



**ENCAPP
2016**

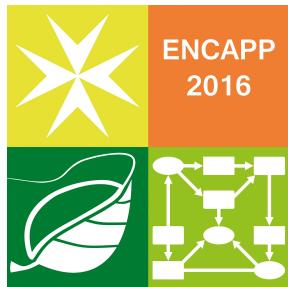
**European
Networks
Conference on
Algal and
Plant
Photosynthesis
2016**

BOOK OF ABSTRACTS



26th to 29th of April 2016

Qawra, Malta



European Networks Conference on Algal and Plant Photosynthesis

Welcome! Merħba!

It is our great pleasure to personally welcome each of you to the European Network Conference on Algal and Plant Photosynthesis (ENCAPP2016) on the beautiful island of Malta. For us, the coordinators of the two Marie-Curie Initial Training Networks (ITNs) PHOTO.COMM and AccliPhot, this event represents an exciting culmination of the scientific activities in our networks. As the final conference of our consortia, we bring together a cohort of young researchers with world leading experts in the field of photosynthesis and microalgal biotechnology research.

This conference has attracted attendees from over 30 countries, working on diverse aspects of algal and plant photosynthesis, synthetic biology and communities. The diversity is reflected in our program, which includes various sessions ranging from electron transport, chloroplast signaling, metabolism, metabolic engineering, to industrial aspects of algal cultivation. An interdisciplinary approach to science is the core foundation of both ITNs which is emphasized in the program by including sessions devoted to the development of mathematical models and addressing innovative advances in biotechnology.

In addition to the scientific program, we have planned a series of social events and get-togethers, because informal discussions and face-to-face meetings outside the formal conference schedule is one of the most important aspects of every scientific meeting. We sincerely hope you will enjoy the scenery and the atmosphere and use this unique opportunity to establish and strengthen your scientific network.

We, as hosts, understand that the success of this conference is down to the many people who have worked tirelessly in planning and executing both the scientific and social programmes. We would like to thank all the fellows of our two networks, PHOTO.COMM and AccliPhot, and particularly our project managers, Stephanie Spelberg, Kathrin Müller, and Kristine Kirkensgaard, who worked night and day to make this event possible. Also we would like to thank the scientific committee and programme chairs for their thorough and timely reviewing of abstracts and, last but not least, our sponsors who have helped us keep down the costs for you.

We hope that you will remember Malta not only as a beautiful and sunny island, but also as the host of an exciting and stimulating scientific conference!

On behalf of the whole Organizing Committee,

Oliver Ebenhöh and Poul Erik Jensen

European Network Conference on Algal and Plant Photosynthesis 2016
Book of Abstracts

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Contributors: Martina Angeleri, Anna Matuszyńska, Fiona Moejes

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Organizing Committee

The European Networks Conference on Algal and Plant Photosynthesis 2016 was brought to you by two ITNs, AccliPhot and PHOTO.COMM:

Represented by the ENCAPP2016 Organising Committee:

Martina Angeleri, *University of Turku (Finland)*
 Anna Matuszyńska, *Heinrich Heine University of Düsseldorf (Germany)*
 Fiona Moejes, *Daithi O'Murchu Marine Research Station (Ireland)*
 Antonella Succurro, *Heinrich Heine University of Düsseldorf (Germany)*

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 Martina Zanella, *ETH Zürich (Switzerland)*
 Julie Zedler, *University of Kent (United Kingdom)*

And a special 'Thank You' to our Maltese contact:

Chris Fenech, *ECMeetings (Malta)*

AccliPhot

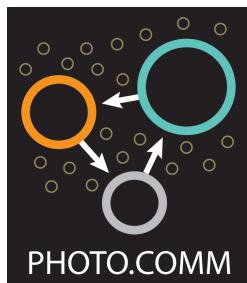
 is a Marie Curie Initial Training Network funded by the European Commission. The main research aim of AccliPhot is to investigate and understand short-term acclimation mechanisms to changes in light conditions in photosynthetic organisms. The AccliPhot network comprises 14 network partners, including 3 private companies. Together the project forms a unique combination of expertise in the area of photosynthetic acclimation. A distinctive feature of AccliPhot is the strong emphasis on interdisciplinarity between experimental and theoretical scientists, combining basic with industrial research. The aim is to generate bright, ambitious and well-trained young researchers with multidisciplinary skills in this field. Therefore the research programme is supplemented by a strong, multidisciplinary training programme in research skills and complementary transferrable skills. Altogether, AccliPhot supports 13 Early Stage Researchers and two Experienced Researchers on their ways to a future scientific career. To learn more please visit us at www.accliphoto.eu.



AccliPhot is funded by the European Union under the Seventh Framework Programme (SP3-People) under the grant agreement number PITN-GA-2012-316427.



PHOTO.COMM



<http://photocomm.ku.dk/>.



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University of Turku

**Imperial College
London**



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Kent**

**UNIVERSITY OF
CAMBRIDGE**



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PHOTO.COMM is funded by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement no 317184.



Sponsors

AccliPhot and PHOTOCOMM are funded by the European Union under the 7th Framework Program



Innovate & Design
Light-Driven
Microbial Communities



The organizing committee would like to thank ENCAPP2016 sponsors



BioSC

Bioeconomy Science Center

<http://www.biosc.de/>



PHYCONET

high value products from microalgae

 **BBSRC** BBSRC Networks in Industrial Biotechnology and Bioenergy (BBSRC NIBB)

<http://www.phyconet.org.uk/>



ISPR

International Society of
Photosynthesis Research

<http://www.photosynthesisresearch.org/>



<https://jxb.oxfordjournals.org/>



<http://www.psi.cz/>



TOTAL

<http://www.total.com/en>



<http://www.labotest.se/>



<http://www.visitmalta.com/>

Useful Information

Welcome to Malta! Situated 80 km south of Sicily, 284 km east of Tunisia, and 333 km north of Libya, Malta boasts an average of 300 days of sunshine a year. With sunshine being a key component of photosynthesis Malta seemed like the ideal choice to hold the ENCAPP2016 conference. The three islands that make up Malta are Malta, Gozo and Comino, with Malta being the largest and the cultural, commercial and administrative centre. The town of Saint Paul's Bay will be the backdrop of ENCAPP2016. It has a rich history – according to the Bible, Saint Paul was shipwrecked on an island which many scholars have now identified as Malta; hence the name Saint Paul's Bay. It lies in the northwest of the island of Malta, 16 km from the capital city Valletta. The average diurnal temperature for April is around 20°C, perfect conditions to speak of photosynthesis as well as to learn more about Maltase culture and explore the main cities or relax on the beach. If you are planning to stay in Malta longer after the conference or your partner is coming with you <http://www.visitmalta.com/> will provide more information on what is possible to do on the island. You should not have any problems finding your way around Malta with one of the two official languages being English and the Maltese people will be happy to help you. Please note that Italian is also widely spoken.

Information for speakers

The allocated time for each talk is 15 minutes + 5 minutes for discussion. Speakers are kindly required to respect this allocated time to allow for the smooth running of the conference. The chairperson will act as time keeper and if you go over the allocated time, the question session will be skipped, leaving you without so crucial feedback from your fellow scientists. So please try and keep within the allocated time! Please approach a member of the ENCAPP2016 Organising Team (in blue polo shirts) prior to the start of your session to upload your talk onto the laptop.

Information for poster presenters

Please put up your poster as soon as you have registered. Each poster has been allocated a number so please find the corresponding posterboard number. Members of the ENCAPP Organising Team will provide you the materials required to hang up your poster.

Welcome Dinner by the sea at Café Del Mar – Tuesday 26th April 2016

Offering stunning views of the Mediterranean Sea and St Paul's island, Café del Mar¹ forms part of the Malta National Aquarium complex and is the ideal place to relax, have a swim and enjoy a drink. Café del Mar is hugely famous around the world for its chill-out compilation albums which have been released annually since 1994, pioneering the chill-out genre. Special guest DJs will entertain us whilst enjoying the surroundings. We have organised the BBQ dinner on the pool deck by the infinity pool that seems to reach out into the clear blue Maltese waters – so don't forget your bathing suits! Café del Mar is about 600 m from San Antonio Hotel and Spa.

A Night of Local Folklore at Razzett L-Antik - Thursday 28th of April

Situated in the heart of the medieval village of Qormi, lies a 300-year-old farmhouse originally built to grind wheat into flour. '1743' Ir-Razzett L-Antik was restored to enshrine a lifestyle almost forgotten by time. With its original features still intact this unique building stages the perfect ambience for distant memories to come alive. The kitchen fuses old and new methods of cooking, incorporating the art of marinating and slow cooking. Ingredients include exotic spices, which the Knights introduced to the Maltese Cuisine. Once we are all seated a duo of strolling musicians takes over to roam around tables and entertain guests throughout dinner. We hope that you will not resist picking up the melody played

¹<http://www.cafedelmar.com.mt/>

by the two musicians and join in and sing your hearts out. Towards the end of the evening, the main stage will take prominence and we will be treated to a selection of musicians and artists performing ethnic music and folklore dance. Coach transfers to Qormi from the hotel leaves at **19:45**, so you don't have to worry about finding the 300-year-old farmhouse almost forgotten by time!

