+}), and the addition of bicarbonate or formate blocked the O_2-dependant decay of $Q_A^{\bullet-}$. These results, together with molecular dynamics simulations and hybrid quantum mechanics/molecular mechanics calculations, indicate that electron transfer from $Q_A^{\bullet-}$ to O_2 occurs when the O_2 is bound to the empty bicarbonate site on Fe2+, were the iron acts as a catalyst. The present findings extend the regulatory role of bicarbonate showing that its release allows O_2 to bind to Fe^{2+} and to oxidize $Q_A^{\bullet-}$. This could be beneficial, by providing a safety valve for $Q_A^{\bullet-}$ and by producing superoxide, a chemical signal for the over-reduced state of the electron transfer chain.

215 In Person Poster

Hydrophobic mismatch controls energy dissipation in light-harvesting complex II proteoliposomes

Dan-Hong Li, Sam Wilson, Alexander Ruban

Queen Mary University of London, London, United Kingdom

In higher plants, the major light-harvesting antenna complex (LHCII) is vital for both light harvesting and photoprotection in photosystem II. The thylakoid lipid bilayer plays an intimate role in the adjustment and stabilisation of the LHCII protein. One potential way the membrane may do so is through a process known as hydrophobic mismatch, wherein alterations in membrane thickness are able to stabilize membrane structures, and affect membrane protein dynamics, conformation, organization, and function. To test this mechanism's applicability to the control of the functionality of LHCII, we designed in vitro proteoliposomal systems from lipids with altered acyl tail length. Here, we show acyl chain length regulates the average fluorescence lifetime of LHCII, showing a 2 ns lifetime at 18 carbon atoms, similar to the thylakoid membrane. Furthermore, LHCII appears to be quenched in proteoliposomes with acyl chains both shorter and longer than 18 carbons. Strikingly, the proteoliposomes show a large structural polymorphism when constructed from short chain length lipids, and from which we were able to isolate two quenched conformations, each with highly divergent fluorescence spectra. One has a broad fluorescence band at 683 nm maxima and another shows a higher contribution to 693 nm. Overall, we have addressed the effects of the lipid/protein hydrophobic mismatch on the light-harvesting activity of LHCII and its orientation and available conformations in the lipid membrane.

216 Pre Recorded Oral

Understanding the role of thylakoid ion transporters for light-induced membrane dynamics

Helmut Kirchhoff, Roma Mukhopadhyay

Washington State University, Pullman, WA, USA

The performance of the photosynthesis machinery in plant tissue, including electron transport, light harvesting, and protein repair, is controlled by reversible ultrastructural changes of the thylakoid membrane architecture inside the chloroplasts. For example, in presence of light the thylakoid lumen can expand and shrink again in the dark. In addition, it was reported that the thickness of the thylakoid membrane bilayer can change in the light. This membrane thinning could regulate photoprotective non-photochemical quenching and protein transport across the thylakoid membrane. The factors that control these swelling/shrinkage processes, however, are unknown. We postulated that the buildup of a proton gradient in the light drives the influx of ions (K+ and/or Cl-) from the stroma to the thylakoid lumen leading to osmotic water influx and swelling. Recent progress in unraveling the molecular identity of thylakoid ion transporter and channels allows to examine their role for swelling and shrinkage processes. Here we used loss-of-function mutants of the K+/+ antiporter KEA3 and the voltage-gated Cl- channel VCCN1 to study their impact on the light-induced swelling/shrinkage process of the thylakoid lumen and membrane thinning. To this end, we develop a semi-automated pipeline to measure the distance of the lumen, the stromal gap, and hydrophobic membrane bilayer thickness obtained from transmission electron microscopic images of dark and light adapted leaf tissues. We present evidence that thylakoid ion transporter control ultrastructural membrane dynamics. The results will be analyzed with a mathematical model describing ion transport across thylakoid membranes in the context of photosynthetic energy transformation.

217 Pre Recorded Poster

ZnO nanopriming improves seedling growth and plant photosynthetic efficiency of wheat (*Triticum aestivum*) through ROS signaling under drought stress

Prabha Rai Kalal[1], Anjana Jajoo[2]

[1]Devi Ahilya University, Indore, Madhya Pradesh, India. [2]Devi Ahilya University, Indore, Madhya Pradesh, India

Drought is one of the most important environmental factors inhibiting photosynthesis and decreasing the productivity of plants. The aim of the present study was to investigate the underlying mechanism of zinc oxide (ZnO) nanopriming in improving germination parameters and maintaining the photosynthetic efficiency of wheat under drought stress. The changes in primed and unprimed wheat plants associated during drought stress were studied by monitoring their physiological and biochemical performance. Results showed ZnO nanopriming prevented chlorophyll degradation under drought conditions thereby improving photosynthetic performance and overall plant growth. Study of Chl *a* fluorescence induction kinetics showed nanopriming to protect the photosynthetic apparatus thereby improving the efficiency of primary photochemistry of PSII under drought stress conditions. Furthermore, the study of antioxidant enzyme activity showed lower levels of reactive oxygen species (ROS) production in nanoprimed drought stressed plants indicating better tolerance to drought stress. We propose the role of ROS (reactive oxygen species) as signaling molecule in enhancing seed germination parameters by inducing various metabolic activities in nanoprimed wheat seeds. It can be concluded that ZnO nanopriming improved seed germination and tolerance potential in wheat by alleviating drought induced inhibition of plant photosynthetic machinery, ultimately resulting in an increase in biomass production as compared to unprimed wheat seeds.

219 Pre Recorded Oral

Effects of Free-Air Carbon Dioxide Enrichment (FACE) on photosynthesis, phenology and yield of winter wheat

Oliver Knopf[1], Antony Castro[1], David Lenzen[1], Juan Quiros[1], Sandra Markwitz[1], Einhard Kleist[1], Michael Quarten[1], Angelina Steier[1], Ralf Pude[2], Hendrik Poorter[1,3], Uwe Rascher[1], Onno Muller[1]

[1]Plant Sciences (IBG-2), Forschungszentrum Jülich GmbH, Jülich, Germany. [2]Field Lab Campus Klein-Altendorf, University of Bonn, Rheinbach, Germany. [3]Department of Biological Sciences, Macquarie University, North Ryde, Australia

Climate change and the associated continuous rise of the atmospheric carbon dioxide concentration [CO2] pose a major challenge for future sustainable wheat production. Alterations in the [CO2] can affect photosynthesis and thus have considerable impacts on crops, e.g. growth, phenology and quality. A free-air CO2 enrichment (FACE) facility was combined with an automated, mobile phenotyping system within the transdisciplinary 'BigBaking' project to get a better understanding of the complex plant response in wheat. Ten different winter wheat (*Triticum aestivum*) cultivars were grown in 2 x 3 m plots in the BreedFACE at ambient (~400 ppm) and elevated [CO2] (~600 ppm). In this study, we aimed to monitor plants throughout the vegetation period with the help of high-throughput photosynthesis measurements. A light-induced fluorescence transient (LIFT) sensor enabled us to observe the seasonal development of relevant traits such as the operating efficiency of photosystem II (Fq'/Fm') at unprecedented speed and under contrasting weather conditions. In addition, RGB data were collected by two PhenoCams continuously monitoring the plots. Winter wheat grown under elevated [CO2] tends to have a higher Fq'/Fm' during vegetative growth at the beginning of the growing period. During the grain-filling phase, plants showed a more cultivar-specific response with a larger variation in Fq'/Fm'. PhenoCam data indicate a delay in the onset of senescence under elevated [CO2] but, in turn, reveal a more pronounced senescence intensity. Furthermore, plants grown under elevated [CO2] accumulated significantly more straw biomass, yield, and increased plant height, suggesting a different resource allocation within winter wheat. Our results indicate that the rise in atmospheric [CO2] has wide-ranging effects on the cultivation of winter wheat and that high-throughput phenotyping can contribute to a better understanding of complex plant adaptation strategies in a highly dynamic environment.

220 Pre Recorded Poster

the role of the photoprotective PsbS protein for the antenna size of Photosystem II in plants

Hui Min Oung[1], Haniyeh Koochak[1,2], Helmut Kirchhoff[1]

[1]Washington State University, Pullman, WA, USA. [2]Sentinel Environmental, Houston, TX, USA

The PsbS protein is the major protein involved in high-energy quenching (qE) and is associated with supramolecular protein reorganizations in stacked grana thylakoid membranes. Yet, the impact of the PsbS protein on the structural and functional organization of the photosystem II (PSII) antenna system including the light harvest complex (LHCII) is unclear. To understand how PSII antenna dynamics is regulated by the PsbS protein under qE conditions, we perform compositional and functional analyses of isolated stacked and unstacked thylakoid domains. We quantify photosynthetic protein compositions such as LHCII, cytochrome (cyt) b_6f, and different structural subspecies of PSII in both stacked and unstacked areas of the thylakoid membrane in wild-type (WT), PsbS protein overexpress (L17, high qE), and knockout (npq4, no qE) mutant plants under qE (30 min, 500 µmol quanta m^{-2} s^{-1}) and dark conditions. Our results showed that the lateral distribution of PSII, PSI, LHCII, and cyt b_6f complex between stacked and unstacked domains is not significantly changed in the qE state compared to dark-adapted plants in all genotypes. Only a slight 12% increase of LHCII trimer per PSII was seen in stacked grana in L17. In contrast, the functional antenna size of PSII in the stacked domains decreased significantly in WT under qE conditions but increased slightly in both mutants. The fact that the LHCII/PSII ratio does not change provides evidence that supramolecular protein reorganization triggers the change in PSII antenna size in WT. Furthermore, the lack of this functional phenotype in the mutants indicates that the PsbS protein is involved in supramolecular reorganizations in grana. To confirm the PsbS-dependent rearrangement of the PSII antenna size under qE conditions, we will measure the antenna size at different pH levels in the mutants and WT. These findings will provide knowledge on how PsbS regulates light harvesting by PSII.

221 In Person Poster

Characterisation of *Synechocystis* sp. PCC 6803 mutants that contain an introduced copy of *psbA* from a cyanobacterial gene cluster induced under far-red light

Kevin J. Sheridan, Julian J. Eaton-Rye, Tina C. Summerfield

University of Otago, Dunedin, New Zealand

Cyanobacteria perform oxygenic photosynthesis using the water:plastoquinone oxidoreductase Photosystem II (PS II), a catalyst responsible for water splitting. A core protein of PS II is D1, encoded by a *psbA* gene family, which provides the majority of the ligands to the water-splitting complex and binds several key cofactors of PS II. Phylogenetic analyses have shown that there are multiple, distinct types of the D1 protein in cyanobacteria which are upregulated in response to a variety of physiological and environmental conditions [Cardona et al. (2015) Mol. Biol. Evol. 32:1310–1328; Sheridan et al. (2020) Photosynth. Res. 145:111–128]. One of these forms of the D1 protein, $D1^{FR}$, is associated with the far-red light photoacclimation (FaRLiP) response which allows cyanobacteria to utilise far-red/near-infrared light, unavailable to the majority of oxygenic photosynthetic organisms. Here, we show that $D1^{FR}$ is incorporated into PS II centres in *Synechocystis* sp. PCC 6803, but retards the performance of PS II. A strain containing only $D1^{FR}$-containing centres was not photoautotrophic, both in the presence and absence of chlorophyll *f* synthase and a variant of the D2 protein associated with the FaRLiP response, $D2^{FR}$. Insertion of characteristic residues associated with the first two helices of the $D1^{FR}$ protein in the native D1 protein of *Synechocystis* sp. PCC 6803 showed that these alterations to the D1 protein severely impact the physiological performance of PS II. Mutations introduced into the first helix of D1 resulted in low PS II assembly and abolished photoautotrophy, while mutations introduced into the second helix of D1 retarded PS II performance.

222 In Person Poster

Mutations targeting Phe345 of CP43 result in elevated rates of bicarbonate-supported oxygen evolution and modified PS II fluorescence in *Synechocystis* sp. PCC 6803

Faiza Arshad, Julian J. Eaton-Rye

Dept. of Biochemistry, Dunedin, Otago, New Zealand

In addition to light harvesting, the CP43 chlorophyll *a*-binding core antenna protein of Photosystem II (PS II) is essential for assembly of the Mn_4CaO_5 oxygen-evolving complex (OEC). The protein comprises six membrane-spanning helices with helices 5 and 6 joined by an extended loop (loop E) of approximately 130 residues and a molecular weight of approximately 15 kDa. Two residues from loop E, Glu341 and Arg344 in *Synechocystis* sp. PCC 6803, (numbered according to PDB:7N8O) form multiple bonds to the OEC and Glu341 also serves as a bidentate ligand coordinating two of the four Mn ions. Glu341 and Arg344 are found in a conserved motif from Gly340 to Phe345 corresponding to GETMRF that provides a cover over the OEC metallic cluster. We have explored the role of Phe345 by creating the F345A, F345H, F345K, F345P, F345W and F345Y mutants. The F345P mutant did not assemble PS II and the F345K strain was also not photoautotrophic although PS II did assemble in this mutant and in F345W cells. While photoautotrophic growth in the F345W cells was somewhat impaired, the F345A, F345H and F345Y cells grew like the control strain with a doubling time of approximately 14 . The F345A, F345H and F345Y strains also assembled PS II at similar levels to control cells. Interestingly, both F345W and F345Y cells exhibited bicarbonate-supported oxygen evolution rates that were 25 to 30% higher than observed in the control strain. The F345Y strain also exhibited an increased fluorescence yield in room temperature fluorescence induction assays and had an elevated emission at 685 and 695 nm in low-temperature fluorescence emission spectra excited at 440 nm. Our data showed hydrophobicity at the 345 amino acid position in CP43 is important for water-splitting activity and mutations on the lumenal side of PS II can influence PS II-specific fluorescence characteristics.