San Antonio Hotel and Spa

San Antonio Hotel and Spa is a 4-star resort located in the popular district of Qawra in Saint Paul's Bay, Malta. Qawra is at walking distance from a couple of sandy beaches, watersport facilities, the National Aquarium, and it is also the site of one of the many towers built by the Knights of Saint John. There are a number of good restaurants a stone's throw from the hotel. For the night owls and fellow party animals, there are plenty of casinos, bars and nightclubs. However, you might not need to even leave the comfort of the hotel! San Antonio Hotel and Spa boasts a restaurant, coffee shop, bistro, a lobby bar, pool bar, and beach bar. There is a large outdoor pool, health centre (including aerobic studio, sauna, indoor heated pool, Jacuzzi and steam bath). There is also a fully equipped fitness centre and a beauty salon.

Travelling around Malta

The GPS Coordinates for San Antonio Hotel and Spa are N35.952928, E14.418354. For your trip back to Malta International Airport (MLA, Luqa), the hotel offers private taxi for transfers from and to the airport at an additional charge. For bookings and inquiries please contact the Hotel Reception by email on reception.dbsanantonio@dbhotelsresorts.com or by phone on (+356) 2158 3434. You can also take a taxi. Estimated drive time is approximately 40 minutes depending on traffic and the price starts from €25. The public transport provides transportation to all localities. Bus X3 from the Bugibba Bus Terminus (100 m from San Antonio Hotel and Spa) takes you to the airport.

On the 1st of January 2008, Malta adopted the Euro as its currency. Exchange offices in Malta International Airport are open 24 hours. Banks on the island remain open until early afternoon from Monday to Friday, and until midday on Saturdays. Banks, cash machines (ATMs) and exchange offices are located throughout the island.

Dress Code

The dress code for the conference and both dinners is smart-casual.

Important Numbers

In case of an emergency, please call 112. This number will connect you to the Maltese police, ambulance service and fire department. The list of embassies and consulates of foreign countries in Malta can be found on the website: <http://foreignaffairs.gov.mt>. You can reach the passport office in Malta on +356 2122 2286. **Members of the ENCAPP2016 Organising Team can be reached on +356 9953 4588.**

Disclaimer

In the event of unforeseen circumstances, the organizers of ENCAPP2016 do not accept responsibility for losses incurred by participants. The information provided in the book of abstracts is the same as provided by the participants at the moment of abstract submission. Minor changes might happen for editing purposes. Errors might result from unclear or delayed submissions. We do not accept any responsibility for the scientific content nor for report errors. The program is correct at the time of publishing and may change.

Career Session

The Career Session will give young researchers the opportunity to ask four experienced researchers daunting questions about various aspects of life as a scientist. Please feel free to ask about anything that you might be worried or curious about, including:

1. Balancing personal life and career
2. Academia vs industry – what are the pros and cons of each career route?
3. Moving abroad – importance of mobility in science
4. Establishing your own working group

The panel of experienced researchers will be represented by:

Oliver Ebenhöh – Junior Professor and head of the Institute of Quantitative and Theoretical Biology at Heinrich Heine University of Düsseldorf (Germany); coordinator of AccliPhot

Oliver completed his PhD in Theoretical Biophysics at the Humboldt University, Berlin (Germany), where he continued to work as a postdoctoral researcher until 2006. He established his research group “Systems Biology and Mathematical Modelling” at the Max-Planck-Institute of Molecular Plant Physiology in Potsdam (Germany) in 2007. He then moved to the University of Aberdeen (United Kingdom) in 2009 where he was appointed Reader in Systems Biology as a joint position of the Institute for Complex Systems and Mathematical Biology and the Institute of Medical Sciences. He was appointed as the coordinator of the Theroetical Systems Biology research program in 2010. In 2014, Oliver began his position as Junior Professor and head of the Institute of Quantitative and Theoretical Biology at Heinrich Heine University of Düsseldorf (Germany) where his research group mainly focuses on the development of mathematical models of plant energy metabolism and photosynthesis.

Julie Maguire – Research Director of Daithi O’Murchu Marine Research Station (Ireland)

Julie was awarded her PhD from University College Cork (UCC) investigating the stress biology of cultured *Pecten maximus* (scallops), although much of the research was carried out at IFREMER in France. Julie worked as a shellfish research officer for the Seafish Industry Authority in Scotland before returning to Ireland as a postdoctoral researcher in an EU project investigating the impact of dredging. In 2002 she became Project Officer for the Environmental Research Institute at UCC which involved the management of over 200 projects. Three years later, she became the Research Director of Daithi O’Murchu Marine Research Station (DOMMRS) – an independent commercial research station that focuses on aquaculture husbandry, macro-algal culture and IMTA, disease and fouling control, biofuel production, minimising waste in the aquaculture and fisheries production process, developing new products from waste, environmental monitoring and harmful algal blooms research and prediction. DOMMRS has co-ordinated a number of EU projects and in November 2013 was awarded the Copernicus Masters Award from the European Space Agency for “Best service for European citizens” (using satellite data) for work carried out on algal blooms.

Yumiko Sakuragi – Associate Professor at the University of Copenhagen (Denmark)

Yumiko grew up in Japan but completed her PhD on cyanobacterial isoprenoid biosynthesis at Penn State University (USA) in 2004. She spent the next three years as a postdoctoral researcher at Harvard Medical School (USA) and at the Royal Veterinary and Agriculture University (Denmark) where she looked into plant cell wall biosynthesis, plant cell wall-microbe interaction and *Pseudomonas aeruginosa* biofilm formation. In 2008, Yumiko joined the University of Copenhagen (Denmark) as an Assistant Professor and Group Leader, and was later promoted to Associate Professor in the Department of Plant

and Environmental Sciences. Her research group is interested in understanding i) the mechanisms of glycoconjugant synthesis and ii) biological functions of the glycoconjugants in photosynthetic organisms, including plant and cyanobacteria.

Patrik Jones – Senior Lecturer at Imperial College London (United Kingdom)

Patrick Jones is an international scientist whose career has taken him all over the world. He completed his PhD in the University of Copenhagen (Denmark) in 2000, working in the field of plant biochemistry. He continued his research in the same field during his first postdoctoral position at the University of Chiba (Japan) after which he switched his interest to wine chemistry during a second postdoctoral position at the Australian Wine Research Institute. In 2005, he moved back to Japan and got his first Principal Investigator position at Fujirebio Inc., Tokyo, where his focus switched to microbial metabolic engineering. From this point on this has been his main focus, expanding his interest to cyanobacteria when he joined the University of Turku (Finland) as a Principal Investigator. He joined Imperial College London as a Senior Lecturer in 2013 where his research group has the objective of understanding and engineering the metabolism of prokaryotes, with particular focus on low-value products such as fuel and fertiliser for which sustainable production methods are needed.

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Scientific Program

Tuesday, 26th April 2016

09:00-09:20 *Opening Session* by Jun.-Prof. Oliver Ebenhöh and Prof. Poul Erik Jensen

Session I: Photosynthetic Electron Flow

Chaired by: Prof. Poul Erik Jensen and Gergana Kostova

09:20-10:00 **Prof. Himadri Pakrasi, Washington University, USA**

Biogenesis of Photosystem II: a membrane bound oxygen evolving molecular machine

10:00-10:20 Artur Włodarczyk, *University of Copenhagen, Denmark*

Cyanobacteria as a photosynthetic factory for the production of plant secondary metabolites

10:20-10:40 Ioannis Dikaios, *University of Verona, Italy*

Functional analysis of PpLHCSR1 chromophore complement and interaction with other LHC subunits by expression in *A. thaliana*

10:40-11:10 **Coffee break**

11:10-11:30 Lucilla Taddei, *Université Pierre et Marie Curie, France*

Acclimation mechanisms to environmental changes in marine diatoms: the study of the Phaeodactylum tricornutum LHCX protein family

11:30-11:50 Gergana Kostova, *Albert Ludwig University of Freiburg, Germany*

The Iron Stress Response of Photosynthetic Cyanobacteria Is Controlled by the sRNA IsaR1

11:50-12:10 Agnieszka Zygałdo Nielsen, *University of Copenhagen, Denmark*

Fusion of ferredoxin and cytochrome P450 enables direct light-driven biosynthesis

12:10-12:30 Deepak Venkanna, *University of Bielefeld, Germany*

Doubling Hydrogen production by knocking down an isoflavone reductase-like protein in *Chlamydomonas reinhardtii*

12:30-14:00 **Lunch Break and Networking**

Session II: Modelling Metabolism

Chaired by: Dr Mark Poolman and Dr Antonella Succurro

14:00-14:40 **Dr Ralf Steuer, Humboldt University Berlin, Germany**

Towards multiscale models of cyanobacterial growth

14:40-15:00 Kailash Adhikari, *Oxford Brookes University, United Kingdom*

Investigation of energy dissipation mechanism under supra optimal light conditions in genome scale metabolic models of *Arabidopsis* and *Chlamydomonas*

15:00-15:20 Brieuc Urbain, *Université de Nantes, France*

Kinetic and energetic analysis of light-limited growth behavior of *Chlamydomonas reinhardtii* in photobioreactors

15:20-15:40 Stefano Magni, *Heinrich Heine University Düsseldorf, Germany*

Dynamical modeling of the heat shock response in *Chlamydomonas Reinhardtii*

15:40-16:10 **Coffee break**

16:10-16:30 Robert J. Fläsig, *Max-Planck-Institut Magdeburg, Germany*

Dynamic flux balance modeling to increase the production of high-value compounds in green microalgae

16:30-16:50 Elahe Radmaneshfar, *University of Aberdeen, United Kingdom*

A systems biology approach to study fatty acid biosynthesis and TAG formation in microalgae

17:00-18:30 **Industrial Exhibition + Poster Session I**

from 19:00 **Welcome BBQ at Café Del Mar**

Wednesday, 27th April 2016

Session III: Light Acclimation

Chaired by: Prof. Michel Goldschmidt-Clermont and Martina Zanella

09:00-09:40	Prof. David M. Kramer, <i>Michigan State University, USA</i> The Limits of Photosynthesis under Dynamic Environmental Conditions: What we can learn when we bridging the gaps between the lab and the world
09:40-10:00	Giulio Rocco Stella, <i>Université Pierre et Marie Curie, France & University of Verona, Italy</i> Regulation and photoprotective role of the (two) Xanthophyll cycle(s) in diatoms
10:00-10:20	Dimitris Petroutsos, <i>CNRS CEA Grenoble, France</i> A blue light photoreceptor mediates the feedback regulation of photosynthesis
10:20-10:40	Guillaume Allorent, <i>University of Geneva, Switzerland</i> Regulation of excess light energy dissipation in Chlamydomonas reinhardtii
10:40-11:00	Anna Matuszyńska, <i>Heinrich Heine University Düsseldorf, Germany</i> A modular model of the light-acclimation responses in photosynthetic eukaryotes

11:00-11:30 **Coffee break**

Session IV: CO₂

Chaired by: Prof. Samuel Zeeman and Kailash Adhikari

11:30-12:10	Prof. Christine Raines, <i>University of Essex, United Kingdom</i> Improving leave photosynthetic carbon metabolism
12:10-12:30	Martina Zanella, <i>ETH Zürich, Switzerland</i> Light-dependent regulation of Calvin-Benson cycle, studying photosynthetic acclimation in Arabidopsis thaliana
12:30-12:50	Dániel Árpád Carrera, <i>ETH Zürich, Switzerland</i> Fructose 1,6-bisphosphate aldolase-the overlooked component of the Calvin-Benson cycle

12:50-14:00 **Lunch Break and Networking**

14:00-14:20	Rémi Willamme, <i>University of Liege, Belgium</i> Impact of the glyoxylate cycle on central carbon metabolism in the green microalga Chlamydomonas reinhardtii cultivated under day/night conditions
14:20-14:40	Shunsuke Adachi, <i>Tokyo University of Agriculture and Technology, Japan</i> Map-based cloning of Carbon Assimilation Rate 8 that increases CO ₂ assimilation rate in rice

Session V: Chloroplast Structure/Assembly

Chaired by: Dr Giovanni Finazzi and Anna Matuszyńska

14:40-15:20	Prof. Francis-André Wollman, <i>Institut de Biologie Physico-Chimique, France</i> Physiology and Biotechnology of electron derivation in Chlamydomonas reinhardtii
15:20-15:40	Federica Cariti, <i>University of Geneva, Switzerland</i> CrPBCP, a new chloroplast phosphatase involved in light acclimation in Chlamydomonas reinhardtii

15:40-16:10 **Coffee break**

16:10-16:30	Sacha Baginsky, <i>Martin-Luther University Halle-Wittenberg, Germany</i> The chloroplast phosphorylation network-Identification of new targets for STN7, STN8 and pCKII
16:30-16:50	Paolo Longoni, <i>University of Geneva, Switzerland</i> Tuning of Light-Harvesting Complex II phosphorylation
16:50-17:10	Serena Flori, <i>CNRS CEA Grenoble, France</i> Revealing the structural bases for light utilization in diatoms
17:10-17:30	Mathias Pribil, <i>University of Copenhagen, Denmark</i> CURT1-mediated thylakoid membrane plasticity is required for efficient photosynthetic performance and plant fitness

17:30 - 19:00 **Industrial Exhibition + Poster Session II**

Thursday, 28th April 2016

Session VI: Communities

Chaired by: Prof. Alison Smith and Martina Angeleri

09:00-09:40	Prof. Jon Clardy, <i>Harvard University, USA</i> Chemical ecology of microalgal-bacterial communities
09:40-10:00	Sebastiana Rocuzzo, <i>University of Sheffield, United Kingdom</i> Industrial Ecology: Exploiting Natural Cues in Algal Biotechnology
10:00-10:20	Anthony Riseley, <i>University of Cambridge, United Kingdom</i> Investigating and Engineering Algal-Bacterial Communities
10:20-10:40	Ulrich Johan Kudahl, <i>University of Cambridge, United Kingdom</i> Identification of bacteria capable of supplying algae with vitamin B12

10:40-11:10 **Coffee break**

11:10-11:30	Fiona Wanjiku Moejes, <i>Daithi O'Murchu Marine Research Station, Ireland & Heinrich Heine University Düsseldorf, Germany</i> Dynamics of the bacterial community associated with phaeodactylum tricornutum cultures
11:30-11:50	Antonella Succurro, <i>Heinrich Heine University Düsseldorf, Germany</i> Modeling ecosystems of standalone organisms and consortia

Session VII: Omics

Chaired by: Prof. Natalia Battchikova and Johan Kudahl

11:50-12:30	Jun.-Prof. Ilka Maria Axmann, <i>Heinrich Heine University Düsseldorf, Germany</i> Daily life of cyanobacteria
12:30-12:50	Ovidiu Popa, <i>Heinrich Heine University Düsseldorf, Germany</i> Functional capabilities of the microbiome associated with cultures of Phaeodactylum tricornutum

12:50-14:00 **Lunch Break and Networking**

14:00-14:20	Martina Angeleri, <i>University of Turku, Finland</i> Phosphoproteins of photosynthetic apparatus in Synechocystis 6803
14:20-14:40	Witold Januszewski, <i>Albert Ludwig University of Freiburg, Germany</i> Metatranscriptomic analysis of an industrial microalgal culture
14:40-15:00	José Flores-Uribe, <i>Technion - Israel Institute of Technology, Israel</i> Metagenomics for Industrial Photosynthesis
15:00-15:20	Dong Wei, <i>South China University of Technology, China</i> Global metabolic regulation of snow alga Chlamydomonas nivalis in response to nitrate or phosphate deprivation by metabolome profile analysis

15:20-15:50 **Coffee break**

Session VIII: Engineering

Chaired by: Dr Patrik Jones and Julie A. Z. Zedler

15:50-16:30	Prof. Birger Lindberg Møller, <i>University of Copenhagen, Denmark</i> Synthetic plant biology: The ultimate way to 'go green' - Lightdriven production of structurally complex diterpenoids
16:30-16:50	Erick M. Ramos-Martinez, <i>University of Copenhagen, Denmark</i> Enhanced secretion of recombinant proteins from Chlamydomonas reinhardtii
16:50-17:10	David Malatszky, <i>Imperial College London, United Kingdom</i> Modelling and engineering Anabaena sp. PCC 7120 for bio-fertilizer of synthetic communities
17:10-17:30	Maria Henriques de Jesus, <i>University of Copenhagen, Denmark</i> Using Tat proteins for pathway organization of light-driven biosynthesis of natural products
17:30-17:50	Payam Mehrshahi, <i>University of Cambridge, United Kingdom</i> Using synthetic biology to engineer medicinal algae

18:00 - 19:00 **Industrial Exhibition + Poster Session III**

from 20:00 **SOCIAL EVENT at Razzett L-Antik**

Friday, 29th April 2016

BioSC Session: Innovative Algae Research for a Bio-based Economy

Chaired by: Prof. Roberto Bassi and Fiona Wanjiku Moejes

- | | |
|-------------|---|
| 09:00-09:10 | Oliver Ebenhöh <i>About BioSC</i> |
| 09:10-09:50 | Dr Laurent Fourage, TOTAL New Energies Division, France
Direct more CO ₂ towards oil through development of new oleaginous microalgae strains |
| 09:50-10:10 | Kirstin Feussner, <i>University of Göttingen, Germany</i>
Oil is on the agenda: metabolism in Phaeodactylum tricornutum |
| 10:10-10:30 | Natanamurugaraj Govindan, <i>University Malaysia Pahang, Malaysia</i>
Different intensity of photosynthetic light energy enhanced biodiesel production on marine algae diatoms |
-

- | | |
|-------------|---------------------|
| 10:30-11:00 | Coffee break |
|-------------|---------------------|
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- | | |
|-------------|--|
| 11:00-11:20 | Tiago Guerra, <i>A4F Algae for Future, Portugal</i>
Mass balance analysis of carbon and nitrogen in industrial scale mixotrophic microalgae cultures |
| 11:20-11:40 | Nodumo Zulu, <i>University of Göttingen, Germany</i>
Improving polyunsaturated fatty acid (PUFA) and triacylglyceride (TAG) accumulation in Phaeodactylum tricornutum |
| 11:40-12:00 | Giorgio Perin, <i>University of Padova, Italy</i>
Forward genetics toward the light-use efficiency improvement of Nannochloropsis gaditana |
| 12:00-12:20 | Weiqi Fu, <i>New York University Abu Dhabi, United Arab Emirates & University of Iceland</i>
Intracellular Spectral Recomposition of Light: A Novel Strategy for Improving Photosynthetic Efficiency of Diatoms |
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|-------------|-----------------------------------|
| 12:20-13:30 | Lunch Break and Networking |
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Session X: Industrial Cultivation