223 In Person Poster

Mutations targeting Arg448 of CP43 disrupt the hydrogen-bond network around the bicarbonate-binding environment of Photosystem II in *Synechocystis* sp. PCC 6803

Ei Phyo Khaing, Julian J. Eaton-Rye

Department of Biochemistry, Otago University, Dunedin, New Zealand

Arg448 is located in the C-terminal region of the CP43 chlorophyll-binding core antenna protein of Photosystem II (PS II). CP43-Arg448 forms hydrogen bonds with Glu242 of the D2 PS II reaction center protein. The D2-Glu242 residue also forms a hydrogen bond with D2-Lys264 which, in turn, contributes to the stabilization of the non-heme iron of PS II and its bound bicarbonate cofactor. In this study, four mutants (R448A, R448D, R448K, and R448P) were assembled to investigate the role of CP43-Arg448 in *Synechocystis* sp. PCC 6803. These mutants, apart from the R448K strain, were highly susceptible to photodamage in the presence of PS II-specific electron acceptors; however, in the presence of bicarbonate, their impaired activity was restored to the level determined before the high light was applied. In addition, acceptor-side electron transfer was altered resulting in slowed forward electron transfer between the primary and secondary plastoquinone electron acceptors Q_A and Q_B. Additionally, all mutants accumulated unassembled CP43 pre-assembly complexes. Furthermore, ^{35}S-Met protein labeling experiments revealed an impaired repair mechanism following photodamage in the R448D mutant during a low-light recovery period. These results indicate that disruption of the hydrogen bonds formed between CP43-Arg448 and D2-Glu242 impairs both PS II assembly and electron transfer as well as the repair mechanism of the photosystem following high-light-induced damage.

224 Pre Recorded Poster

Origin of the misses during photosynthetic water oxidation

Guangye Han[1], Petko Chernev[2], Stenbjörn Styring[2], Johannes Messinger[2,3], Fikret Mamedov[2]

[1]Chinese Academy of Sciences, Beijing, China. [2]Uppsala University, Uppsala, Sweden. [3]Umeå University, Umeå, Sweden

Photosynthetic water oxidation is catalyzed by photosystem II, large protein-pigment comlex in the thylakoid membrane of plants, alga and cyanobacteria. The reaction is defined by so-called S cycle with five intermediate S states, S_{0-4}. The transition efficiency of these intermediates is commonly described by the miss factor of the Kok model. In this study, we used electron paramagnetic resonance signals, which probe all S states in the same sample during S cycle advancement, to quantify the turnover efficiency in photosystem II membrane preparations from spinach in the presence of exogenous electron acceptor. Measurements were performed at selected temperatures between -10 °C and +20 °C and at flash frequencies of 1.25, 5 and 10 Hz. The results show that at optimal conditions the majority of the misses originate from the reactions occurring in the water oxidizing complex, allowing the extraction of their S state dependence. At all temperatures, the lowest miss was found for the S_1-S_2, and the highest miss was connected to the S_2-S_3 state transition. The S_3-S_0 and S_0-S_1 transitions were found to occur with an intermediate miss parameter. The miss in the S_1-S_2 transition increased with temperature, while the misses arising from the other transitions showed the opposite trend. The electron paramagnetic resonance results in the photosystem II membranes were confirmed by measurements of flash-induced oxygen release patterns in thylakoid membranes. The origin of the S state and temperature dependences of misses are discussed and correlated, for the first time, to structural changes at the $CaMn_4O_5$-cluster during completer S state advancement that were determined recently by femtosecond X-ray crystallography. Thereby, possible “molecular errors” connected to the e- transfer, + transfer, H_2O binding and O_2 release are identified [Han G. et al. (2022) Chem. Sci., *in press*].

225 Pre Recorded Poster

Comparison of electron transfer in the photosystem I in solution and deposited on conducting glass electrode

Alice Goyal[1], Sebastian Szewczyk[2], Gotard Burdziński[1], Mateusz Abram[3], Joanna Kargul[3], Krzysztof Gibasiewicz[1]

[1]Faculty of Physics, Adam Mickiewicz University, Poznan, Wielkopolskie, Poland. [2]Faculty of Physics, Adam Mickiewicz University, Poznan, wielkopolskie, Poland. [3]University of Warsaw, Warsaw, Mazowieckie, Poland

In our work, we studied and compared the electron transfer (ET) reactions inside and outside the photosystem I (PSI) complexes isolated from red alga Cyanidioschyzon merolae suspended in solution and immobilized on conducting glass (fluorine-doped tin oxide; FTO), thus forming a bio-photoelectrode. The photovoltaic efficiency of the electrochemical system comprising such a bio-photoelectrode turned out to be low (0.47%). The comparison of the results of the photocurrent measurements with the transient absorption data, monitoring the redox state of the primary electron donor in the immobilized PSI, was utilized to assess contribution of different factors to the limited efficiency of PSI-based system. The experiments were performed at a range of electric potentials applied to the multi-layer PSI film and at different compositions of electrolyte solution being in contact with the film. Our studies demonstrate that electrodeposition of PSI complexes on FTO significantly affects their ability to support full natural forward electron transfer. It was observed that 65%-90% of immobilized PSI particles remain inactive, depending on the potential applied and the presence or absence of redox mediators. The emergence of such large fraction of permanently inactive PSI is unclear. On the other hand, the substantial fraction of photoactive complexes (10-35%) did not translate into an equally effective generation of photocurrent, whose internal quantum efficiency was less than 0.5% at any conditions. This mismatch indicates that the charge recombination reactions are generally much more effective than the light-induced, PSI-mediated ET working and counter electrode in the studied system.

226 Pre Recorded Poster

The far-red light absorbing light-harvesting complexes: red-shifted phycobiliprotein complexes from *Halomicronema hongdechloris*

Min Chen

University of Sydney, Sydney, NSW, Australia

Phycobilisomes (PBS) are light-harvesting antenna complexes of cyanobacteria and red algae and PBS core structures are made up of allophycocyanin subunits. *Halomicronema hongdechloris*, a chlorophyll *f*-producing cyanobacterium, contains a Far-Red Light Photoacclimation gene cluster (FaRLiP). There are multiple copies of allophycocyanin encoding gene, including five genes encoding allophycocyanin (APC) in the FaRLiP gene cluster, reported from *H. hongdechloris* genome. The different expression level of allophycocyanin subunits play important role in far-red light photoacclimation processes. Using heterologous expressed in an *Escherichia coli* reconstitution system, we characterized photochemical properties of reconstituted allophycocyanin proteins. Cysteine is the residue that binds chromophores. Several mutants of conserved cystine residues in allophycocyanin subunits were constructed. The changed chromophores binding properties including shifted absorptions were observed. Additionally, five annotated phycobiliprotein lyase-encoding genes (*cpcS*) from the *H. hongdechloris* genome were phylogenetically classified and experimentally tested for their catalytic properties including their contribution to the shifted absorption of phycobiliprotein complexes. We will discuss some hypotheses towards understanding the roles of the specialized allophycocyanin subunits and the involvement of phycobiliprotein lyases.

227 Pre Recorded Poster

Chromatic acclimation processes and their relationships with phycobiliprotein complexes

Fanyue Wang, Min Chen

University of Sydney, Sydney, NSW, Australia

Chromatic acclimation (CA) is a widespread mechanism for optimizing the composition of phycobiliprotein complexes to maximize the cyanobacterial light capture efficiency. We hypothesized that bilin-based GAF (cGMP phosphodiesterase/Adenylate cyclase/FhlA) domain-containing photoreceptors are vital functional domains in charge of different CA pathways, There are seven CA types, CA1-CA7, classified according to various photoregulatory pathways. Here we use sequence analyses and bioinformatics to predict the presence of CA types according to three GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA)-containing photoreceptors, CcaS (Cyanobacterial chromatic acclimation sensor), RcaE (Regulator of chromatic adaptation), and RfpA (Regulator for far-red photoacclimation) across selected cyanobacteria with public accessible genomic information. Combining with genomic information of phycobilisome compositions, the CA capability of various cyanobacteria were predicted. Screening 65 accessible cyanobacterial genome databases we defined 19 cyanobacteria that have the capability to perform Far-red light photoacclimation (FaRLiP) under control of RfpA. Forty out of 65 cyanobacteria have the capability to perform green/red light photoacclimation although they use different photoreceptors (RcaE and/or CcaS) and photoregulatory pathways. The reversible response of photoreceptors in CA regulation pathways trigged by changed light conditions reflects the flexibility of photoregulatory mechanism in cyanobacteria.

228 Pre Recorded Oral

Evolution and evolvability of Photosystem II

Tom D. Kim[1], Thomas Oliver[1,2], Joko P. Trinugroho[1,3], Violeta Cordón-Preciado[1], Giorgio Bianchini[4], A. William Rutherford[1], Patricia Sánchez-Baracaldo[4], Tanai Cardona[1]

[1]Department of Life Sciences, Imperial College London, London, United Kingdom. [2]Department of Physics and Astronomy, Vrije Universiteit, Amsterdam, Netherlands. [3]Bioinformatics and Data Science Research Centre, Binus University, Jakarta, Indonesia. [4]School of Geographical Sciences, University of Bristol, Bristol, United Kingdom

Photosystem II is the water-oxidizing and O_2-evolving enzyme of photosynthesis. How and when it originated are questions that have remained difficult to answer. In this talk, I will present advances in our understanding of the origin and evolution of photosystem II and oxygenic photosynthesis. The evolution of photosystem II indicates that water oxidation originated early in the history of life, long before the diversification of cyanobacteria and other major groups of prokaryotes. It challenges current paradigms that describe the origin and early evolution of life occurring in the absence of light and without the involvement of bioavailable O_2. I will show how the continuous duplication process of the D1 subunit of photosystem II, which controls photochemistry and binds the catalytic Mn_4CaO_5 water-splitting cluster, grants the enzyme adaptability to variable environmental conditions, and even the capacity to innovate enzymatic functions beyond water oxidation. I argue that this evolvability can be exploited using directed evolution to develop novel light-powered enzymes with the capacity to carry out complex multi-step oxidative transformations. We will show our progress developing directed evolution approaches for in vivo and in vitro applications of photosystem II. I will report the isolation of photosystem II variants where Mn in the growth media have been replaced with Ru. These variants appear to show photosynthetic growth, including a quadruple mutant where the redox active TyrZ, Y161, has been replaced by cysteine. A first of its kind. The isolation of such variants demonstrate that the system is evolvable and suggests that the remarkable properties of photosystem II can be harnessed for bespoke sustainable biocatalysis.

229 Pre Recorded Oral

Metabolite interactions in the bacterial Calvin cycle and implications for flux regulation

Paul Hudson

KTH Royal Institute of Technology, Stockholm, Sweden

Metabolite-level regulation of enzyme activity is important for coping with environmental shifts in bacteria. Knowledge of such regulation can also guide attempts to engineer strains for biochemical production. Recently developed proteomics methods allow for mapping of post-translational interactions, including metabolite-protein interactions, that may be relevant for quickly regulating pathway activity. While metabolite-level regulation in glycolysis has been investigated, there is relatively little study of metabolite-level regulation in the Calvin cycle, particularly in bacteria. We applied limited proteolysis small molecule mapping (LiP-SMap) to identify and compare metabolite-protein interactions in the proteomes of four bacteria that fix CO2 using the Calvin cycle: the photoautotrophic cyanobacteria Synechocystis sp. PCC 6803 and Synechococcus elongatus PCC 7942 and chemoautotrophs Cupriavidus necator and Hydrogenoflava pseudoflava. Clustering analysis of the hundreds of detected interactions showed that some metabolites interacted in a species-specific manner, such as interactions of glucose-6-phosphate in Cupriavidus and of glyoxylate in Synechocystis. Metabolite interactions with the Calvin cycle enzymes fructose-1,6/sedoheptulose-1,7-bisphosphatase (F/SBPase) and transketolase were tested for effects on catalytic activity using kinetic assays. The Calvin cycle intermediate GAP activated both Synechocystis and Cupriavidus F/SBPase, which suggests a feed-forward activation of the Calvin cycle in both photoautotrophs and chemolithoautotrophs. LiP-SMap is a promising technique to explore and uncover novel post-translational metabolic regulation, although the method could benefit from improved sensitivity and specificity.

230 In Person Poster

Removal of Photosystem II-specific lipoproteins modifies the phenotype of a ΔPsbJ deletion strain of *Synechocystis* sp. PCC 6803

Oliver J. S. Craig, Julian J. Eaton-Rye

University of Otago, Dunedin, New Zealand

The Photosystem II (PS II) complexes of cyanobacteria possess lumenal lipoproteins which assist in biogenesis, repair, and stabilization of the oxygen-evolving complex. In *Synechocystis* sp. PCC 6803 these include PsbP (CyanoP), PsbQ (CyanoQ), Psb27, and Ycf48. The PsbP, PsbQ and Psb27 proteins are lipidated at a conserved eubacterial lipobox sequence found near their N-terminus while the lipobox sequence for Ycf48 is distinct but is conserved in many cyanobacteria. Single deletion mutants for each of these proteins show only slight reduction in PS II function; however, these strains showed altered phenotypes in a *psbJ* deletion background. The *psbJ* gene encodes a 4 kDa membrane-spanning subunit of PS II with no established function. Oxygen evolution in the ΔPsbJ strain is reduced compared to wild type in the presence of 2,6-dimethoxy-1,4-benzoquinone (DMBQ), but in the presence of bicarbonate the effect is reversed with the ΔPsbJ strain outperforming wild type [Choo et al. (2022) Photosynth. Res. 151:103–111]. The bicarbonate-supported rate of oxygen evolution was similar to wild type in the ΔPsbJ:ΔPsbP and ΔPsbJ:ΔPsbQ double mutants but remained elevated in the ΔPsbJ:ΔPsb27 strain. Low-temperature fluorescence emission spectroscopy exhibited an increased peak at 685 nm upon 580 nm excitation in the ΔPsbJ:ΔPsbP and ΔPsbJ:ΔPsbQ strains, suggesting energy transfer from the phycobilisome may be impaired. Fluorescence emission following excitation at 440 nm, however, suggested similar levels of assembled PS II were present. The ΔPsbJ strain, but not the other single mutants, was highly susceptible to photodamage but recovered its oxygen-evolving ability under low light in the presence of DMBQ. Likewise the ΔPsbJ:ΔPsbP, ΔPsbJ:ΔPsbQ and ΔPsbJ:ΔPsb27 mutants were sensitive to high-light conditions; however, they recovered more slowly than the ΔPsbJ strain. In addition, while all other strains were photoautotrophic, the ΔPsbJ:ΔYcf48 mutant was an obligate photoheterotroph.