Chaired by: Dr Vitor Verdelho and Artur Włodarczyk

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|-------------|---|
| 13:30-13:40 | Chairman Introduction |
| 13:40-14:00 | Valeria Villanova, <i>Fermentalg, France</i>
Characterisation and optimisation of mixotrophic growth in Phaeodactylum tricornutum |
| 14:00-14:20 | Dipali Singh, <i>Oxford Brookes University, United Kingdom</i>
Modelling metabolism of the diatom Phaeodactylum tricornutum |
| 14:20-14:40 | Doris Gangl, <i>University of Kent, United Kingdom</i>
Expression of an active plant cytochrome P450 in the chloroplast of the green alga Chlamydomonas reinhardtii and cultivation of transgenic strains in an industrial setting |
| 14:40-15:00 | Julie A. Z. Zedler, <i>University of Kent, United Kingdom</i>
Chlamydomonas reinhardtii as a platform for sustainable diterpene production |
| 15:00-15:10 | <i>Closing Session</i> |
| 15:10-15:30 | Coffee break |
| 15:30-18:00 | Afternoon Career Session: designed for early stage researchers |
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Session I: Photosynthetic Electron Flow

Chaired by:

Prof. Poul Erik Jensen

University of Copenhagen, Denmark

Gergana Kostova

Albert Ludwig University of Freiburg, Germany

This session will present the recent advancements in the field of photosynthesis, photosynthetic linear and cyclic electron flow, regulation of photosynthesis, dissipation of excessive energy and effects of changing environmental conditions. It will expand on state of the art methods and techniques used in photosynthesis research. Approaches to direct electrons from photosynthesis towards enhanced production of high value compounds will also be reviewed. The session will also discuss the generation and neutralization of reactive oxygen species (ROS), associated with photosynthesis.

Biogenesis of Photosystem II: a membrane bound oxygen evolving molecular machine

Keynote Speaker: Prof. Himadri Pakrasi

Daniel A. Weisz, Michael L. Gross, Himadri B. Pakrasi

Washington University, USA

Photosystem II (PSII), a large multisubunit pigment-protein complex in the thylakoid membranes in cyanobacteria and chloroplasts, catalyzes light-mediated oxidation of water to molecular oxygen, and is responsible for half of the bioenergy production in photosynthetic organisms. Because of the unusual redox environments in which this unique metalloenzyme operates, it often undergoes damages and degradation, followed by resynthesis and assembly processes that involve multiple intermediate stages. During the past 15 years, the molecular structure of the fully functional PSII complex has been elucidated by x-ray crystallography and spectroscopic methods. Such a complex contains at least 20 polypeptide components, the majority of which are integral membrane proteins. In addition, it contains numerous cofactors such as pigments, metals and quinone molecules. In comparison, the structural details of the PSII assembly intermediate complexes remain poorly understood. During recent years, we have used the powerful tools of molecular genetics and mass spectrometry to begin to unravel such details of some of the protein factors that are transiently present in PSII assembly intermediate complexes in cyanobacteria. During this presentation, we will discuss such studies that have helped us understand the life history of Photosystem II.

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Cyanobacteria as a photosynthetic factory for the production of plant secondary metabolites

presented by Artur Włodarczyk

Artur Włodarczyk, Thiagarajan Gnanasekaran, Agnieszka Zygadło Nielsen, Birger Lindberg Møller and Poul Erik Jensen

University of Copenhagen, Denmark

Cyanobacteria, the evolutionary ancestor of plant chloroplasts, are prokaryotic photosynthetic organisms able to convert inorganic carbon, nitrogen and phosphorous compounds into biomass and a wide variety of different metabolites using only water and light as resources. Recently they have been gaining renewed interest as chassis for metabolic engineering and synthetic biology approaches. Expression of plant pathways employing cytochrome P450s in foreign hosts such as *E. coli* often causes problems, due to difficulties with proper folding, post-translational modifications and targeting of these proteins to the membranes. As a proof of concept, we engineered the dhurrin pathway from Sorghum bicolor comprising two membrane bound cytochromes P450 (CYP79A1 and CYP71E1) and a soluble glycosyltransferase (UGT85B1) into a cyanobacterium *Synechocystis* sp. PCC 6803 as the heterologous expression platform. By expressing the cytochromes P450 in the thylakoid membranes of cyanobacteria, we demonstrated that photosystem I and ferredoxin can replace the native P450 oxidoreductase (POR) as supply for the electrons required for the P450 catalysis. We demonstrated that photosynthetic reducing power can be used for the biosynthesis of bioactive compounds. We are currently pursuing this approach to express phenylpropanoid pathway in order to produce phenolic compounds and their glucosides, which can be used for fragrance and food applications. By using cyanobacteria as a expression host we aim to find a way to directly convert sunlight energy and carbon dioxide into high value chemicals, as an alternative to the conventional methods.

Functional analysis of PpLHCSR1 chromophore complement and interaction with other LHC subunits by expression in *A. thaliana*

presented by Ioannis Dikaios

Ioannis Dikaios¹, Alessandro Alboresi² and Roberto Bassi¹

¹University of Verona, Italy; ²University of Padova, Italy

Non-photochemical quenching (NPQ) of chlorophyll fluorescence is a process essential for the regulation of photosynthesis and plant protection from light stress. In vascular plants this process is triggered by a luminal pH sensor, the PSBS protein, which transduces chloroplast lumen acidification, induced by excess light, into a quenching reaction occurring within specific interacting chromophore-bound light-harvesting proteins (Lhc). In algae, such as *Chlamydomonas reinhardtii*, stress-related light-harvesting proteins (LHCSR) fulfill both pH sensing and quenching reactions, due to their capacity of binding chlorophylls and xanthophylls [1]. The moss *Physcomitrella patens*, an evolutionary intermediate between algae and plants, has both PSBS and LHCSR active in quenching [2] with LHCSR working in a direct zeaxanthin-dependent manner [3]. Plant and mosses have a very similar organization of thylakoid membranes thus suggesting LHCSR might be active in plants. To verify this hypothesis, we overexpressed lhcsr gene into *Arabidopsis thaliana* psbS mutant, npq4, and screened transformants by fluorescence video-imaging, resulting to the isolation of *A. thaliana* plants which accumulate a pigment binding, NPQ-active LHCSR1 in thylakoid membranes. In the context of functional and structural analysis of LHCSR protein a series of in vivo transformations was performed using *A.t.* mutants altered in xanthophyll content or lacking specific Lhc subunits. For this reason the double mutant npq1npq4 - unable to convert violaxanthin into zeaxanthin - was complemented in order to verify the direct dependence of LHCSR on zeaxanthin, mutant lut2npq4 was used due to its complete lack of lutein and antenna mutants nomnpq4 and ch1lhcb5 were used due to their lack of either minor antennas or the complete antenna system respectively, all of them overexpressing LHCSR1 in different levels. Finally, for complementation of the NPQ phenotype we have also expressed PSBS from *P. patens* in *A. thaliana* npq4 plants, generating plants that activate NPQ in cases up to the level of *A. thaliana* wild type.

References

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3. A. Pinnola, L. Dall'Osto, C. Gerotto, T. Morosinotto, R. Bassi, A. Alboresi, Plant Cell (2013)

Acclimation mechanisms to environmental changes in marine diatoms: the study of the *Phaeodactylum tricornutum* LHCX protein family

presented by Lucilla Taddei

Lucilla Taddei¹, Olga Chukutsina^{2,3}, Giulio Rocco Stella^{1,4}, Bernard Lepetit⁵, Herbert Van Amerongen², Marianne Jaubert¹, Giovanni Finazzi⁶, Angela Falciatore¹

¹ Université Pierre et Marie Curie, France; ² Wageningen University, The Netherlands; ³ Vrije Universiteit Amsterdam, The Netherlands; ⁴ University of Verona, Italy; ⁵ University of Konstanz, Germany; ⁶ CNRS CEA Grenoble, France

Photosynthetic organisms are able to adjust light absorption to electron flow capacity by dissipating the excessive energy as heat, to avoid photodamage. This process is named the Non-Photochemical Quenching of chlorophyll fluorescence (NPQ). Marine diatoms are prominent phytoplanktonic organisms in the ocean that successfully manage highly variable light conditions also thanks to an impressive NPQ capacity. Molecular studies in the diatom *Phaeodactylum tricornutum* have revealed the involvement of LHCX1, a member of the light-harvesting protein family, in the NPQ process. Here, using genetics, time-resolved spectroscopy and purification of photosynthetic complexes from *P. tricornutum* wild-type and knock-down lines, we show that LHCX1 mediates a constitutive quenching process mostly occurring in the reactions center of Photosystem II. However, upon exposure to high light, a different quenching mechanism is triggered, occurring mainly in the antenna complexes. Expression analysis of the different LHCX family members in cells exposed to HL suggests that this antenna-related quenching mechanism could involve the LHCX3 isoform. Overall, by revealing the existence of different NPQ sites with different targets and regulators, our data provide a solid molecular interpretation for the extreme flexibility of diatoms to respond to the highly variable ocean environment.

The Iron Stress Response of Photosynthetic Cyanobacteria Is Controlled by the sRNA IsaR1

presented by Gergana Kostova

Jens Georg¹, Verena Schön¹, Christian Weingärtner¹, Wolfgang R. Hess¹, Linda Vuorijoki², Tuomas Huokko², Martina Angeleri², Eva-Mari Aro²

¹Albert Ludwig University of Freiburg, Germany; ²University of Turku, Finland

Iron is an important cofactor for many proteins involved in the photosynthetic electron transfer and respiration. One of the most Fe-rich cellular systems is the photosynthetic apparatus, leading to an about 10-fold higher Fe quota per cell in photosynthetic compared to other bacteria. Therefore, photosynthesis is highly vulnerable to Fe stress. The sRNA IsaR1 was identified as Fe stress induced ncRNA (Syr22, NC-181) through transcriptomic analysis of *Synechocystis* sp. PCC6803 and it is conserved also in other cyanobacteria. Computational target predictions suggested that the expression of several mRNAs for Fe cofactor-containing proteins could be controlled by IsaR1, which was supported by the results of transcriptome profiling experiments upon pulsed overexpression of IsaR1. We verified regulatory interactions between IsaR1 and the 5'UTRs of mRNAs encoding the major ferredoxin, the enzymes aconitate hydratase and superoxide dismutase in a heterologous reporter assay. Proteins affected by IsaR1 were confirmed independently in a targeted proteomics approach using suitable mutants. These mutants were also characterized biophysically. Furthermore, we analyzed the changes in the half-lives of RNAs in *Synechocystis* in standard and Fe limiting conditions. Together, our results prove that IsaR1 acts as a global regulator in the regulatory network of cyanobacterial gene expression under iron limitation.

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Fusion of ferredoxin and cytochrome P450 enables direct light-driven biosynthesis

presented by Agnieszka Zygadlo Nielsen

Silas Busck Mellor, Agnieszka Zygadlo Nielsen, Birger Lindberg Møller, Poul Erik Jensen
University of Copenhagen, Denmark

Plants produce a multitude of specialized metabolites with use in the medicinal, fragrance and flavor industries. Cytochromes P450 are key enzymes in biosynthetic pathways of these products. These enzymes are difficult to express heterologously in active form, and due to their requirement for reducing power in the form of NADPH their use for in vitro and whole-cell production is complicated. We recently showed that plant P450s can be expressed in chloroplasts of tobacco plants and in cyanobacteria, where they will insert into the thylakoid membrane and that photosynthesis can support P450 catalytic activity independent of NADPH through the action ferredoxin. In the current study, we report the fusion of ferredoxin with the plant P450 CYP79A1, which catalyzes the initial step of the pathway leading to biosynthesis of the cyanogenic glucoside dhurrin. Fusion with ferredoxin allows the cytochrome P450 enzyme to obtain electrons for catalysis directly from photosynthesis by interacting with photosystem I. Furthermore, the electrons captured by the fused ferredoxin domain are directed more effectively towards the P450 in competition with other ferredoxin requiring enzymes. As a result, it partially overcomes the problem of competition for reduced ferredoxin by electron sinks coupled to endogenous metabolic pathways. The ferredoxin-P450 fusion enzyme obtains reducing power solely from its fused ferredoxin, but maintains a similar level of catalytic activity compared to unfused CYP79A1 at in vivo concentrations of soluble ferredoxin. This demonstrates that electron transfer from photosystem I to CYP79A1 is greatly accelerated as a consequence of the fusion. The fusion strategy reported here therefore forms the basis for increased partitioning of photosynthetic reducing power towards P450-dependent biosynthesis of important natural products. This approach has high potential for stably engineering cyanobacteria that enables high-level cytochrome P450-dependent production of high-value natural products in a light-driven manner.

Doubling Hydrogen production by knocking down an isoflavone reductase-like protein in *Chlamydomonas reinhardtii*

presented by Deepak Venkanna

Deepak Venkanna, Lisa Schierenbeck, Lutz Wobbe and Olaf Kruse

University of Bielefeld, Germany

The unicellular green algae *Chlamydomonas reinhardtii* belongs to a select group of microorganisms, which are capable of solar-driven hydrogen production. Sulphur starvation induces photoinhibition of PSII which lowers oxygen evolution rates below that of mitochondrial consumption rates leading to establishment of an anaerobic environment and induction of hydrogen production by an oxygen sensitive hydrogenase. Though the induction of anaerobiosis requires a diminished water splitting activity at PSII, numerous studies underscore the importance of residual PSII activity that is required for high hydrogen yields. In the following study a reverse genetics approach using artificial microRNA (amiRNA) was applied to assess the role of Isoflavone Reductase like Protein (IRL). Transcriptome analyses suggested an increase in the expression of IRL under H₂ production. A comparison between a high hydrogen producer stm6glc4 (mt) and parental strain cc406 (wt) showed that the expression of IRL was higher in the wt which is a low H₂ producer and vice versa. An amiRNA targeting IRL was constructed and transformed into *C. reinhardtii* strains cc124 (wt) and high H₂ producer stm6 (mt). The resulting mutants showed a reduction in IRL and stayed green during sulphur starvation. IRL knockdown led to a 100% increase in H₂ yields due to a prolonged production phase. Our findings suggest the main reason for this prolongation being a stabilized PSII which is less susceptible to photoinhibition under sulphur depletion. IRL is a promising and novel target for engineering approaches aiming at increasing hydrogen production in *C. reinhardtii*.

References

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Session II: Modelling Metabolism

Chaired by:

Dr Mark Poolman

Oxford Brookes University, United Kingdom

Dr Antonella Succurro

Heinrich Heine University Düsseldorf, Germany

The session on “Modelling Metabolism” will cover different approaches to investigate the potential behaviour of plant metabolism using a variety of computational methods, including kinetic (ODE based) and structural (constraint based) techniques, applied to both small and large models. A special focus is given to the study of representative organisms of green algae, diatoms and plants, to identify areas of metabolism involved in growth, adaptation and production of important biochemical compounds. Presentation of investigations that either integrate modelling with existing experimental data or that suggest, or could direct, new practical investigation at the laboratory or industrial scale are particularly encouraged.

Towards multiscale models of cyanobacterial growth

Keynote Speaker: Dr Ralf Steuer

Ralf Steuer¹, Marco Rügen¹, Stefanie Westermark¹, Christian Beck¹, Alexandra Reimers² and Henning Knoop¹

¹*Humboldt University Berlin, Germany;* ²*Freie Universität Berlin, Germany*

While many aspects of phototrophic growth are well understood, it still remains a considerable challenge to understand the dependencies and interconnections between the diverse cellular processes that together give rise to cellular growth. Computational modeling allows us to quantitatively describe the individual cellular processes relevant for growth. As yet, however, such computational models are mostly confined to the inner workings of individual processes, rather than describing the interactions between them in the context of a living cell. This contribution seeks to summarize existing computational models that are relevant to describe cyanobacterial growth and outlines how these models can be integrated into a coherent whole. Our ultimate aim is to understand cellular functioning and growth as the emergent outcome of individual yet interconnected cellular processes. In particular, we are interested in the temporal resource allocation problem of phototrophic growth. Based on a comprehensive account of intracellular interconversions and constraints, we are able to describe the processes and fluxes required to synthesize new cellular components. We demonstrate that appropriately constructed “whole-cell models”, based only on a narrow set of well-defined parameters and assumptions, allow us to derive several emergent properties related to cyanobacterial growth and functioning, such as the temporal dynamics of glycogen synthesis.

References

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Investigation of energy dissipation mechanism under supra optimal light conditions in genome scale metabolic models of Arabidopsis and Chlamydomonas

presented by Kailash Adhikari

Kailash Adhikari, David Fell, Mark Poolman

Oxford Brookes University, United Kingdom

A Genome Scale Metabolic Model (GSM) represents the entire metabolic capabilities of an organism and is built from data typically extracted from annotated genome databases. With aid of other computational tools, GSM can be used as an in-silico lab, allowing us to simulate metabolic behaviors of an organism under given environmental conditions. Here we present, fully compartmentalised GSMS of *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*, reconstructed using the latest annotations in the BioCyc database. As both the models are based on a recent release of the database, with more genome annotations, they includes large coverage of metabolic networks compared to their predecessors, hence gives more opportunity for developing newer insight about organism's metabolic behaviors. Under natural environment, plants and algae are exposed to various light conditions which affects their photosynthetic productivity. Under high light conditions, absorption of light energy becomes excessive, compared to that required by photosynthetic activity, thus generating excess energy in the system, which is harmful to these organisms. We have used the light scan analysis technique, to analyse the reactions particularly responding to the high input of photon flux in the models and identified potential metabolic cycles, that could act as energy dissipation modes under supra optimal light conditions. Moreover, we present explanations on how the metabolic network in these organisms are rerouted, in response to the increasing input of light, thus acting as photoprotective mechanism.

References

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2. Roger L Chang et al., Mol Syst Biol (2011)

Kinetic and energetic analysis of light-limited growth behavior of *Chlamydomonas reinhardtii* in photobioreactors

presented by Brieuc Urbain

Brieuc Urbain, Guillaume Cogne, Mariana Titica, Jack Legrand

Université de Nantes, France

A biochemically-based structured model for the autotrophic growth of *Chlamydomonas reinhardtii* in photobioreactors (PBRs) is developed through the knowledge of a detailed underlying metabolic network. The model is reduced to a minimal set of 6 reactions derived from metabolic investigations of light-limited growing cell behaviors in PBRs. Structuration of the model including a fully detailed description of cellular energetics leads to the formulation of only two kinetic equations for fixing the degree-of-freedom of the system to zero, namely photon uptake rate and kinetics of a maintenance process involving the light-dependent regulation of the oxidative pentose-phosphate pathway. The model involves the introduction of only 3 parameters that have to be identified from experimental data. After parameter identification, the current model shows good agreement with previous experimental results for continuously growing cultures under different illumination conditions. A parameter sensitivity analysis is performed to check the model robustness. Each parameter is varied around its nominal value, identified on experimental data, under different lighting conditions. This analysis was used as an indicator of the most influencing parameters and provides valuable information on the quality of identification. An experimental platform is developed to assess energetic parameters in PBR cultures under various conditions.