231 In Person Poster

Phycourobilin and the evolution of the Florideophyte phycobilisome

Adrian I. S. Smith-Beech, Erin Delargy, Peter K. Dearden, Chun Shen Lim, Sigurd M. Wilbanks

Department of Biochemistry, University of Otago, Dunedin, New Zealand

Despite impressive structural characterisation of the rhodophyte phycobilisome, there is little known about its biogenesis. The addition of phycourobilin (PUB) in the β-subunit of phycoerythrin (PE) improves the light-harvesting capacity of late-diverging, intertidal/subtidal Rhodophyta. It has a similar function to the PUB utilised by the open ocean picocyanobacteria, but is evolutionarily distinct. It is in these cyanobacteria that phycobilin attachment and interconversion is firmly understood. We present data on the chromophore composition of individual PE subunits and their tryptic chromopeptides from *Griffithsia sp*. We provide evidence for minimal variation of phycobilin composition between seasons. Our sequencing of *Griffithsia* has provided novel sequences encoding the rhodophyte phycobilin lyases, including the putative phycobilin lyase/isomerase, RpeF. This provides the tools to test whether this enzyme is responsible for the occurrence of phycourobilin on β-phycoerythrin in members of the Florideophyceae. These data may yet unify recent advancements in the architecture of rhodophyte phycobilisomes with current understanding of cyanobacterial phycobilin lyases. Furthermore, describing the phylogeny of the phycobilin lyases will contribute to understanding the macroevolutionary trajectory of the Rhodophyta and its primary plastid.

232 In Person Poster

Contributions of the DE loop of the D2 protein to the bicarbonate-binding environment of Photosystem II

Victor Zhong, Julian J. Eaton-Rye

University of Otago, Dunedin, New Zealand

The sequence ^{240}AEETYSMVTAN^{250} in the DE loop of the D2 protein of Photosystem II (PS II) represents a conserved motif surrounding the bicarbonate ligand to the non-heme iron (NHI) between the plastoquinone electron acceptors Q_A and Q_B. We have targeted Met246 and Asn250 since both residues contribute to hydrogen bonding in the bicarbonate-binding environment. Using *Synechocystis* sp. PCC 6803, we have created six mutants (M246A, M246F, M246K, N250A, N250D, and N250H). Photoautotrophic growth was retarded, and oxygen evolution reduced, in N250A and N250H cells in the presence of 2,5-dimethyl-1,4-benzoquinone (DMBQ) but oxygen evolution recovered in the presence of bicarbonate. In contrast, photoautotrophic growth and oxygen evolution were abolished in N250D cells. In addition, chlorophyll fluorescence revealed inhibited electron transfer in the N250A and N250H strains and indicated N250D cells lacked active PS II. While M246F cells grew like control, M246A cells were impaired and the M246K mutant was an obligate photoheterotroph. DMBQ-supported oxygen evolution was reduced in the M246A strain but slightly improved in the presence of bicarbonate while fluorescence yield assays indicated a reduced level of active PS II in the M246A strain and no active centers in the M246K strain. In addition, we have created the R265A and R265D mutants to probe the role of Arg265 which also contributes to the hydrogen bond network in the bicarbonate-binding environment but is not part of the conserved motif. The R265A and R265D strains grew similarly to the control; however, oxygen evolution in the presence of DMBQ was impaired. In contrast, oxygen evolution was supported in the presence of bicarbonate. In addition, fluorescence induction and decay measurements revealed inhibited electron transfer between Q_A and Q_B. Our data support key roles for Met246, Asn250 and Arg265 in the hydrogen-bond network that stabilizes the bicarbonate-binding environment in the quinone-iron-acceptor complex of PS II.

233 Pre Recorded Poster

Multiple quantum-coherent energy transfer pathways in photosynthesis: electronic-vibrational mixing within the Photosystem II CP43 and CP47 antenna complexes

Valentin Maffeis[1,2], Patricia Lorente[3], Rafael Picorel[3], Elisabet Romero[1]

[1]Institute of Chemical Research of Catalonia (ICIQ), Tarragona, Spain. [2]Université Lyon, Lyon, France. [3]Department of Plant Biology, Estacion Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas, Zaragoza, Spain

Photosynthesis, the biological process whereby the energy of the Sun is stored into biochemical energy, starts with energy absorption by light-harvesting complexes. These pigment-protein complexes are able to absorb and transfer energy with high speed and efficiency to the reaction center, the site of solar-energy conversion. To understand how these complexes work allows to elucidate strategies to design robust bio-inspired solar-energy conversion systems. Here, we report a two-dimensional electronic spectroscopy study of the photosystem II core antenna complexes, CP43 and CP47, and of monomeric chlorophyll *a* at cryogenic temperature aiming to investigate both the mechanism and the pathways of energy transfer with a focus on quantum-coherent phenomena. Our results demonstrate that multiple energy transfer pathways are active within CP43 and CP47 and that electron-vibrational mixing promotes energy transfer via a vibronic-coherent mechanism. This work provides unprecedented detail on the energy transfer process within CP43 and CP47 and contributes to our understanding of the natural light-harvesting design principles. Therefore, we envision that our findings will pave the way to implement quantum-coherent energy transfer in artificial light-harvesting complexes to develop robust, fast and efficient solar-energy conversion systems.

234 Pre Recorded Poster

Probing the release of protons by Photosystem II in the S_1 to S_2 high-spin transition and in the D1/R323E mutant

Alain Boussac[1], Julien Sellés[2], Miwa Sugiura[3]

[1]I2BC, UMR CNRS 9198, CEA Saclay, Gif sur Yvette, France. [2]IBPC, UMR CNRS 7141 and Sorbonne Université, Paris, France. [3]Proteo-Science Research Center, and Department of Chemistry, Graduate School of Science and Technology, Ehime University, Matsuyama, Japan

Stoichiometries and kinetics of the proton releases were investigated in the S-state cycle of Photosystem II from *Thermosynechococcus elongatus* containing either a Mn_4CaO_5 or a Mn_4SrO_5 cluster. The measurements were done at pH 6.0 and 7.0 knowing that, in PSII/Ca at pH 6.0 and 7.0 and in PSII/Sr at pH 6.0, the flash-induced S_2-state is in a low-spin configuration whereas in PSII/Sr at pH 7.0, the S_2-state is in a high-spin configuration in half of the centers. The fittings of the period 4 oscillations indicate that one proton is released in the S_1 to S_2^{HS} transition in PSII/Sr at pH 7.0. We had suggested that the proton release in the S_2^{LS} to S_3 transition took place in a $S_2^{LS}Tyr_Z^{\bullet}$ to $S_2^{HS}Tyr_Z^{\bullet}$ transition before the electron transfer from the cluster to $Tyr_Z^{\bullet}$ occurs. The release of a proton in the $S_1Tyr_Z^{\bullet}$ to $S_2^{HS}Tyr_Z$ transition would imply that a proton release is missing in the $S_2^{HS}Tyr_Z^{\bullet}$ to S_3Tyr_Z transition. Instead, the proton release in the S_1 to S_2^{HS} transition was mainly done at the expense of the proton release in the S_3 to S_0 to S_1 transitions. This complex link between proton movements in, and immediately around, the Mn_4 cluster and the mechanism leading to the release of protons into the bulk will be briefly discussed. It was proposed that the D1/R323 residue could contribute to regulate a proton egress pathway from the Mn_4CaO_5 cluster and Tyr_Z via a proton channel identified from the 3D structure. To test this suggestion, a PsbA3/R323E site-directed mutant has been constructed. Rather than participating in the egress of protons to the lumen, we found that this channel, by interacting with the bulk, is more likely involved in the relaxations of the -bonds around Tyr_Z, thus tuning the driving force required for Tyr_Z oxidation.

235 Pre Recorded Oral

Olle Bjorkman, a pioneer and continuing inspiration for C_4 photosynthetic research

Rowan Sage, Tammy Sage, Matt Stata, Arthur Leung

EEB Department, University of Toronto, Toronto, ON, Canada

The pathway of C_4 photosynthesis was elucidated in the mid-1960s, and quickly led to the realization that warm season grasses and halophytic chenopods exhibited ecological patterns which could be explained by the newly discovered C_4 physiology. Among the first to study the environmental significance of the C_4 pathway was Olle Bjorkman, whose leadership was instrumental in the rapid advances that by the early 1970's had identified most of the ecological advantages of the C_4 pathway. Of unique significance was his collaboration with Malcolm Nobs on crossing C_3 and C_4 Atriplex species, which led to the first C_3 x C_4 hybrids and the dissection of essential traits specific to the C_4 pathway. This work represented the first concerted effort to address how C_4 plants evolved, and became the foundation for subsequent work on C_4 evolution. Today, Bjorkman's C_4 legacy remains an inspiration for current research on how, when and where C_4 photosynthesis evolved. In this talk, I will discuss the legacy of Olle Bjorkman's C_4 contributions and provide an overview of the latest understanding of the C_4 evolutionary process.

236 Pre Recorded Poster

Light acclimation interacts with thylakoid ion transport to govern the dynamics of photosynthesis in Arabidopsis

Thekla von Bismarck[1], Karin Köhl[1], Peter Jahns[2], David M. Kramer[3], Ute Armbruster[1]

[1]Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany. [2]Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany. [3]Michigan State University, East Lansing, USA

Light intensity fluctuates strongly in nature and constrains the maximum yield of plants in the field. Understanding the mechanistic interactions of the fast response to light intensity changes and long-term light acclimation to the prevailing condition is crucial for an in-depth understanding of dynamic photosynthesis in nature.

In this study, we address this by systematically characterizing light-environmental effects on the thylakoid ion transport-mediated short-term responses during light fluctuations. We grew *Arabidopsis thaliana* wild-type and mutants of the photosynthesis regulators K^+/ $^+$ antiporter KEA3 and the Cl^- channel VCCN1 under eight different light environments to stepwise bridge the gap between widely used lab conditions and natural light environments. We determined the effect of the growth condition on photosynthesis-related parameters in steady-state as well as dynamic conditions. For a detailed characterization of selected light conditions, we monitored ion flux dynamics at an unprecedented high temporal resolution using a modified spectroscopy approach. Our analysis revealed light intensity as the main light acclimatory driver to sculpt photosynthetic capacity, having negative and positive effects on the activities of VCCN1 and KEA3, respectively, during a high light pulse. We found that fluctuations in light intensity increase the levels of photoprotective zeaxanthin (Zx), which slows down the relaxation of the pH-dependent NPQ component qE, and a new role of KEA3 in suppressing Zx during the day. This gives KEA3 a dual role in accelerating NPQ upon a shift to light limiting conditions, i.e. directly via the downregulation of lumenal pH and thereby qE, and indirectly on a longer time scale via lowering Zx levels.

In summary, both light environment factors, intensity and fluctuations, strongly shape the fast response by modulating thylakoid ion transport with distinct effects on lumen pH, Zx accumulation, photoprotection and photosynthetic efficiency.

237 Pre Recorded Poster

Light harvesting strategies in far-red light

Thomas Oliver, Eduard Elias, Roberta Croce

Vrije University, Amsterdam, Netherlands

Oxygenic photosynthesis is normally powered by photons in the visible portion of the spectrum (400-700 nm). By constitutively producing the red-shifted chlorophyll d, the unique cyanobacterium, *Acaryochloris marina*, can harvest far-red light (700-800 nm). Interestingly, the *A. marina* MBIC11017 strain uses both soluble phycobilisomes and membrane bound Pcb antenna to harvest light [Chen et al. (2002) FEBS Lett. 514:149–152]. Acclimation of *A. marina* to far-red light (~730 nm) induces changes in this light harvesting machinery, with a reduction in the phycobilisome content [Duxbury et al. (2009) Photosynthesis Research, 101:69–75].

Additionally, certain cyanobacteria have evolved the ability to harvest far-red in a process called far-red light photoacclimation (FaRLiP) [Gan et al. (2014) Science, 345:1312–1317]. When exposed to far-red light, these cyanobacteria synthesise the red-shifted chlorophylls, chlorophyll f and chlorophyll d, and express PSI and PSII subunits that incorporate these chlorophylls. Since the discovery of this process, it has been proposed that a far-red utilising crop plant could be engineered to extend their photosynthetic spectrum and improve yields [Chen and Blankenship (2011) Trends Plant Sci. 16:427–431].

Here, we investigate the ability of *A. marina* to harvest light when acclimated to either visible or far-red light, using time-resolved and steady-state fluorescence measurements. We also present an approach for the incorporation of chlorophyll f into an engineered cyanobacteria.

238 Pre Recorded Poster

Mutations in cytochrome b_{559} targeting interactions with phosphatidylglycerol impair PS II assembly and increase light sensitivity in *Synechocystis* sp. PCC 6803

Bharat Kumar Majhi, Julian J. Eaton-Rye

University of Otago, Dunedin, Otago, New Zealand

Photosystem II (PS II) catalyses the oxidation of water and reduction of plastoquinone in oxygenic photosynthesis. In cyanobacteria, the PS II complex is found as a dimer where each monomer consists of approximately 20 protein subunits: these consist of the D1 and D2 subunits that contain the reaction centre, two chlorophyll-binding antenna proteins CP43 and CP47, 13 low-molecular-weight (LMW) proteins, and three or four lumenal hydrophilic subunits. Cytochrome b_{559} (Cyt b_{559}) is composed of two membrane-spanning LMW subunits: PsbE (the α-subunit), and PsbF (the β-subunit). This cytochrome is in proximity to PsbJ, a LMW protein composed of one membrane-spanning helix, the mobile plastoquinone electron acceptor (Q_B), β-carotene, and phosphatidylglycerol (PG). This study was conducted to determine how the interactions of Cyt b_{559} with PG impact PS II activity in *Synechocystis* sp. PCC 6803. Mutations were introduced into PsbE in the vicinity of the acceptor side of PS II targeting Phe10 and Ser11 since these residues possess hydrogen bonds with PG. The F10A and S11A mutants remained photoautotrophic although variable chlorophyll fluorescence induction and blue-native polyacrylamide gel electrophoresis indicated the level of assembled PS II was reduced. In addition, forward electron transfer between the primary quinone acceptor Q_A and Q_B was impaired, and oxygen-evolving activity was reduced. Notably, the mutation at Phe10 introduced light sensitivity where recovery from photodamage required protein synthesis and light. In fact, recovery was completely prevented when the cells were transferred into dark following high-light exposure (2000 µmol photon m^{-2} s^{-1}) but a full recovery of activity was observed once the cells were returned to low light (30 µmol photon m^{-2} s^{-1}). Based on the findings we suggest a model for recovery from photodamage that requires protein synthesis during high-light exposure followed by a low-light-driven assembly process.

239

Improve Rubisco, improve plant productivity

Spencer Whitney

Australian National University, Canberra, ACT, Australia

Screens of natural Rubisco catalytic diversity over the last decade have revealed significant plasticity in carboxylation potential that challenge the antiquated views of kinetic trade-offs in speed and CO_2-selectivitiy that underpin scepticism that the inherent potential for improving Rubisco performance is constrained. In this talk I will discuss the use of directed evolution as a feasible pathway for generating 'better-than-nature' step changes in Rubisco performance that are needed to visibly improve plant growth.

240 Pre Recorded Oral

The electron-proton bottleneck of photosynthetic oxygen evolution

Paul Greife[1], Matthias Schönborn[1], Matteo Capone[2,3], Ricardo Assunção[1], Daniele Narzi[3], Leonardo Guidoni[3], Holger Dau[1]

[1]Freie Univ. Berlin; Dept. of Physics, Berlin, Germany. [2]Univ. of L'Aquila; Dept. of Information Engineering, Computer Science and Mathematics, L'Aquila, Italy. [3]Univ. of L'Aquil; Dept. of Physical and Chemical Sciences, L'Aquila, Italy

Photosynthesis fuels life on Earth by storing solar energy in chemical form, inspiring technological schemes for sustainable fuel production. Today's oxygen-rich atmosphere results from photosynthetic O2-production during water-splitting at the protein-bound manganese cluster of photosystem II. Formation of the O2 molecule starts from a state with four accumulated electron holes, the S4-state, postulated half a century ago1 and remaining enigmatic ever since. Here we resolve this missing key element in photosynthetic O2-formation and its crucial mechanistic role. We tracked 230,000 excitation cycles of dark-adapted photosystems with microsecond infrared spectroscopy. Combing these results with computational chemistry reveals that in S4 not only are four electron holes accumulated by metal ion and protein sidechain oxidation, but also a crucial proton vacancy is created through gated sidechain deprotonation. Subsequently, a reactive oxygen radical is formed in an astonishing single-electron multi-proton transfer event. This is the slowest step in photosynthetic O2-formation – despite its low energetic barrier – due to entropic slowdown. In conjunction with previous breakthroughs in experimental and computational investigations, a compelling atomistic picture of photosynthetic O2-formation emerges. Our results provide insight into a biological process that has probably operated in the same unique way for three billion years and are expected to support the knowledge-based design of artificial water-splitting systems.

Greife et al. (2022) The electron-proton bottleneck of photosynthetic oxygen evolution, 03 Feb 2022, PREPRINT available at Research Square, https://doi.org/10.21203/rs.3.rs-1210297/v1

241 In Person Poster

Deletion of RubA in combination with low-molecular-weight proteins disrupts Photosystem II assembly in *Synechocystis* sp. PCC 6803

Toby J. Brown, Julian J. Eaton-Rye

University of Otago, Dunedin, New Zealand

Rubredoxin A (RubA) is a Photosystem II (PSII) assembly factor with a key role in assembly of the PSII reaction centre. In the cyanobacterium *Synechocystis* sp. PCC 6803 the *rubA* gene is found in a cluster with *ycf48*, encoding another assembly factor required for reaction centre biogenesis, and the *psbEFLJ* operon encoding four of thirteen membrane-spanning low-molecular-weight (LMW) proteins present in the mature photosystem. The PsbE and PsbF proteins form cytochrome b_{559} which is essential for the initiation of PSII biogenesis and PsbL likely affects a later stage. Many of the remaining LMW proteins are known to be part of pre-assembly complexes incorporating the PSII CP43 and CP47 chlorophyll-binding core antenna proteins. PSII biogenesis involves a stepwise progression that brings together the separate pre-assembly complexes; however, it is not clear at which step the LMW peptides PsbJ, PsbX and PsbY join the process. To address this question the ΔRubA:ΔPsbX, ΔRubA:ΔPsbY and ΔRubA:ΔPsbJ double deletion mutants were produced. Interestingly, ΔRubA:ΔPsbX and ΔRubA:ΔPsbY cells were photoautotrophic; however, the ΔRubA:ΔPsbJ strain was not. In contrast, all four single deletion mutants were photoautotrophic. Among the double mutants, the ΔRubA:ΔPsbY strain assembled the most PSII complexes and the ΔRubA:ΔPsbJ strain assembled the least. This was shown by low-temperature fluorescence emission spectra and by fluorescence induction experiments in the presence or absence of diuron. In addition, activity measurements in the presence of the PSII-specific electron acceptor 2,5-dimethyl-1,4-benzoquinone revealed the ΔRubA:ΔPsbJ and ΔRubA:ΔPsbX strains were incapable of oxygen evolution; whereas, ΔRubA:ΔPsbY cells evolved oxygen at a rate similar to that observed in the ΔRubA strain. Our current data suggest that PsbJ may impact PSII biogenesis at an earlier step than indicated by current models that suggest PsbJ binds during the final stages of PS II assembly.

242 Pre Recorded Poster

3D visualisation of non-photochemical quenching in photosynthetic organisms

Mattia Storti[1], Haythem Hsine[1], Clarisse Uwizeye[1], Fabien Chevalier[1], Gilles Curien[1], Daniel Beal[2], Dimitri Tolleter[1], Giovanni Finazzi[1]

[1]CEA\CNRS\INRAE\UGA, Grenoble, France. [2]JBeamBio, La Rochelle, France

Determining cellular/subcellular photoprotective responses in complex photosynthetic systems is a bottleneck in plant biology. Here we describe a new approach for single cell photophysiology based on tailoring a confocal microscopy customised for pulse modulated amplitude (PAM) imaging. This method allows to collect 3D photophysiology information in living samples characterised by increasing complexity, such as developing tissues, photosymbiontic organisms and plant cells with C3 an C4 anatomies. The method bypasses artifacts associated with 2D single cell analysis and its simplicity will contribute to its widespread use in plant photoacclimation studies especially for NPQ.

243 In Person Oral

Modeling and mutagenesis of the CO_2 uptake NDH-1 complexes in cyanobacteria

Robert Burnap[1], Juliana Artier[1], Neil Miller[1], Clark Jett[1], Dean Price[2], Ross Walker[1]

[1]Oklahoma State University, Stillwater, OK, USA. [2]Australian National University, Canberra, FCT, Australia

Cyanobacteria have evolved a CO_2-concentrating mechanism (CCM), which efficiently supplies CO_2 to the photosynthetic mechanism. Essentially, it functions as a 'supercharger' for CO_2 by concentrating it within the cell, thereby saturating the active sites of the CO_2-fixation enzyme, Rubisco, thereby increasing the efficiency of photosynthesis. This includes specialized Type-1 NDH complexes that function to couple photosynthetic redox energy to CO_2 hydration forming the bicarbonate that accumulates to high cytoplasmic concentrations during the operation of the CCM, required for effective carbon fixation. We used a *Synechococcus* PCC7942 expression system to investigate the role of conserved histidine and cysteine residues in the CupB (also designated, ChpX) protein, which has been hypothesized to participate in a vectoral CO_2 hydration reaction near the interface between CupB protein and the proton-pumping subunits of the NDH-1 complex. A homology model has been constructed and most of the targeted conserved residues are in the vicinity of a Zn ion modeled to form the catalytic site of deprotonation and CO_2 hydration. Growth and CO_2 uptake assays show that the most severe defects with substitutions of the predicted Zn ligand, CupB-His86, but also with a second sphere ligand CupB-Glu95, which coordinates the unusual Arg Zn ligand. CupB-Arg91. Mutations at other sites produced intermediate effects. Proteomic analysis revealed that some amino acid substitution mutations of CupB caused the induction of bicarbonate uptake proteins to a greater extent than complete deletion of CupB, despite growth under CO_2-enriched conditions. Mutagenesis of residues hypothesized to be involved in the transmembrane proton pumping activity also affect CO_2 uptake. The results are discussed in the context of a possible mechanism that couples transmembrane proton pumping to CO_2-hydration activity in the cyanobacterial NDH-1 complexes.

Funded by U.S. Department of Energy Basic Sciences, grant number DE-FG02-08ER15968

245 Pre Recorded Oral

Molecular architecture of spinach thylakoid membranes visualized by cryo-electron tomography

Wojciech Wietrzynski[1,2], Lorenz Lamm[2,3], Willaim J. Wood[4], Lorna A. Malone[4], Matina-Jasemi Loukeri[5], Matthew P. Johnson[4], Benjamin D. Engel[1,2]

[1]Biozentrum, University of Basel, Basel, Switzerland. [2]Helmholtz Pioneer Campus, Neuherberg, Germany. [3]Helmholtz AI, Neuherberg, Germany. [4]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom. [5]Max Planck Institute for Biochemistry, Martinsried, Germany

Thylakoid membranes of cyanobacteria and chloroplasts scaffold an assortment of large protein complexes that work together to harness energy from light and convert it into a biologically-useful, chemical form. In vascular plants, thylakoids divide into grana and stroma lamellae – two membrane sub-compartments with different protein compositions. Photosynthetic complexes and the two membrane domains are tightly intertwined and interact at spatial and temporal scales.

Although extensively studied with a range of methods, it has been a longstanding challenge to visualize how the thylakoid network organizes at molecular level to assert its morphology and to fine tune the photosynthetic reactions.

Here, we use Cryo-electron Tomography (Cryo-ET) coupled with Focused Ion Beam Milling (FIB) to visualize intact Spinacia oleracea (Spinach) chloroplasts with single-complex resolution. Our tomograms allow us to analyze the architecture and composition of grana and stroma lamellae as well as interaction and transitions between them. With the help of novel, AI-based particle-detection methods1 we are able to chart the appressed membranes to, for the first time, demonstrate the spatial interaction of Photosystems II (PSII) within the entire granum in quasi-native condition. This approach will provide the means for high throughput analysis of photosynthetic samples under different environmental conditions to breach the in vivo and in vitro structural methods.

246 Pre Recorded Oral

Synthetic biology tools to understand and control subcellular biocatalysis

Claudia Vickers

Eden Brew, Brisbane, Queensland, Australia. Queensland University of Technology, Brisbane, Queensland, Australia. Griffith Universtiy, Brisbane, Queensland, Australia

Effective redirection of carbon at specific metabolic nodes requires a suite of tools that can deliver useful outcomes under a wide variety of different conditions. Metabolic flux control mechanisms are commonly exerted at pre-translational levels; however, many metabolic conditions demand protein-mediated solutions that more directly and/or more rapidly influence catalytic conditions. We use isoprenoid (terpene/terpenoid) production as a model system to investigate these challenges. Isoprenoids are an extremely large and diverse group of natural compounds with myriad industrial uses, ranging from specialized applications (e.g. pharmaceuticals, fine chemicals, additives) through to bulk chemicals (e.g., colourants, fragrances, industrial polymers, agricultural chemicals, and fuel replacements). To control carbon flux at specific metabolic nodes for delivery of different classes of isoprenoids we have developed a variety of molecular tools that can be used to redirect metabolism at metabolic nodes. These include co-location of pathway enzymes using scaffolds (including nanocompartments) to alleviate toxicity effects, increase titres, and alter the product profile of promiscuous enzymes, and protein-based biosensors to understand metabolite accumulation. Here we will explore these tools and their application for metabolic engineering of isoprenoid production pathways. Using these approaches, we can effectively control and balance metabolism to deliver g/L titres of target isoprenoids.

247 Pre Recorded Oral

Structural basis for light harvesting regulation through state transitions in green algae

Xiaowei Pan[1,2], Ryutaro Tokutsu[3], Anjie Li[1], Kenji Takizawa[3], Chihong Song[4], Kazuyoshi Murata[4], Tomohito Yamasaki[5], Zhenfeng Liu[1], Jun Minagawa[3], Mei Li[1]

[1]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China. [2]College of Life Science, Capital Normal University, Beijing, China. [3]National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan. [4]Exploratory Research Centre on Life and Living Systems, National Institutes of Natural Sciences, Okazaki, Japan. [5]Science and Technology Department, Natural Science Cluster, Kochi University, Kochi, Japan

During photosynthesis in green algae and plants, light-harvesting complexes (LHCs) absorb photon energy and supply excitation energy for subsequent charge separation processes occurring in photosystems I and II (PSI and PSII, respectively). PSI and PSII exhibit different absorption characteristics, with the former having a broad absorption peak in the far-red region and PSII favoring red light. An imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate constantly. To ensure optimal photosynthetic efficiency, plants and algae have developed a short-term light-acclimation mechanism, termed state transitions, to balance energy distribution between the two photosystems. During the process, a portion of light-harvesting complex II (LHCII) is phosphorylated, dissociated from PSII and binds with PSI to form the supercomplex PSI–LHCI–LHCII. In green algae, state transitions show stronger amplitude and play a wider photoprotective role than the process in plants. Here, we report high-resolution structures of PSI–LHCI–LHCII from *Chlamydomonas reinhardtii*, revealing the mechanism of assembly between the PSI–LHCI complex and two phosphorylated LHCII trimers containing all four types of LhcbM protein. Two specific LhcbM isoforms, namely LhcbM1 and LhcbM5, directly interact with the PSI core through their phosphorylated amino terminal regions. Furthermore, biochemical and functional studies on mutant strains lacking either LhcbM1 or LhcbM5 indicate that only LhcbM5 is indispensable in supercomplex formation. The results unravel the specific interactions and potential excitation energy transfer routes between green algal PSI and two phosphorylated LHCIIs.

Pan X. et al. (2021) Nat. Plants 7: 1119-1131.