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Dynamical modeling of the heat shock response in Chlamydomonas reinhartii

presented by Stefano Magni

Stefano Magni¹, Antonella Succurro¹, Aleksander Skupin², Oliver Ebenhöh¹

¹*Heinrich Heine University Düsseldorf, Germany;* ²*University of Luxembourg*

Organisms exposed to temperatures higher than usual can activate a heat shock response (HSR) allowing them to react to the new conditions. We focus on Chlamydomonas reinhartii which, beside being a model organism for green algae, is also interesting for the production of biofuels and hydrogen. Processes involved in the HSR are highly conserved among species, thus similar mechanisms might be at work in crop plants, subject to heat stress due to global warming. Here, we build a data driven mathematical model for the HSR in C. reinhartii, employing ODEs based on simple mass action kinetics and involving few non-linear terms. The signaling network structure is extracted from various experimental data available in the literature, which we further use to validate the model. Temperature variations are sensed via the accumulation of unfolded proteins, which activate a heat shock factor. This in turn activates the expression of genes coding for heat shock proteins able to repair the unfolded proteins. The model allows to analyze the response on different signal levels and to various stimuli not easily accessible through experiments. In C. reinhardtii the HSR has been shown to be elicited also by light, via an independent regulatory pathway which involves intermediates of Chlorophyll biosynthesis as Mg-Protoporphyrin IX (MgProto). We thus extend our model to include the description that we are developing of this activation mechanism. This comprehends light activating the release from the Chloroplast of MgProto, and the activation of the HEMA gene necessary for the first step of Chlorophyll biosynthesis.

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Dynamic flux balance modeling to increase the production of high-value compounds in green microalgae

presented by Robert J. Flassig

R. J. Flassig, M. Fachet, K. Höffner, K. Sundmacher

Max-Planck-Institut Magdeburg, Germany

Photosynthetic organisms can be used for renewable and sustainable production of fuels and high-value compounds from natural resources. Costs for design and operation of large-scale algae cultivation systems can be reduced if data from laboratory scale cultivations are combined with detailed mathematical models to evaluate and optimize the process. In this work we present a modeling formulation for accumulation of high-value storage molecules in microalgae that provides quantitative predictions under various light and nutrient conditions. The modeling approach is based on dynamic flux balance analysis (DFBA) and includes regulatory models to predict the accumulation of pigment molecules. The accuracy of the model predictions is validated through independent experimental data followed by a subsequent model-based fed-batch optimization. In our experimentally validated fed-batch optimization study we increase biomass and β -carotene density by factors of about 2.5 and 2.1, respectively. Our study shows that a model-based approach can be used to develop and significantly improve biotechnological processes for biofuels and pigments.

References

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A systems biology approach to study fatty acid biosynthesis and TAG formation in microalgae

presented by Elahe Radmaneshfar

Elahe Radmaneshfar¹, Oliver Ebenhoeh^{1,2}

¹ University of Aberdeen, United Kingdom; ² Heinrich Heine University Düsseldorf, Germany

Omega-3 fatty acids have vital roles in human health. Fish oils are a rich source of omega-3. However, due to the depletion of wild fish stocks, a different source of omega-3 is required. In the food chain, microalgae are the primary producers of omega-3 and are good alternatives to fish oils. The production of omega-3 involves chain elongation and desaturation of primer fatty acids, which are then being incorporated and stored in many different types of lipids. However, the unique properties of edible oils from different sources utilized for food is dependent on the fatty acid composition of triacylglycerol (TAG). Therefore, we are interested to direct the fatty acids to be stored in TAG. Although many components of the this pathways have been identified, the production of omega-3 in industrial scale is still challenging and requires a platform to study the impact of kinetic properties of the enzymes on the production of omega-3. In this talk, I will present a mathematical model, which addresses the combinatorial explosion of intermediates in the synthesis pathway of fatty acids. This complexity arises from the unspecificity of some enzymes. Our model simulates the distribution of various fatty acids in TAG over time. The model predicts the total number of free fatty acids in the endoplasmic reticulum, and also the average degree of desaturation and the length distribution of fatty acids as functions of the enzymatic rate constants.

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3. Arao T. and Yamada, Phytochem. (1994)

Session III: Light Acclimation

Chaired by:

Prof. Michel Goldschmidt-Clermont

University of Geneva, Switzerland

Martina Zanella

ETH Zürich, Switzerland

Plants and algae must optimally perform under varying environmental conditions, among which light changes are critical for optimum photosynthesis. During this session we will integrate a broad range of disciplines to set the theoretical framework for testing current hypothesis on light absorption, utilisation and dissipation, and to decipher the molecular mechanisms behind these processes. Presentation of results of *in silico* experiments with biologically significant predictions, is encouraged.

The Limits of Photosynthesis under Dynamic Environmental Conditions: What we can learn when we bridging the gaps between the lab and the world

Keynote Speaker: Prof. David M. Kramer

David M. Kramer and Atsuko Kanazawa

Michigan State University, USA

The talk will cover the following connected topics:

1. New phenotyping technologies: PhotosynQ.org - Community-driven plant phenotyping platform for data-driven innovations in science and agriculture; MultispeQ – A PhotosynQ-enabled tool for large-scale field phenotyping; CoralspeQ - A field-deployable phenotyping platform for corals, algae and cyanobacteria; Dynamic Environmental Phenotype Imager (DEPI)- A platform for high throughput dynamic plant phenotyping, replays complex, dynamic environmental conditions while measuring detailed photosynthetic and growth responses;
2. Using these tools to understand the responses and limitations of photosynthesis in dynamic environments: The functions of genes of unknown function; The biophysical ‘Achilles Heal’ of photosynthesis that involves the interactions of electronmotivs and protonmotive energy storage systems of chloroplasts;
3. Using enabling tools to improve the breeding and management of crops, especially in the developing world;

The talk will be followed by hands-on demonstrations and discussions of the capabilities and applications of the platforms and how you can get involved, contribute to the platforms and have access to all these tools!

Regulation and photoprotective role of the (two) Xanthophyll cycle(s) in diatoms

presented by Giulio Rocco Stella

Giulio Rocco Stella^{1,2}, Jean-Pierre Bouly¹, Matteo Ballottari², Roberto Bassi², Angela Falciatore¹

¹Université Pierre et Marie Curie, France; ²University of Verona, Italy

Photosynthetic organisms have developed different strategies to adapt their photosynthetic apparatus to changing light conditions. Diatoms are prominent phytoplankton organisms in the ocean, particularly adapted to highly stressful and variable environmental conditions. They possess a peculiar set of light harvesting and photoprotective pigments compared to plants and green algae, but the basic mechanisms of diatom light acclimation are still largely unknown. In response to high light, green algae and plants de-epoxidize violaxanthin (Vx) to zeaxanthin (Zx) in a photoprotective process called the xanthophyll cycle (XC), while the reverse reaction happens in low light. In diatoms, an additional XC is also found, converting diadinoxanthin (Ddx) to diatoxanthin (Dtx) under high-light. Diatoms have expanded the genes involved in the XC, and the model species *Phaeodactylum tricornutum* possess four putative (violaxanthin) de-epoxidases and three putative (zeaxanthin) epoxidases. So far, only the involvement of the Violaxanthin de-epoxidase VDE in the XC and Non-Photochemical Quenching photoprotection regulation has been demonstrated in *P. tricornutum*. Here, the function of two other de-epoxidases, Violaxanthin de-epoxidase-like 2 (VDL2) and Violaxanthin de-epoxidase-related (VDR), has been examined. In particular, knock-down lines for both genes show (i) a reduced photoprotection capacity and (ii) a strong imbalance between the Ddx-Dtx and the Vx-Zx pool compared to wild type cells. These results suggest that VDL2 and VDR can modulate the activity of two XC cycles in *P. tricornutum* and provide novel elements to further characterize the biosynthetic pathways and specific function of the double XC in diatoms.

A blue light photoreceptor mediates the feedback regulation of photosynthesis

presented by Dimitris Petroutsos

Dimitris Petroutsos¹, Ryutaro Tokutsu², Shinichiro Maruyama³, Serena Flori¹, Andre Greiner⁴, Leonardo Magneschi^{1,5}, Loic Cusant¹, Tilman Kottke⁶, Maria Mittag⁷, Peter Hegemann⁴, Jun Minagawa², Giovanni Finazzi¹

¹CNRS CEA Grenoble, France; ²National Institute for Basic Biology, Okazaki, Japan; ³Tohoku University, Japan; ⁴Humboldt University Berlin, Germany; ⁵University of Münster, Germany; ⁶University of Bielefeld, Germany; ⁷Friedrich Schiller University Jena, Germany

In plants and algae, light serves both as the energy source for photosynthesis and as a biological signal triggering cellular responses via specific photoreceptors. Red light is perceived by phytochromes whereas blue light by cryptochromes (CRYs) and/or phototropins (PHOTs). Photoperception spans several orders of light intensity ranging from far below the threshold for photosynthesis to values beyond the capacity of photosynthetic CO₂ assimilation. Excess light may cause oxidative damage and cell death unless it is prevented by enhanced thermal dissipation (energy quenching, qE), a key photoprotective response. Here, we show the existence of a molecular link between photoreception, photosynthesis, and photoprotection in the green alga *Chlamydomonas reinhardtii*. We show that PHOT controls qE by inducing the expression of the qE effector protein LHCSR3 in high light. This control requires blue light perception by the photosensory domains (LOV) of PHOT, LHCSR3 induction through PHOT kinase, and light dissipation in photosystem II via LHCSR3. phot mutants display severely reduced fitness under excessive light conditions, indicating that light sensing, utilization, and dissipation is a concerted process playing a vital role in microalgal acclimation to environments of variable light intensities.

Regulation of excess light energy dissipation in Chlamydomonas reinhardtii

presented by Guillaume Allorent

Allorent Guillaume, Legendre-Lefebvre Linnka, Chappuis Richard, Goldschmidt-Clermont Michel
University of Geneva, Switzerland

When photosynthetic organisms are exposed to high light, a wide range of mechanisms are activated to dissipate excess absorbed energy and protect the photosynthetic machinery from photodamage. These processes, mainly associated with photosystem II (PSII), are referred to as Non-Photochemical Quenching mechanisms (or NPQ). The most prominent NPQ component in plants and green algae is qE, the energy-dependent component of NPQ, which corresponds to a thermal dissipation of excess energy. In plants, the PSII-subunit PSBS is known to be the main protein involved in qE and acts as a pH-driven amplifier of NPQ. In the green alga *Chlamydomonas reinhardtii*, thermal dissipation is facilitated by the LHCSR3 protein. In contrast to PSBS in plants, the LHCSR3 protein is not constitutively expressed and is only induced after prolonged exposure of the algae to high light. The npq4 mutant, impaired in the accumulation of the LHCSR3 protein, is more sensitive to high light, demonstrating that this protein is the key effector of the high-light response in *Chlamydomonas*. The regulation of qE by LHCSR3 and its signaling pathway has been thoroughly investigated, but the question of the function of other qE-related proteins remains open. In this project, we show that by changing light-stress conditions, we can modulate and modify the qE response in *Chlamydomonas*. Our data reveal a new regulatory pathway for the NPQ response in *Chlamydomonas*.

A modular model of the light-acclimation responses in photosynthetic eukaryotes

presented by Anna Matuszyńska

Anna Matuszyńska¹, Giovanni Finazzi², Federica Cariti³, Michel Goldschmidt-Clermont³, Oliver Ebenhöh¹

¹*Heinrich Heine University Düsseldorf, Germany;* ²*CNRS CEA Grenoble, France;* ³*University of Geneva, Switzerland*

During evolution, photosynthetic organisms acquired numerous sophisticated methods to dynamically react to light fluctuations, which resulted in various common acclimation strategies. On one hand, through a process called state transitions, they are capable of equally distributing the energy between the two photosystems. On the other hand, dissipation of excitation energy as heat in the light-harvesting complexes protects them against excessive light. The underlying molecular mechanisms of light-acclimation differ between organisms, making it difficult to provide a generalised description of the processes. Theoretical approaches serve as powerful tools to discover common, organisational principles governing the design of biological systems. By reducing the complexity to the essential features, we can provide a simple, yet robust model, that is not specially tailored for only one model organism, but can describe a variety of species. Here we present a mathematical model of the photosynthetic electron transport chain, that incorporates both state transitions and heat dissipation mechanisms allowing to reflect the dynamics of photosynthetic acclimation. Due to its modular design, the model resembles a LEGO® style system: you can add or remove components of the photoprotective mechanisms, thus simulating various mutants or organisms. The effect of regulatory acclimation mechanisms can be easily monitored in a minimally invasive way by chlorophyll fluorescence measurements. Our model can quantitatively reproduce the dynamics of fluorescence under various light conditions for the higher plant *Arabidopsis thaliana* and the green alga *Chlamydomonas reinhardtii*, thus making it an ideal platform to test various hypotheses regarding the photo-regulatory mechanisms in various species.

Session IV: CO₂

Chaired by:

Prof. Samuel Zeeman

ETH Zürich, Switzerland

Kailash Adhikari

Oxford Brookes University, United Kingdom

This session intends to encourage scientific discussion on the structure and function of primary carbon metabolism with an aim to increase the photosynthetic productivity in plants and algae. Relative importance of individual enzymes in the Calvin cycle, their regulation and contribution for photosynthetic efficiency will be highlighted. Discovery of potential alternate and efficient carbon fixation pathways to produce high value compounds, transgenic manipulation of carbon metabolism to identify the factors that affect the photosynthesis and yield, will be among other topics of discussion.

Improving leave photosynthetic carbon metabolism

Keynote Speaker: Prof. Christine Raines

Christine Raines¹, Andrew Simkin, Kenny Brown, Stuart Fisk, Patricia Lopez, Tracy Lawson

¹ University of Essex, United Kingdom

Increasing demands of the growing world population for food and fuel are putting ever greater pressure on the need to develop higher yielding crop varieties. It has been estimated that increases of 50% will be required in the yield of grain crops such as wheat and rice if food supply is to meet the demands of the increasing world population. The primary determinant of crop yield is the cumulative rate of photosynthesis over the growing season which is the result of the crop's ability to capture light, the efficiency by which this light is converted to biomass and how much biomass is converted into the usable product e.g. grain in the case of wheat and rice. There is compelling evidence from transgenic studies that the manipulation of the Calvin-Benson cycle enzyme SBPase could increase yield in a range of species, including wheat, tobacco and Arabidopsis. More recently we have shown that overexpression of SBPase in combination with FBPaldolase, ictB (from cyanobacteria) or a component of the algal electron transport chain can lead to a further increase in photosynthesis and biomass.

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Light-dependent regulation of Calvin-Benson cycle, studying photosynthetic acclimation in *Arabidopsis thaliana*

presented by Martina Zanella

M. Zanella, S.C. Zeeman

ETH Zürich, Switzerland

As photoautotrophic organisms plants use light to convert water and atmospheric CO₂ into carbohydrates, which serve as primary energy source not only for the plant itself but also for heterotrophic organisms. Photosynthesis dominates leaf metabolism and it is divided into energy-capturing reactions (light reactions), where the photochemical electron transport chain provides energy for ATP synthesis and NADPH reduction; and carbon-fixing reactions (Calvin-Benson cycle) where ATP and NADPH are used to fix CO₂ into sugars phosphates. An intimate coordination between CO₂ assimilation and light-harvesting, necessary for an effective metabolism, relies on numerous regulatory mechanisms. The light-dependent redox signaling, which modulates the activity of specific steps in the Calvin-Benson cycle, is one of the more studied and yet not completely understood mode of this tuning. Our aim is to investigate the effects, in terms of photosynthetic performance, of uncoupling the redox-regulation of Calvin-Benson cycle. Redox-insensitive forms of fructose 1,6-bisphosphatase (FBPase), sedoheptulose 1,7-bisphosphatase (SBPase), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK), have been generated and transformed into *Arabidopsis thaliana* lines lacking the endogenous genes, providing redox-insensitive plants unable to inactivate the Calvin-Benson cycle enzymes upon light/dark transition and in response to light intensity variations. Also, observations on the single knock-outs raised awareness on possible alternative carbon fluxes potentially emerging in specific physiological conditions. This project will provide insights into fundamental aspects of central carbon metabolism of potential great value not only for the scientific community, but also for applied fields like agronomy and crop science, currently facing looming challenges in terms of providing food, fibre and fuel for an increasing world population.

Fructose 1,6-bisphosphate aldolase – the overlooked component of the Calvin-Benson cycle

presented by Dániel Árpád Carrera

Dániel Árpád Carrera, Gavin George, Samuel C. Zeeman, Sebastian Streb
ETH Zürich, Switzerland

Fructose 1,6-bisphosphate aldolase (FBA) enzymes have key functions in sucrose biosynthesis, glycolysis, gluconeogenesis and the Calvin-Benson cycle. They catalyze the reversible condensation of triose-phosphates to fructose 1,6-bisphosphate. Additionally, plastidial FBAs (pFBA) are known to catalyze the condensation of erythrose 4-phosphate and dihydroxyacetone phosphate to sedoheptulose 1,7-bisphosphate. Therefore, pFBAs have dual substrate specificity, with potential to control photosynthetic carbon flux through the cycle. A high degree of redundancy exists among FBA isoforms in plants; there are eight annotated FBA genes in *Arabidopsis thaliana*. Three of the proteins encoded by this family (FBA 1-3), which have very conserved protein sequences, are localized to the plastid. The respective single knock-out homozygous *Arabidopsis thaliana* mutants were isolated and investigated. While they are thought to catalyse the same reactions and occupy the same subcellular compartment, surprisingly the mutants show a distinct developmental and molecular phenotypes suggesting varying contributions in the Calvin-Benson cycle. *Fba1* mutants are indistinguishable from wild-type plants, but *fba2* mutant plants have a reduced growth and starch synthesis. In contrast *fba3* mutants show a severe reduced-growth phenotype with high sugar and starch contents, even though reduction in total FBA activity, like in the *fba2* mutants, could not be detected. Our working hypothesis is that FBA1 and mainly FBA2 catalyze the two proposed Calvin-Benson cycle reactions. FBA3 seems to have a different role critical for the plant's utilization of assimilated carbon for growth and development.