248 Pre Recorded Oral

Combining atomic force microscopy and mass spectrometry to determine the supramolecular organization of cyanobacterial thylakoid membrane complexes

Andrew Hitchcock

University of Sheffield, Sheffield, United Kingdom

Cyanobacteria are ubiquitous in nature and have developed numerous strategies that allow them to live in a diverse range of environments. Certain cyanobacteria synthesize chlorophylls *d* and *f* to acclimate to niches enriched in far-red light (FRL) and incorporate paralogous photosynthetic proteins into their photosynthetic apparatus in a process called FRL-induced photoacclimation (FaRLiP). We have characterized the macromolecular changes involved in FRL-driven photosynthesis and used atomic force microscopy (AFM) to examine the supramolecular organization of photosystem I (PSI) associated with FaRLiP in three cyanobacterial species, *Chroococcidiopsis thermalis* PCC 7203, *Chlorogloeopsis fritschii* PCC 9212 and *Synechococcus* sp. PCC 7335 [MacGregor-Chatwin et al. (2022) Sci. Adv. 8: eabj4437]. Mass spectrometry (MS) analysis determines the changes in the proteome of *C. thermalis* 7203 that accompany FaRLiP, and fluorescence lifetime imaging microscopy and electron microscopy reveal an altered cellular distribution of photosystem complexes and the cell-to-cell variability of the FaRLiP response.

We had previously used AFM to show that ordered macromolecular arrays of trimeric PSI are present in thylakoids from the model species *Thermosynechococcus elongatus*, *Synechococcus* sp. PCC 7002, and *Synechocystis* sp. PCC 6803 [MacGregor-Chatwin et al. (2017) Plant Cell 29:1119-1136]. Quantitative MS analysis of *Synechocystis* thylakoid membranes now reveals the copy number per cell of more than 1000 proteins, including subunits of PSI and other complexes of the photosynthetic electron transport chain [Jackson et al. (2022) Photosynth. Res. under review].

249 In Person Oral

Excitation Energy Transfer Processes in the Photosystem II Supercomplex

Thanh Nhut Do[1], Hoang Long Nguyen[1], Parveen Akhtar[2], Kai Zhong[3], Thomas L. C. Jansen[3], Jasper Knoester[3], Stefano Caffarri[4], Petar Lambrev[2], Howe-Siang Tan[1]

[1]Nanyang Technological University, Singapore, Singapore. [2]Biological Research Center, Szeged, Hungary. [3]University of Groningen, Zernike Institute for Advanced Materials, Groningen, Netherlands. [4]Aix Marseille Université, Marseille, France

The Photosystem II (PSII) supercomplexes exist in a dimeric form with the core complex (CC) surrounded by several peripheral antenna complexes that harvest sunlight and transfer the excitation energy into the reaction center. Understanding the ultrafast excitation energy transfer (EET) dynamics within PSII is important to get further insights about the overall photosynthetic mechanism [Croce R. and van Amerongen H. (2020) Science 369: eaay2058]. We report on our recent Ultrafast Two-Dimensional Electronic Spectroscopy (2DES) studies of the various subdomains of the PSII supercomplex: (1) The ultrafast EET dynamics in the LHCII-CP29-CP24 subdomain of PSII. With comparison study of LHCII, we show that the 'bottleneck' states in LHCII exhibit faster effective dynamics in the LHCII-CP24-CP29 assembly. Simulations indicate that this is due to inter-complex EET and/or structural distortion of LHCII in the bigger assembly [Do T.N. et al. (2022) J. Phys. Chem. Lett. 13:4263-4271]. (2) The EET dynamics of the LHCII-CP26-CC subdomain. We directly observe the tens of picosecond EET process from the LHCII-CP26 antenna complexes to the CC.

250 Pre Recorded Oral

Cryo-EM structure of the photosynthetic complex from *Gemmatimonas phototrophica* at 2.4 Å

Michal Koblizek[1], Alastair T. Gardiner[1], Nupur[1], Pavel Hrouzek[1], Marek Kuzma[2], Tomas Polivka[3], Christopher J. Russo[4], C. Neil Hunter[5], Pu Qian[6]

[1]Center Algatech, Institute of Microbiology, Trebon, Czech Republic. [2]Lab of Molecular Structure, Prague, Czech Republic. [3]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic. [4]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom. [5]Dept of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield, United Kingdom. [6]Thermo Fisher Scientific, Eindhoven, Netherlands

Phototrophic Gemmatimonadetes evolved the ability to use solar energy following horizontal transfer of photosynthesis-related genes from an ancient phototrophic proteobacterium [1]. The electron cryomicroscopy structure of the *Gemmatimonas phototrophica* photosystem at 2.4 Å reveals a unique, double-ring complex. Two unique membrane-extrinsic polypeptides, RC-S and RC-U, hold the central type-2 reaction center within an inner 16-subunit light-harvesting 1 (LH1) ring, which is encircled by an outer 24-subunit antenna ring (LHh) that adds light-gathering capacity. Each LH subunit is populated by one novel ketocarotenoid called gemmatoxanthin [2]. The outer LHh antenna contains 24 bacteriochlorophyll molecules absorbing at 800 nm and 24 bacteriochlorophyll pairs absorbing at 816 nm. The inner antenna is formed by 16 bacteriochlorophyll pairs absorbing at 868 nm. Since the pigments in the outer ring have higher energy than the pigments in the inner antenna the whole complex serves as a funnel. The energy absorbed by the pigments in the LHh antenna is transferred within several picoseconds down the energy gradient to the reaction center. This structural and functional study shows that *Gem. phototrophica* has independently evolved its own compact, robust and highly effective architecture for harvesting and trapping solar energy [3].

[1] Zeng et al. (2014) Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. Proc. Natl. Acad. Sci. USA 111:7795-7800.
[2] Nupur et al. (2021) Structure elucidation of the novel carotenoid gemmatoxanthin from the photosynthetic complex of *Gemmatimonas phototrophica* AP64. Sci. Rep. 11: 15964.
[3] Qian P et al. (2022) 2.4-Å structure of the double-ring *Gemmatimonas phototrophica* photosystem. Sci. Adv. 8: eabk3139. https://doi.org/10.1126/sciadv.abk3139

251 Pre Recorded Oral

The costs and benefits of photorespiration

Florian Busch

University of Birmingham, Birmingham, United Kingdom

Photorespiratory metabolism is essential for plants to maintain functional photosynthesis in an oxygen-containing environment. Because it is associated with the loss of previously fixed carbon, photorespiration is often considered a wasteful process, making the photorespiratory pathway a popular target for improving photosynthesis in crop plants. However, this pathway has also many positive aspects, as it is well integrated within other metabolic processes such as nitrogen assimilation and the C_1 metabolism. As such, the photorespiratory pathway does not only supply valuable metabolites, but does so – at least under some conditions – with a net carbon benefit to the plant. This prompts the question how costly photorespiration is to the plant, and whether it might even be beneficial. To tally up the net cost/benefit of photorespiration a key aspect to consider is the diffusion of CO_2 through the stomata and the mesophyll. Low diffusion conductances, such as much closed stomata, increase the internal recycling of photorespiratory CO_2 and therefore decrease the relative impact of photorespiration on net carbon uptake. I will outline the distinction between Rubisco oxygenation and photorespiratory CO_2 release and how to quantify both as a basis to evaluate the costs and benefits of photorespiration.

252 Pre Recorded Oral

Dynamic regulation of photosynthesis in unicellular and filamentous cyanobacteria by flavodiiron proteins

Yagut Allahverdiyeva

University of Turku, Turku, Finland

Cyanobacteria and microalgae evolved several auxiliary electron transport (AET) pathways that allow photosynthetic cells to survive in fluctuating natural habitats. Flavodiiron proteins (FDPs, Flv) function as a powerful sink of excess electrons downstream of Photosystem I, thus safeguarding photosynthesis. *Synechocystis* sp. PCC6803 possess 4 isoforms of FDPs: while the SynFlv1/Flv3 hetero-oligomer is mainly responsible for the initial fast and transient O_2 photoreduction during a sudden increase in light intensity, SynFlv2/Flv4 catalyses steady O_2 photoreduction under illumination at air-level CO_2. Importantly, the single knockout of any FDP strongly diminishes the O_2 photoreduction, indicating that the hetero-oligomeric FDP forms working in an interdependent manner. Moreover, SynFlv3 homooligomers, being unable photoreduce O_2 *in vivo*, serve an as of yet unknown physiological function. Absence of SynFlv1/3 results in impaired oxidation of the Fd pool in dark-adapted cells upon illumination, while NADPH redox kinetics are unaltered. This suggests that Fd is the primary electron donor to SynFlv1/3 hetero-oligomers *in vivo*.

Heterocystous, N_2-fixing *Anabaena* sp. PCC7120 possesses 6 genes encoding FDPs. The two additional Anabaena FDP proteins, AnaFlv1B and AnaFlv3B, are exclusively localized in the heterocysts. AnaFlv3B mediates O_2 photoreduction as a homo-oligomer, playing an important role in maintaining micro-oxic conditions inside heterocysts under illumination, which is crucial for functioning of oxygen-sensitive nitrogenase. We demonstrate that, different from SynFlv3, vegetative-cell specific AnaFlv3A is responsible for moderate O_2 photoreduction independently of AnaFlv1A, but only in presence of AnaFlv2 and AnaFlv4. Strikingly, the lack of vegetative-cell specific AnaFlv3A resulted in strong downregulation of the heterocyst-specific uptake hydrogenase, which led to enhanced H2 photoproduction under both oxic and micro-oxic conditions. These results reveal a novel regulatory network between the Mehler-like reaction and the diazotrophic metabolism, which is of great interest for future biotechnological applications.

253 In Person Oral

The initial events in photochemical charge separation in homodimeric and heterodimeric Type I reaction centers

John Golbeck

Penn State University, University Park, PA, USA

The mechanism of initial charge separation in photosynthetic reaction centers (RCs) remains the topic of intense study. All known RCs, Type I and Type II, consist of a core of six chlorin, (iso)bacteriochlorin or pheophytin molecules arranged in two quasi-symmetrical branches with either perfect or pseudo C_2 symmetry. With the availability of 3-dimensional structures for heterodimeric Type I RCs from cyanobacteria and plants and more recently for homodimeric Type I RCs from *Heliomicrobium modesticaldum*, *Chlorobaculum tepidum*, and *Chloracidobacterium thermophilum*, we are in a position to find commonalities among Type I RCs as well as to compare them with Type II RCs. It has recently been recognized that in both homodimeric and heterodimeric Type I RCs, the relative orientation and distances between the secondary and tertiary (bacterio)chlorophyll molecules classify them as a dimer, and that combined, they constitute the acceptor 'A_0'. To probe the electronic structure of the core chlorophyll(s) in the A-branch of Photosystem I, our group and K.V. Lakshmi (RPI) employed two-dimensional hyperfine sublevel spectroscopy in conjunction with density functional theory to show electron delocalization over both Chl_{2A} and Chl_{3A} when RCs are in the state P_{700} A_{0A}^-. In a fs and ps time-resolved study, our group and D. Cherepanov and A. Semenov (Moscow State) showed that P_{700} is electronically coupled with Chl_{2A} and Chl_{2B} to produce a symmetric tetrameric exciplex $Chl_{2A}P_AP_BChl_{2B}$ in which the excited state $(Chl_{2A}P_AP_BChl_{2B})^*$ is mixed with two charge-transfer states $P_{700}^+Chl_{2A}^-$ and $P_{700}^+Chl_{2B}^-$ before generating the charge-separated states $P_{700}^+A_{0A}^-$ and P_{700}^+ A_{0B}^-. Thus, a thermodynamically driven, smooth migration of electron density occurs after photon absorption. We suggest that the presence of highly coupled donors and acceptors allows for extensive delocalization of the electron density that prolongs the lifetime of the charge-separated state, thereby contributing to the extraordinarily high quantum yield of 1.0.

254 Pre Recorded Oral

Molecular asymmetry of the photosynthetic supercomplex in *Chlorobaculum tepidum*

Ryan Puskar[1], Chloe Du Truong[1], Kyle Swain[1], Shan Li[2], Kai-Wen Cheng[2], Ting Yu Wang[2], Yu-Ping Poh[1], Yuval Mazor[1], Haijun Liu[3], Tsui-Fen Chou[2], Brent Nannenga[1], Po-Lin Chiu[1]

[1]Arizona State University, Tempe, AZ, USA. [2]California Institute of Technology, Pasadena, CA, USA. [3]Washington University, St. Louis, MO, USA

Green sulfur bacteria (GSB) are photoautotrophs with a highly efficient light-harvesting and transfer system for surviving under low-light conditions. Its photochemical reaction center (RC) features a dimeric architecture for charge separation across the membrane. Trimeric Fenna-Matthews-Olson (FMO) protein complexes attach to the RC cytoplasmic surface and mediate light energy transfer from the light-harvesting chlorosome to the RC. To understand how these proteins organize to assemble a highly efficient machinery, we determined high-resolution structures of the photosynthetic supercomplex from the GSB *Chlorobaculum tepidum* using single-particle cryogenic electron microscopy (cryo-EM). The structure shows two FMO trimers, two cytochrome c subunits (PscC), and small membrane subunits that are assembled on the symmetric RC core (PscA). The structure reveals two novel accessory protein subunits (PscE and PscF), bound with the symmetric RC core, presenting an asymmetric form of the overall supercomplex and implicating a bias in energy transfer along the two FMO-PscA branches. We also discovered a new linker bacteriochlorophyll (BChl) located in one of the two FMO-PscA interfaces, possibly mediating exciton transfer from the FMO trimer to the RC core. Our structure of the GSB photosynthetic supercomplex provides mechanistic insights into the routes for light excitation energy transfer and a possible evolutionary transition intermediate of the bacterial photosynthetic supercomplex from the primitive homodimeric RC.

255 Pre Recorded Poster

Molecular-dynamics simulations of Photosystem II crystals combined with time-resolved XFEL serial crystallography

Margaret Doyle[1], Asmit Bhowmick[1], David Wych[2], Louise Lassalle[1], Philipp Simon[1], James Holton[1,3,4], Nicholas Sauter[1], Vittal Yachandra[1], Jan Kern[1], Junko Yano[1], Michael Wall[2]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [2]Los Alamos National Laboratory, Los Alamos, NM, USA. [3]University of California, San Francisco, San Francisco, CA, USA. [4]SSRL, SLAC National Accelerator Laboratory, Menlo Park, CA, USA

We calculated electron density maps from crystalline molecular dynamics (MD) simulation of Photosystem II (PS II) using the room temperature XFEL structure of the dark stable S1 state as the starting point. The agreement with the XFEL data for the crystallographic water positions around the active site, including inside the various channels, is 85%. From the calculated maps, the O4 and Cl channels are found to have several hydrogen bond networks extending all the way to the bulk water, while the O1 channel contains the most mobile waters on average. Rare events which aid the transport of waters/ions across gaps in the water network of the channels that show up as additional density in our analysis are also identified. By constructing electron density maps using crystalline MD over time and symmetrically equivalent copies, our approach can help interpret time-resolved serial crystallography data and thus build our fundamental understanding of the physical transport processes taking place during the water splitting reaction in PS II. equivalent copies, our approach can help interpret time-resolved serial crystallography data and thus build our fundamental understanding of the physical transport processes taking place during the water splitting reaction in PS II.

256 Pre Recorded Poster

Coupling between the Mn_4Ca cluster and residues within the O1 and O4 proton-transfer/substrate-access pathways as probed with FTIR difference spectroscopy

Richard J. Debus

University of California, Riverside, CA, USA

The extensive network of -bonds surrounding the Mn_4Ca cluster is sensitive to structural changes accompanying the S_1 to S_2 transition. This structural coupling between the cluster and specific domains of this network is manifested as changes to the vibrational modes of amino acid side chains, -bonded H_2O molecules, and the polypeptide backbone [e.g., see Table 1 in Debus R (2021) Biochemistry 60:3841-3855]. The extent of this coupling extends over at least 25 Å, from the D1-E65/D1-R334/D2-E312 triad in the Cl1 (Broad) proton egress/substrate access pathway to D1-N298 near Y_Z. To determine if this structural coupling extends significantly into the H-bond networks that comprise the O4 (Narrow) or O1 (Large) proton egress/substrate access pathways, we analyzed the FTIR difference spectra of PSII core complexes purified from the mutants D1-N338A, CP43-V410N, and CP43-T412A. The residue D1-N338 is located deep within the O4 pathway, whereas the residues CP43-V410 and CP43-T412 are located deep within the O1 pathway. Our data show that the CP43-V410N and CP43-T412A mutations show nearly all of the same spectral alterations that are produced by mutations of residues that are structurally coupled to the Mn_4Ca cluster, whereas the D1-N338A mutation shows none of these spectral alterations. In addition, none of these three mutations alters the vibrational features of weakly -bonded H_2O molecules during the S_2 to S_3, S_3 to S_0, or S_0 to S_1 transitions. We conclude that structural coupling with the Mn_4Ca cluster extends deep into the O1 pathway but not into the O4 pathway and that neither D1-N338, CP43-V410, nor CP43-T412 interacts with the H_2O molecules that deprotonate or form stronger H-bonds during the S_2 to S_3, S_3 to S_0, or S_0 to S_1 transitions.

*Work supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences (Grant DE-SC0005291).

257 Pre Recorded Oral

Quinone-mediated electron transfer in Type II reaction centers

Hiroshi Ishikita

The University of Tokyo, Tokyo, Japan

Photosystem II (PSII) and the photosynthetic bacterial reaction centers form purple bacteria (PbRC) share structural similarities and are classified as Type-II reaction centers. The photoinduced electron transfer is terminated by quinone, Q_B. Q_B accepts two electrons and two protons. In PbRC, the first proton is transferred to the distal carbonyl O site of Q_B from the protein bulk region via Asp-L213 and Ser-L223 [Alexov, E. G. and Gunner, M. R. (1999) Biochemistry 38:8253-8270]. The corresponding proton transfer pathway is conserved as D1-His252 and D1-Ser264 in PSII [Ishikita H. and Knapp E.-W. (2005) J. Am. Chem. Soc. 127:14714-14720]. The second proton is transferred to the proximal carbonyl O site from D1-His215 at the non-heme Fe complex in PSII [Saito K. et al. (2013) Proc. Natl. Acad. Sci. USA 110:954-959] and from His-L190 at the non-heme Fe complex in PbRC [Sugo Y. et al. (2021) Proc. Natl. Acad. Sci. USA 118, e2103203118]. These structurally conserved components play a role in proton-coupled electron transfer. However, some discrepancies are also observed between the two reaction centers: for example: (i) Glu-L212, a key residue that exhibits uptake of 0.3–0.8 H^+ during the electron transfer in PbRC, is replaced with D1-Ala251 in PSII; (ii) Glu-M234 at the non-heme Fe complex in PbRC (*Rhodobacter sphaeroides*) is replaced with bicarbonate in PSII [Eaton-Rye J. J. and Govindjee. (1988) Biochim. Biophys. Acta 935:248-257]. These might be a key to understanding why the Type-II reaction centers show notable structural differences not only on the lumenal oxygen-evolving side but also the stromal electron acceptor side.

258 Pre Recorded Oral

The influence of carbonic anhydrase and phospho*enol*pyruvate carboxylase on CO_2 assimilation in C_4 plants

Asaph Cousins

School of Biological Sciences, Washington State University, Pullman, WA, USA

There are key enzymes involved in the uptake and assimilation of atmospheric carbon dioxide (CO_2) that influence carbon assimilation in both C_3 and C_4 plants. For example, carbonic anhydrase (CA) is generally found in high concentrations in the chloroplast of C_3 plants and has been suggested to be important for facilitating CO_2 availability to Rubisco. Alternatively, CA performs the first enzymatic step of C_4 photosynthesis by catalyzing the reversible hydration of dissolved CO_2 providing the bicarbonate used by phospho*enol*pyruvate carboxylase (PEPC) in the first commented step of the carbon concentrating mechanism (CCM). Our work has shown natural variation in CA and PEPC activity across diverse C_4 grasses that has important implications in coordinating the trade-off in leaf level CO_2 and water exchange. Furthermore, reduced CA activity can limit rates of C_4 photosynthesis at high temperatures, and C_4 plants with approximately 0.2% of wild-type leaf CA activity had reduced photosynthetic parameters and could only survive at elevated pCO_2. These results suggest that leaf CA activity is required to sustain an efficient CCM in C_4 plants. Comparisons of C_4 and non-photosynthetic PEPC isozymes suggest that the enzyme's kinetic properties have undergone selection to function during C_4 photosynthesis. For example, the C_4 PEPC isozyme generally has a lower affinity for phospho*enol*pyruvate (PEP) and different sensitivity to allosteric regulation compared to non-photosynthetic isozymes. Additionally, an increase in PEPC's affinity for bicarbonate has a selective advantage for maintaining high rates of C_4 photosynthesis. Our research has focused on understanding the diversity of PEPCs allosteric regulation and the potential tradeoff between its kinetic properties for PEP and bicarbonate. Collectively, this research demonstrates that variation in CA and PEPC activity can have important impacts on rates of leaf CO_2 assimilation in C_4 plants. This research is supported by U.S. Department of Energy, Grant #DE- SC0001685.

259 Pre Recorded Oral

Regulation vs. acclimation in response to fluctuating light conditions in the moss *Physcomitrium patens*

Alessandro Alboresi, Claudia Beraldo, Eleonora Traverso, Shunling Tan, Antoni Mateu Vera Vives, Mattia Storti, Tomas Morosinotto

Department of Biology, University of Padova, Italy, Padova, Italy

Photosynthesis works in highly variable environmental conditions and multiple mechanisms regulate light use efficiency and photosynthetic electron transport in response to dynamic metabolic and environmental constraints. Acclimation for example involves changes in the composition of the photosynthetic apparatus while regulation that includes processes that alter the activity of protein complexes without changing their abundance.

All photosynthetic organisms have a panel of specific regulatory mechanisms that was defined by their evolutionary trajectories and for this reason the exploration of biodiversity gives valuable insights into their functioning and biological role. Here we will discuss mechanisms of response to light fluctuation using the model moss *Physcomitrium patens*, that diverged from vascular plants early after land colonization, thus providing insights on how photosynthetic organisms adapted to the challenges of new environmental conditions associated with life out of the water.

Multiple mechanisms for responses to light fluctuations have been identified to be active in this organism. Non-Photochemical Quenching (NPQ) which consists in the thermal dissipation, protecting PSII from ROS generation. This protection is enhanced by the xanthophyll cycle, converting violaxanthin into zeaxanthin under strong illumination. Pseudo-cyclic electron flow (PCEF) catalyzed by Flavodiiron proteins, instead, protects PSI from over-reduction under fluctuating light conditions. Mitochondrial respiration also impacts modulation of photosynthetic electron transport and its response.

The modulation of these mechanisms in response to prolonged exposition to different growing conditions, namely light intensities, will also be described to discuss how they are modulated during acclimation.

260 In Person Poster

Towards structure determination of the of the photosynthetic super-complex to understand the regulation of sustained cyclic electron flow (CEF) in the photopsychrophile *Chlamydomonas* sp. UWO241

Jay-How Yang[1], Jackson Carrion[1], Isha Kalra[2], Rachael Morgan-Kiss[2], Petra Fromme[1]

[1]Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA.
[2]Department of Microbiology, Miami University, Oxford, OH, USA

The abstract reports on the structural aspects of the project, which aims to determine the structure of the super-complex with Cryo-EM. The overall goal of this project is to describe the function of sustained cyclic electron flow (CEF) and assembly of the PSI super-complex in the Antarctic psychrophile *Chlamydomonas sp*. UWO241 (UWO241) and the model *Chlamydomonas reinhardtii* acclimated to long-term salinity stress. Major objectives are: 1) determine the functional role of sustained CEF and impacts on downstream carbon metabolism in UWO241 acclimated to variable environmental stressors; 2) dissect the structure of the UWO241 PSI super-complex through proteomic and structural studies; 3) determine whether *C. reinhardtii* utilizes “UWO-like” super-complex to support sustained CEF during long-term stress acclimation. Outcomes of this project will support research focused on meeting future energy and food needs by advancing our understanding of how extremophilic phototrophs use sustained CEF and rewired carbon metabolism to survive long-term exposure to environmental stressors, such as excessive light, low temperature, and high salinity. The single-particle cryo-electron microscopy (cryo-EM) techniques is used to determining the high-resolution structures of the PSI super-complex in the Antarctic *Chlamydomonas sp*. UWO24. We aim to gain detailed information on the interaction of the proteins and cofactors in the super-complex at molecular resolution. The goal is to identify the key amino acids in the formation and stabilization of the super-complex including details on the cofactor interactions that will reveal the electron transfer pathways that drive the cyclic electron transfer in the super-complex.

[Kalra I. (2020) Plant Physiol. 183:588-601]

261 Pre Recorded Oral

The power of spectral sensing for faster identification of high-performing crop cultivars

Caitlin Moore

University of Western Australia, Crawley, WA, Australia. University of Illinois Urbana-Champaign, Urbana, IL, USA

Ensuring cropping systems remain resilient to climate change is a global challenge facing humanity. To mitigate projected crop yield decline due to increased climate pressure, we need to develop better crop varieties that can withstand living in a hotter world. This talk will discuss the power of spectral sensing techniques for building and testing high-throughput phenotyping applications aimed at accelerating the way we detect photosynthesis in the field. Specifically, how hyperspectral cameras and optical spectrometers can be used in combination with existing portable photosynthesis instruments to link with photosynthetic performance in food and fuel crops, such as tobacco, soybean, maize and sorghum. Our ability to detect photosynthesis, particularly differences in photosynthetic performance within crop cultivars and across cropping systems exposed to different climate stressors, will be an important strategy in our efforts to ensure crop yields continue to increase and remain resilient to climate change through this century.

262 Pre Recorded Oral

Architecture, biogenesis, and dynamics of cyanobacterial thylakoid membranes

Luning Liu

University of Liverpool, Liverpool, United Kingdom

Thylakoid membranes are the specialized internal membrane system in plants, algae, and cyanobacteria, which can perform oxygenic photosynthesis to convert sunlight to chemical energy for most life activities on Earth. In cyanobacteria, thylakoid membranes locate between the plasma membrane and the central cytoplasm, leading to intricate cellular compartmentalization. In addition, cyanobacterial thylakoid membranes represent the active sites for both photosynthetic and respiratory electron transport.

In this talk, I will introduce the recent studies on deciphering the native architecture, biosynthesis process, and dynamics of photosynthetically functional thylakoid membranes in cyanobacteria at the molecular detail using multidisciplinary techniques. In particular, we probe the native organization and interactions of photosynthetic protein supercomplexes within cyanobacterial thylakoid membranes using high-resolution atomic force microscopy, and characterize the spatial-temporal stepwise biogenesis process of thylakoid membranes in the model cyanobacterium *Synechococcus elongatus* PCC 7942, using electron microscopy/cryo-electron tomography, fluorescence microscopy, and mass spectroscopy. These studies provide mechanistic insight into how photoautotrophs generate the photosynthetic machinery in nature and manipulate thylakoid membrane organization and function in response to environmental changes, and may inform the design and engineering of artificial photosynthetic systems.

1 Huokko, T. et al. (2021) Probing the biogenesis pathway and dynamics of thylakoid membranes. Nat. Commun. 12: 3475.
2 Zhao, L.S. et al. (2020) Structural variability, coordination, and adaptation of a native photosynthetic machinery. Nat. Plants 6:869-882.
3 Zhao, L.S. et al. (2022) Native architecture and acclimation of photosynthetic membranes in a fast-growing cyanobacterium. Plant Physiol. accepted.
4 Mullineaux, C.W. and Liu, L.N. (2020) Membrane dynamics in phototrophic bacteria. Annu. Rev. Microbiol. 74:633-654.

263 Pre Recorded Oral

Correlating spectroscopy with structure in the oxygen-evolving complex of Photosystem II

Dimitrios Pantazis

Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany

Spectroscopic methods are crucial for studying the intermediate states of the oxygen-evolving complex (OEC) of photosystem II. They report on the electronic/geometric structure of the manganese cluster and on chemical changes that occur in distinct catalytic steps (S-states). Magnetic resonance techniques (such as EPR, ENDOR, EDNMR) are particularly suitable for probing the local and electronic structure of the OEC in all semi-stable S-states, providing rich, sensitive, and targeted information on spin-dependent properties. The exceptionally rich data obtained by these methods describe a dynamic exchange-coupled transition metal cluster that responds sensitively to external perturbations and provides a large variety of spectroscopic signatures. Perplexingly, almost always two or more types of EPR signal can be observed for one and the same S-state. However, the complicated nature of the system and the complexity of the data preclude direct interpretations in terms of precise atomistic models. This task is undertaken by modern spectroscopy-oriented quantum chemistry. Here I will provide an overview of recent and ongoing efforts to create computational structural models that are consistent with spectroscopic data, highlighting specific issues relating to structural heterogeneity in the S1-S2-S3 states, to the potential relevance of intra-state isomerism for catalytic progression, and to the possibilities for reconciling spectroscopic data with current crystallographic models of S-state intermediates.

264 Pre Recorded Oral

Improving Rubisco by consulting ancestors living in higher CO_2

Myat Lin, Vishal Chaudhari, Maureen Hanson

Cornell University, Ithaca, NY, USA

Photosynthetic efficiency of C3 plants suffers from the slow catalytic rate and the reaction of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) with O_2 instead of CO_2, leading to the costly process of photorespiration. In flowering plants, Rubisco found in the chloroplasts is a hexadecamer composed of eight large subunits expressed from a single chloroplast gene and eight small subunits expressed from a multigene family in the nucleus and imported into the chloroplasts for assembly with the large subunits with the aid of multiple chaperones. In order to identify Rubisco enzymes more suitable for CO_2 levels in the present and future atmosphere, we evaluated ancestral Rubiscos that existed when atmospheric CO_2 was higher than in the more recent past. The properties of the predicted ancestral Rubisco enzymes were determined following expression in *E. coli*. Our data indicate that the predicted ancestors of C3 Rubiscos are more efficient and are similar to C4 Rubisco, providing a source of enzymes better adapted to a changed climate [Lin et al. (2022) Science Advances 8(15):eabm6871]. The effect of ancestral Rubiscos on plant growth and photosynthetic efficiency can be tested in tobacco by incorporation of genes for both the large and small subunit into the chloroplast genome.

265 Pre Recorded Poster

Lysine residues on the acceptor side *Cereibacter sphaeroides* allow for selective orientation and a cathodic current

Kamil Woronowicz[1,2], Allen Gong[1,3], Riley McHale[1], Enoch Nagelli[1], Kenneth J. McDonald[1], F. John Burpo[1]

[1]United States Military Academy, West Point, NY, USA. [2]Wagner College, Staten Island, NY, USA. [3]Washington State University, Seattle, WA, USA

Reaction centers (RCs) from *Cereibacter sphaeroides* (also known as *Rhodobacter sphaeroides*) have been previously coupled to electrodes to generate anodic ight-dependent current, with only a few examples of selective orientation allowing cathodic current. Here we present a novel approach incorporating structural analysis of distribution of lysine residues on the surface of RC. Although 2-3 lysine residues are present on each of the 14 dimers of the light harvesting complex 1 (LH1) surounding the RC forming a hockey puck-like structure, they appear to be involved in salt bridges required to stabilize the dimer interface or the inter-dimer interactions. Our hypothesis is that this leaves only the lysine residues on the acceptor side of LH1-RC surface available for attachment onto the graphene oxide electrodes. Potentiometric evidence for this selective orientation is presented. The light-dependent current generated exceeds cathodic currents reported in the literature previously and approaches the robustness of anodic light-dependent currents generated by LH1-RCs observed by several labs. Interestingly, the light-dependent current measured includes a diffusion-limited component sensitive to quinone (Q0) concentration.

266 Pre Recorded Poster

Room temperature XFEL crystallography reveals asymmetry in the vicinity of the two phylloquinones in photosystem I

Hiroki Makita[1], Stephen Keable[1], Philipp Simon[1], Adrian Kolsch[2], Shigeki Owada[3], Jaehyun Park[4], Alexander Batyuk[5], Athina Zouni[2], Nicholas Sauter[1], Vittal Yachandra[1], Junko Yano[1], Jan Kern[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [2]Humboldt-Universität zu Berlin, Berlin, Germany. [3]Japan Synchrotron Radiation Research Institute, Kouto, Japan. [4]Pohang Accelerator Laboratory, Pohang, Korea, Republic of. [5]SLAC National Accelerator Laboratory, Menlo Park, CA, USA

Photosystem I (PSI) is a large multi-subunit pigment-protein complex in oxygenic photosynthetic organisms that catalyzes light-driven electron transfer reactions across thylakoid membrane. PSI has a symmetric structure with two highly similar branches of pigments at the center that are involved in electron transfer. Despite its structural similarity, yield and kinetics of electron transfer along these two branches differ significantly. To understand the origin of bidirectionality and biphasicity of PSI electron transfer, we have determined the structure of cyanobacterial PSI at room temperature (RT) using femtosecond X-ray pulses from X-ray free electron laser (XFEL). Compared to previous cryogenic structures, the RT structure shows a clear expansion of the entire protein complex in the direction of the membrane plane. At the reaction center, we observe conformational differences between the two branches near the secondary electron acceptors A_{1A} and A_{1B}, which are both phylloquinones. In the A-branch, the Phe residue is oriented parallel to the quinone rings, imposing a pi-stacking interaction. In the B-branch, however, the equivalent Phe residue is rotated to an almost perpendicular confirmation with respect to the quinone ring. Additionally, the symmetry breaking Trp positioned between A_{1A}, A_{1B}, and F_X is tilted further away from A_{1A} compared to the cryogenic structures. These changes increase the asymmetry between the branches and may provide insight to the preferential directionality of electron transfer.

267 Pre Recorded Oral

The role of VIPP1 in thylakoid biogenesis and maintenance

Tilak Gupta[1], Sven Klumpe[1], Karin Gries[2], Steffen Heinz[3], Wojciech Wietrzynski[4], Norikazu Ohnishi[5], Wataru Sakamoto[5], Jörg Nickelsen[3], Jan Schuller[6], Michael Schroda[2], Benjamin Engel[4]

[1]Max Planck Institute of Biochemistry, Martinsried, Germany. [2]Technical University of Kaiserslautern, Kaiserslautern, Germany. [3]LMU Munich, Munich, Germany. [4]Biozentrum, University of Basel, Basel, Switzerland. [5]Okayama University, Okayama, Japan. [6]Philipps-University Marburg, Marburg, Germany

In this talk, I will describe our group's recent studies of Vesicle-Inducing Protein in Plastids 1 (VIPP1), the "photosynthetic ESCRT-III" [Gupta et al. (2021) Cell 184:3643-3659.e23]. VIPP1 is a conserved membrane-remodeling protein that builds the thylakoid membranes of cyanobacteria, algae, and plants. These photosynthetic membranes harness the power of sunlight to generate the biochemical energy and oxygen that sustain most of the life on Earth. For decades, it has remained a mystery how VIPP1 performs its vital membrane-remodeling functions. We used cryo-electron microscopy to determine structures of cyanobacterial VIPP1 rings, revealing how VIPP1 monomers flex and interweave to form basket-like assemblies of different symmetries. Three VIPP1 monomers together coordinate a non-canonical nucleotide binding pocket on one end of the ring. Inside the ring's lumen, amphipathic helices from each monomer align to form large hydrophobic columns, enabling VIPP1 to bind and curve membranes. In vivo mutations in these hydrophobic surfaces cause extreme thylakoid swelling under high light, indicating an essential role of VIPP1 lipid binding in resisting stress-induced damage. Using cryo-correlative light and electron microscopy (cryo-CLEM), we observed oligomeric VIPP1 coats encapsulating membrane tubules within the native *Chlamydomonas* chloroplast, and even caught VIPP1 in the act of transferring lipid between the chloroplast envelope and thylakoids. Our work provides a structural foundation for exploring how VIPP1 directs thylakoid biogenesis and maintenance.

Gupta T.K., Klumpe S., Gries K., Heinz S., Wietrzynski W., Ohnishi N., Niemeyer J., Spaniol B., Schaffer M., Rast A., Ostermeier M., Strauss M., Plitzko J.M., Baumeister W., Rudack T., Sakamoto W., Nickelsen J., Schuller J.M., Schroda M., Engel B.D. (2021). Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. Cell 184:3643-3659.e23.

269 Pre Recorded Oral

Understanding and altering NPQ: from ecophysiology to gene editing

Dhruv Patel-Tupper[1], Armen Kelikian[1], Anna Leipertz[1], Michelle Tjahjadi[2], Myeong-Je Cho[2], Krishna Niyogi[1,2,3,4]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA. [2]Innovative Genomics Institute, University of California, Berkeley, CA, USA. [3]Howard Hughes Medical Institute, Berkeley, CA, USA. [4]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Photosynthetic light harvesting in plants is regulated by non-photochemical quenching (NPQ) mechanisms that safely dissipate excess absorbed sunlight. Ecophysiological studies have identified several types of NPQ that operate on different timescales. Changes in NPQ can be fast but are not instantaneous, so they often lag behind fluctuations in light intensity that occur in nature and lead to losses in photosynthetic efficiency because of competition between NPQ and photochemistry. We have used molecular genetics and ultrafast spectroscopy to understand how NPQ mechanisms work in model organisms. More recently, we have used this fundamental knowledge to accelerate NPQ kinetics through transgenic expression of three NPQ-related proteins (VDE, PsbS, and ZEP), and in some plants this has increased photosynthetic efficiency and crop productivity. Because all plants have genes encoding these three proteins, we are investigating whether we can use gene editing to generate transgene-free crop plants with altered NPQ kinetics and increased productivity.

270 Pre Recorded Oral

The genetics of photosynthesis efficiency, what can we learn from nature to improve crop photosynthesis?

Thu-Phuong Nguyen[1], Tom Theeuwen[1], Roel van Bezouw[1], Francesco Garassino[1], Louise Logie[1], René Boesten[1], Jeremy Harbinson[2], Mark G. M. Aarts[1]

[1]Laboratory of Genetics, Wageningen University & Research, Wageningen, Netherlands. [2]Laboratory of Biophysics, Wageningen University & Research, Wageningen, Netherlands

The ability to perform photosynthesis is a major driver of plant evolution. Despite a very long history of selection for optimal photosynthesis, evolution is still ongoing, as evidenced by the genetic variation for photosynthesis efficiency that can be observed when comparing different plant species or different genotypes of the same species. Such variation may be selected for, to improve crop photosynthesis and, potentially, to improve crop yield. We have explored the opportunities to characterize the genetic variation for photosynthesis efficiency in the model species *Arabidopsis thaliana*. One of the challenges in investigating such genetic variation is the ability to adequately phenotype photosynthesis parameters. For this purpose, we developed and used the Phenovator, a phenotyping platform for high-throughput imaging of light use efficiency of photosystem II electron transport (ΦPSII or Fq'/Fm') through chlorophyll fluorescence measurements. It demonstrated to be very efficient in phenotyping several, large, genetically segregating *A. thaliana* populations and diversity panels, under different growth conditions. The observed genotypic variation was used to identify quantitative trait loci (QTL) for ΦPSII, reflecting genetic variation residing on the nuclear genome. In addition, we have investigated the effect of variation residing on the chloroplast genome of *A. thaliana*, through analysis of so-called cybrids, or cytoplasmic swaps, which carry new combinations of nuclear and cytoplasmic genomes, not found in nature. Because *A. thaliana* is not a particularly high-photosynthesis species, we recently initiated the research of *Hirschfeldia incana*, a related Brassicaceae species, with a much higher maximum rate of CO_2 assimilation than *A. thaliana*.

271 In Person Poster

Characterisation of a spontaneous revertant of the *Synechocystis* sp. PCC 6803 mutant lacking the Photosystem II proteins PsbV and CyanoQ

Kevin J. Sheridan[1,2], Lisa Biiri[1,2], Briony Smith[1], James Gorrie[1], Julian J. Eaton-Rye[2] and Tina C. Summerfield[1]

[1]Department of Botany, University of Otago, Dunedin, NZ, [2]Department of Biochemistry, University of Otago, Dunedin, NZ

A Photosystem II double mutant lacking PsbV and CyanoQ was unable to grow photoautotrophically at pH 7.5, but growth was restored at pH 10. Maintaining the ΔPsbV:ΔCyanoQ strain at pH 7.5 enabled in isolation of a pseudorevertant strain. This pseudorevertant strain exhibited photoautotrophic growth at pH 7.5. When cells were grown in BG-11 medium buffered at pH 7.5, assembly PSII centers in the pseudorevertant was increased compared to the ΔPsbV:ΔCyanoQ strain. Furthermore, fluorescence induction showed restoration of the P rise in the pseudorevertant compared to original ΔPsbV:ΔCyanoQ strain and rates of oxygen evolution were higher. We investigated the genetic basis of restoration of the growth by sequencing the genome of the pseudorevertant strain. Mutations in the pseudorevertant strain included a point mutation that altered an amino acid residue in a thioredoxin. Comparison of 200 cyanobacterial genomes showed a conserved amino acid was altered in the pseudorevertant. We investigated the role of this thioredoxin by introducing the pseudorevertant copy of the gene into the ΔPsbV:ΔCyanoQ strain.

272 In Person Poster

Investigating D2 protein diversity in *Prochlorococcus* spp. strains using the model strain *Synechocystis* sp. PCC 6803

Kevin J. Sheridan[1,2], Joshua T. Clegg[1,2], Julian J. Eaton-Rye[2] and Tina C. Summerfield[1]

[1]Department of Botany, University of Otago, Dunedin, NZ, [2]Department of Biochemistry, University of Otago, Dunedin, NZ

The reaction centre of Photosystem II is formed by the D1 and D2 heterodimer. The majority of the cofactors bound to the heterodimer are associated with D1. However, the quinone, Q_A is bound to D2. Many cyanobacteria have two copies of the *psbD* gene which typically encode identical D2 proteins. In contrast, *Prochlorococcus* strains contain one *psbD* copy. Genome comparisons indicate the D2 protein of some *Prochlorococcus* strains contains a 7 amino acid insertion in the DE-loop of D2. The insert was noted to be present in strains lacking the extrinsic proteins PsbU and PsbV [Ting et al. (2009) Photosynth. Res. 101:1–19]. Our analysis shows the majority of sequenced *Prochlorococcus* strains (>40) have an insertion in D2 and lack PsbU, PsbV and CyanoQ. Analysis of phylogenetically diverse cyanobacteria shows this D2 insertion is only found in *Prochlorococcus* strains and only these strains lack both PsbU, PsbV and CyanoQ. We identify two insertions sequences: one found in high light *Prochlorococcus* ecotypes, and one found in low light ecotypes. Using the strain *Synechocystis* sp. PCC 6803 we are investigating the role of the DE-loop of D2 and whether the requirement for the extrinsic proteins is altered by the addition of the insertion in this loop.

273 Pre Recorded Oral

Diversity in crop plant photosynthetic responses to shade

Sam Taylor

Lancaster University, Lancaster, UK

Rubisco activity responds to shading of leaves, an inevitable and frequent reality in sunlit crop canopies. Rubisco activity is maximised in saturating light and decreases in shade, but while transitions between sunlight and shade in crop canopies are effectively instantaneous, changes in Rubisco activity depend on slower responding aspects of chloroplast biochemistry. This means there are biochemistry-dependent lags between changes in light and Rubisco activity and suggests opportunities for identifying natural variation relevant to e.g., crop improvement. The complexity of leaf and chloroplast physiology makes characterising these opportunities difficult, but our research in cowpea suggests promising new targets. Using results from brassicas and cowpea, I will show how we are identifying new opportunities for improving photosynthesis.

274 Pre Recorded Oral

Improving the Calvin Benson cycle to increase crop yields

Professor Christine Raines

University of Essex, Colchester, UK

The year on year increases in crop yield that have been achieved over the last 50 years are now reaching a plateau in many of our major crops. Given the continued growth in the world population, estimated to be 9 billion by 2050, it is essential that new solutions are found to produce higher yielding crops. Over the 5-10 years evidence has accumulated demonstrating that improving the photosynthetic process is one approach that can contribute to increasing in crop yields. The work in my laboratory has focussed on approaches to improve the Calvin Benson cycle either directly or indirectly using genetic engineering strategies. We have had a number of successes in the greenhouse and in the field, although not all of the manipulations work in all species under all growing conditions. I will provide an overview of our past and current work and consider future directions.

275 Pre Recorded Oral

Targeted metabolite profiling of C_3, intermediates and C_4 plant species to uncover diversity in the Calvin-Benson cycle

Stephanie Arrivault

Max Planck Institute of Molecular Plant Physiology. Potsdam, Germany

The Calvin-Benson cycle (CBC) evolved over 2 billion years ago and there is evidence for variation in how the CBC operates (for example between photosynthetic organisms that do and don´t have a CO_2 concentration mechanism, CCM). While most studies of CBC diversity have focused on single aspects such as Rubisco kinetics and regulation, we are using quantitative CBC metabolite profiling as a top-down strategy to uncover inter-species diversity in CBC operation.

To investigate how CBC operation was modified during the evolutionary progression from C_3 to C_4 photosynthesis, we compared CBC metabolite profiles in nine *Flaveria* species, and several C_3 and C_4 species from outside the genus.

The C_3 *Flaveria* species have a CBC profile close to that of the other C_3 species examined, especially rice and wheat. Intermediate *Flaveria* species lie on the path between C_3 and C_4 species, with the C_3–C_4 intermediate species being closer to C_3 species and C_4-like species closer to complete C_4 species. These results indicate that CBC operation had already started to adapt to enhanced internal CO_2 status in the C_3–C_4 *Flaveria* species and that this adaptation progressed furthest in C_4-like and C_4 species. Additional analyses will be presented to point out the specific differences in CBC operation between the species investigated.

276 In Person Oral

A cross-scale analysis to understand and quantify effects of photosynthetic enhancement on crop growth and yield

Alex Wu[1], Jason Brider[1], Florian A. Busch[2,3,4], Min Chen[5], Karine Chenu[1], Victoria C. Clarke[2], Brian Collins[1], Maria Ermakova[2], John R. Evans[2], Graham D. Farquhar[2], Britta Forster[2], Robert T. Furbank[2], Michael Gorszmann[2], Miguel A. Hernandez[5], Benedict M. Long[2], Greg Mclean[1], Andries Potgieter[1], G. Dean Price[2], Robert E. Sharwood[6], Michael Stower[1], Erik van Oosterom[1], Susanne von Caemmerer[2], Spencer M. Whitney[2], Graeme L. Hammer[1]

[1]ARC Centre of Excellence for Translational Photosynthesis, Centre for Crop Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland, Australia. [2]ARC Centre of Excellence for Translational Photosynthesis, Division of Plant Science, Research School of Biology, The Australian National University, Acton, Australian Capital Territory, 2601, Australia. [3]School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK. [4]Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK. [5]ARC Centre of Excellence for Translational Photosynthesis, School of Life and Environmental Science, Faculty of Science, University of Sydney, Sydney NSW2006, Australia. [6]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, 2154, Australia

Photosynthetic manipulation provides new opportunities for enhancing crop yield. However, understanding and quantifying effectively how the seasonal growth and yield dynamics of target crops might be affected over a wide range of environments is limited. Using a state-of-the-art cross-scale model we predicted crop-level impacts of a broad list of promising photosynthesis manipulation strategies for C_3 wheat and C_4 sorghum. The manipulation targets have varying effects on the enzyme-limited (A_c) and electron transport-limited (A_j) rates of photosynthesis. In the top decile of seasonal outcomes, yield gains with the list of manipulations were predicted to be modest, ranging between 0 and 8%, depending on the crop type and manipulation. To achieve the higher yield gains, large increases in both A_c and A_j are needed. This could likely be achieved by stacking Rubisco function and electron transport chain enhancements or installing a full CO_2 concentrating system. However, photosynthetic enhancement influences the timing and severity of water and nitrogen stress on the crop, confounding yield outcomes. Strategies enhancing A_c alone offers more consistent but smaller yield gains across environments, A_j enhancement alone offers higher gains but is undesirable in less favourable environments. Understanding and quantifying complex cross-scale interactions between photosynthesis and crop yield will challenge and stimulate photosynthesis and crop research.

277 Pre Recorded Poster

Unique compositions of the light-harvesting antenna systems in Mesostigma viride which is the earliest known divergent streptophytes.

Atsushi Takabayashi, Michiki Aso, Renon Matsumae, Shinsa Kameo, and Ryouichi Tanaka
Institute of Low Temperature Science, Hokkaido University, N19 W8 Kita-Ku, Sapporo 060-0819, Japan

Green algae comprise two major lineages: streptophytes and chlorophytes, the former of which consists of charophytes and land plants. It is widely accepted that land plants evolved from a single lineage of charophytes. To understand the evolutionary divergence of the light-harvesting antenna systems during the evolution of streptophytes, we identified light-harvesting chlorophyll-a/b complex (LHC) proteins in Mesostigma viride which is one of the earliest known divergent stretophytes by Iso-form sequencing using a PacBio sequel next-generation sequence and compared its composition with those of a model charophyte Klebsormidium flaccidum and land plants. We found that M. viride possesses three LHCs (LHCP, algae-type LHCA2 and LHC9) that are not found in other streptophytes but are found in chlorophytes, in addition to the LHC proteins common to K. flaccidum and land plants. On the other hand, LHCB6, an LHC protein conserved among land plants, was only identified in K. flaccidum, but not in M. viride. These data suggested that the loss of the three LHCs and the acquisition of LHCB6 occurred after M. viride was diverged from the other streptophytes. Subsequently, we analyzed the composition of M. viride photosystems by clear-native-PAGE and LC-MS/MS. The results suggested that algae-type LHCA2 and LHCA9 bound PSI. We also found that LHCP bound PSII, which is in contrast to a previous study showing that LHCP seemed to be bound to PSI in Ostreococcus tauri These data suggests that M. viride has unique photosystems that are possibly inherited from the common ancestral green algae.

278 Pre Recorded Oral

Revealing the sites of chlorophylls *d* and *f* in far-red light-acclimated photosystem II

Christopher J. Gisriel[1], Gaozhong Shen[2], Ming-Yang Ho[2,3], Vasily Kurashov[2], David A. Flesher[4], Jimin Wang[4], Muhamed Amin[5], John H. Golbeck[2,6], M. R. Gunner[7], Tanai Cardona[8], Gary W. Brudvig[1,4], and Donald A. Bryant[2]

[1]*Department of Chemistry, Yale University, New Haven, CT 06520, USA.*
[2]*Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.*
[3]*Department of Life Science, National Taiwan University, Taipei 10617, Taiwan.*
[4]*Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.*
[5]*Department of Sciences, University of Groningen, 9718 BG Groningen, The Netherlands.*
[6]*Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA.*
[7]*Department of Physics, City College of New York, New York, NY 100031, USA.*
[8]*Department of Life Sciences, Imperial College London, London SW7 2AZ, UK.*

Far-red light (FRL) photoacclimation (FaRLiP) provides a selective growth advantage for some terrestrial cyanobacteria by allowing photons in the far-red wavelength range (700-800 nm) to initiate electron transfer in the photosystems. Photosystem II (PSII) is altered during FaRLiP; PsbA (D1), PsbB (CP47), PsbC (CP43), PsbD (D2), and PsbH are replaced by alternate subunit isoforms and five of the sites that normally contain chlorophyll (Chl) *a* instead contain Chls *d* or *f*. The new Chls effectively lower the energy previously thought to define the "red limit" for light required to drive photochemical catalysis of water oxidation. Expanding the spectral range that can be used by PSII could enhance crop production; therefore, understanding the structural basis of FRL-absorbing PSII, especially determining the sites occupied by Chls *d* and *f*, is highly desirable. To achieve this, we have solved molecular structures of FRL-absorbing PSII from *Synechococcus* sp. PCC 7335 using cryo-electron microscopy (cryo-EM)[1]. Due to the minor structural differences between Chls *a*, *d*, and *f*, we developed quantitative methods for distinguishing different Chl types in cryo-EM maps[2]. These allowed us to confidently assign a Chl *d* molecule in the Chl_{D1} site of the electron transfer chain, three Chl *f* molecules bound to the PsbB subunit (CP47), and one Chl *f* molecule bound to the PsbC subunit (CP43) of FRL-absorbing PSII. Based on robust sequence alignments, it is likely that the Chl *d* molecule in the electron transfer chain, and at least one Chl *f* molecule in each of the major antenna-coordinating subunits, are conserved in FRL-absorbing PSII among all FaRLiP-capable cyanobacteria[3]. The structures of FRL-absorbing PSII provide a basis for understanding the lower energy limit required to drive water oxidation, which is the gateway for most solar energy utilization on Earth.

References

1. Gisriel, C. J. *et al.* Structure of a monomeric photosystem II core complex from a cyanobacterium acclimated to far-red light reveals the functions of chlorophylls *d* and *f*. *J. Biol. Chem.* **298**, 101424 (2022).
2. Gisriel, C. J. *et al.* Quantitative assessment of chlorophyll types in cryo-EM maps of photosystem I acclimated to far-red light. *BBA Adv.* **1**, 100019 (2021).
3. Gisriel, C. J., Cardona, T., Bryant, D. A. & Brudvig, G. W. Molecular evolution of far-red light-acclimated photosystem II. *Microorganisms* **10**, (2022).

279 Pre record

Steady state models of C4 photosynthesis

Chandra Bellasio[1,2]

[1]Australian National University, Canberra, ACT, Australia; [2]University of the Balearic Islands, Palma, Balearic Islands, Spain

Models are simplified descriptions of systems. Mathematical models are widely applicable because they output numbers. Mechanistic models are modular, meaning that systems are subdivided in processes, further resolved with any desired level of detail; they allow to determine the cause of an observed phenotype, and measure how much it is not known about it, but have assumptions limiting their validity. Stock and flow models are mechanistic models that describe the evolution of systems over time; they are computationally demanding and generally cannot be solved analytically. Under the assumption that a system has stabilised, it is possible to derive simpler steady state models, which can generally be solved analytically, therefore are easier to use and suitable as submodels for large scale simulations. The basic empirical model of Prioul and Chartier (1977) describes assimilation as a function of CO2 concentration in the substomatal cavity; because it does not have assumptions it can be useful to compare across photosynthetic types. The C4 model of Berry and Farquhar (1978) was developed to study the CO2 concentrating pump and photorespiration suppression; it has submodels of light reactions producing ATP, ATP and enzyme limited C4 metabolism. The model of Bellasio and Farquhar (2019) is a sophisticated tool developed to study energetics; it has a submodel of light reactions producing ATP and NADPH, ATP, NADPH, and enzyme limited metabolism, with variable activity of the C2 and C4 cycles, and a hydromechanical/biochemical stomatal function. The model of Bellasio (2017) is a platform to study the fluxes, reaction rates, ATP and NADPH demand of any type of photosynthetic metabolism, based solely on stoichiometric constraints. The model of Bellasio and Ermakova (2022) links the optical properties of leaf compartments with electron transport, the relative rate of ATP and NADPH generation, fluxes through the carbon metabolism and between leaf compartments.

280 Pre record

Modulation of Photosynthetic Potential of Arabidopsis thaliana by the C4 Carbonic Anhydrase

Deepika Kandoi,[1] Kamal Ruhil,[1] Govindjee Govindjee,[2] Baishnab C Tripathy[1]

[1]Jawaharlal Nehru University, New Delhi, New Delhi, India; [2]University of Illinois, Urbana, Champaign, India

An important method to improve photosynthesis in C3 crops, such as rice and wheat, is to transfer efficient C4 characters to them. Here, cytosolic carbonic anhydrase (CA: βCA3) of the C4 Flaveria bidentis (Fb) was overexpressed under the control of 35S promoter in Arabidopsis thaliana, a C3 plant, to enhance its photosynthetic efficiency. Overexpression of CA resulted in a better supply of the substrate HCO3- for the endogenous phosphoenolpyruvate carboxylase in the cytosol of the overexpressers, and increased its activity for generating malate that feeds into the tricarboxylic acid cycle. This provided additional carbon skeleton for increased synthesis of amino acids aspartate, asparagine, glutamate and glutamine. Increased amino acids contributed to higher protein content in the transgenics. Further, expression of FbβCA3 in Arabidopsis led to a better growth due to expression of several genes leading to higher chlorophyll content, electron transport and photosynthetic carbon assimilation in the transformants. Enhanced CO2 assimilation resulted in increased sugar and starch content, and plant dry weight. In addition, transgenic plants had lower stomatal conductance, reduced transpiration rate and higher water use efficiency. These results, taken together, show that expression of C4 CA in the cytosol of a C3 plant can indeed improve its photosynthetic capacity with enhanced water use efficiency.