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Impact of the glyoxylate cycle on central carbon metabolism in the green microalga *Chlamydomonas reinhardtii* cultivated under day/night conditions

presented by Willamme Rémi

Willamme Rémi, Claire Remacle, Emilie Perez

University of Liege, Belgium

Biomass of microalgae can be used as sources for various products such as biofuels, biosourced polymers, antioxidants, and proteins for food and feed. As low light conditions are frequently encountered in our regions, it is of interest to add an organic carbon source to boost growth especially when cells are cultivated under day/night conditions, such as in the case of large-scale open-air cultivation projects. The model microalga *Chlamydomonas reinhardtii* is able to metabolize acetate via the glyoxylate cycle (GC), a shunt of the Krebs cycle which leads to synthesis of amino acids and glucose. In our work, we determine the diurnal accumulation profiles of major metabolites in cells adapted to light and dark intervals. We also take advantage of a mutant strain deficient for isocitrate lyase, one of the 2 specific enzymes of the GC, to investigate the role of this cycle on carbon metabolism. In addition to metabolomic data, we also present here results about our RNA-Seq-based transcriptome study on both mutant and control strains, allowing us to gain insight into the impact of diurnal gene expression regulation on central carbon metabolism through the glyoxylate cycle.

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Map-based cloning of Carbon Assimilation Rate 8 that increases CO₂ assimilation rate in rice

presented by Shunsuke Adachi

Shunsuke Adachi^{1,2,3}, Kazuaki Yoshikawa¹, Utako Yamanouchi⁴, Takanari Tanabata⁵, Jian Sun⁴, Toshio Yamamoto⁴, Rowan Sage^{2,6}, Tadashi Hirasawa^{1,2}, Junichi Yonemaru⁴

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⁴ National Institute of Agrobiological Sciences, Japan; ⁵ Kazusa DNA Research Institute, Japan; ⁶ University of Toronto, Canada

Increasing the CO₂ assimilation rate (A) of individual leaf is one important approach for increasing crop productivity. Exploiting the wide variation in A in natural genetic resources using quantitative genetics is one promising means to identify genes contributing to higher photosynthesis. Using a rice (*Oryza sativa*) population derived from a cross between a high-yielding indica cultivar, ‘Habataki’, and a Japanese commercial cultivar, ‘Koshihikari’, we compared A of the flag leaves under an ambient CO₂ concentration and saturated light in the paddy field. We then found Carbon Assimilation Rate 8 (CAR8) on the short arm of chromosome 8. CAR8 encodes a putative OsHAP3 subunit of a CCAAT-box-binding transcription factor called OsHAP3H. Sequencing analysis revealed that the ‘Habataki’ allele of CAR8 has a 1-bp deletion at 322 bp from the start codon, resulting in a truncated protein of 125 amino acids. The indica allele of CAR8, which increases A, is functionally changed by genetic complementation analysis. The increase of A is largely due to an increase of RuBP regeneration rate via increased leaf nitrogen content, and partially explained by reduced stomatal limitation via increased stomatal conductance relative to A. This allele also increases hydraulic conductivity, which would promote higher stomatal conductance. Detailed analysis of molecular function of CAR8 demonstrates specific genetic mechanisms that can be exploited to improve photosynthesis in rice and potentially other crops.

Session V: Chloroplast Structure/Assembly

Chaired by:

Dr Giovanni Finazzi

CNRS CEA Grenoble, France

Anna Matuszyńska

Heinrich Heine University Düsseldorf, Germany

Within this session, dedicated to chloroplast structure and assembly, the discussion will be focused on the interplay between structure and function in the plastidial physiology. We will bring together specialists in diatoms and microalgae physiology, investigating the adaptation capacity of these organisms and scientists involved in elucidate the shaping role of phosphorylation for the photosynthetic acclimation in *Arabidopsis thaliana*. Last but not least an insight about potential industrial and biotechnological applications of photosynthetic organisms as machinery for complex molecules production will be presented by our speakers.

Physiology and Biotechnology of electron derivation in Chlamydomonas reinhardtii

Keynote Speaker: Prof. Francis-André Wollman

Francis-André Wollman¹, Hiroko Takahashi, Wojciech Nawrocki, Han-Yi Fu, Yves Choquet, Manon Guille-Collignon, Frédéric Lemaitre, Guillaume Longatte, Daniel Picot, Fabrice Rappaport, Olivier Vallon

¹*Institut de Biologie Physico-Chimique, France*

Photosynthetic electron flow in organisms performing oxygenic photosynthesis requires extra ATP production for CO₂ fixation. Cyclic electron flow points to a branched pathway at the PSI acceptor side that is critical for phototrophic growth. Microalgae, such as Chlamydomonas reinhardtii, developed specific traits for photosynthetic electron derivation, as illustrated by algae-specific proteins the contribution of which will be discussed. Aside from the physiology of electron derivation, biotechnological strategies for using the redox power generated by photosynthesis are rapidly developing. A strategy for electron derivation that preserve algal phototrophy will be presented.

CrPBCP, a new chloroplast phosphatase involved in light acclimation in *Chlamydomonas reinhardtii*

presented by Federica Cariti

Federica Cariti¹, Marie Chazaux², Paolo Longoni¹, Xenie Johnson², Michel Goldschmidt-Clermont¹

¹ University of Geneva, Switzerland; ² CNRS - AMU, CEA Cadarache, France

In the green alga *Chlamydomonas reinhardtii*, a major component of short-term acclimation of the photosynthetic apparatus to changing light is the ability to distribute energy between photosystem II (PSII) and photosystem I (PSI). In response to changes in the redox state, light quality or quantity, the light harvesting complex (LHCII) can migrate between PSII and PSI in a process called “state transitions”. LHCII allocation to PSI is triggered by threonine phosphorylation; the kinase responsible for this process, Stt7, was identified more than a decade ago [Depège et al. 2003]. In *Arabidopsis thaliana* a kinase homologous to Stt7 (STN7) phosphorylates the LHCII while a single phosphatase (PPH1/TAP38) is mainly responsible for LHCII de-phosphorylation which favors its association to PSII. Another antagonistic pair of kinase (STN8) and phosphatase (PBCP), is involved in light acclimation in *Arabidopsis*. They are mainly involved in the phosphorylation / de phosphorylation of PS II core proteins (D1, D2, CP43 and PsbH). Here we show that a mutant in a putative CrPBCP orthologue is affected in different targets compared to *Arabidopsis*. Besides PSII core proteins, some LHCII components appear constitutively more phosphorylated in the Crpbcp mutant. Our preliminary results indicate that the roles of PBCP for light acclimation in *Chlamydomonas* differ from those of the plant orthologue, suggesting a different scenario for the regulation of LHCII phosphorylation in the green algae. The next questions we are addressing aim to elucidate the consequences of constitutive antenna phosphorylation and the role of PBCP phosphatase in *Chlamydomonas*.

The chloroplast phosphorylation network – Identification of new targets for STN7, STN8 and pCKII

presented by Sacha Baginsky

Sacha Baginsky

Martin-Luther University Halle-Wittenberg, Germany

We previously catalogued the chloroplast phosphoproteome under different environmental conditions and in different *Arabidopsis* genotypes. This work established kinase targets and provided insights into kinase-substrate relationships. Here, we give an update on our ongoing work to decipher this network and its regulation. One of the tools we established towards this goal is a phosphopeptide array (named ChlороPhos1.0) that was designed based on identified phosphorylation sites in chloroplast proteins. Using this array, we identified new *in vitro* substrates of plastid casein kinase II (pCKII) among them Rubisco activase (RCA), a pacemaker of the Calvin cycle. Using *pckII* knockdown plants, we confirmed the pCKII-dependent phosphorylation of RCA and some other targets *in vivo* and went ahead to study the physiological consequences of pCKII-depletion by metabolome profiling. We identified a significant re-organization of the metabolome in mutants compared to wildtype, with some of the metabolic differences being consistent with a change in RCA activity. Crosstalk between the regulation of photosynthesis and plastid gene expression is catalyzed by the thylakoid-associated kinases STN7 and STN8. Comparative phosphoproteomics with affinity enriched samples of the chloroplast genetic system identified several proteins involved in chloroplast RNA metabolism as new targets for STN7/STN8. We discuss the implications of these findings for our understanding of long-term acclimation and present our revised view on the chloroplast phosphorylation network.

Tuning of Light-Harvesting Complex II phosphorylation presented by Paolo Longoni

Paolo Longoni, Michel Goldschmidt-Clermont
University of Geneva, Switzerland

The major light-harvesting complex (LHCII) is a crucial component of the photosynthetic machinery, playing a central role in light capture and acclimation responses to changing light conditions. In *Arabidopsis thaliana* the major LHCII is constituted of homo- and hetero-trimmers composed of three classes of isoforms: Lhcb1, Lhcb2, and Lhcb3. These have different relative abundance, with Lhcb1 estimated to represent two thirds of the total, Lhcb2 one fourth and Lhcb3 approximately one tenth. The presence of these isoforms varies in different LHCII trimers associated to photosystems I and II, suggesting a specific role for each of them. Moreover, the two phosphorylatable isoforms Lhcb1 and Lhcb2, display distinct patterns and extent of phosphorylation, and play different roles in photosynthesis acclimation. In particular, phosphorylated Lhcb2 plays a central role of in stabilizing the PSI-LHCII supercomplex. The phosphorylated fraction for each isoform can be determined via a Phos-Tag based gel system. This information allows to quantify the extent of phosphorylation of Lhcb1 and Lhcb2 in different light condition. To further address the regulation of the (de)phosphorylation of each isoform, knock-out mutants of the LHCII-specific phosphatase PPH1/TAP38 and mutants affected in the formation of photosynthetic supercomplexes were analyzed. The results show that the regulation of LHCII phosphorylation level is partially independent of the presence of PPH1/TAP38 phosphatase and can be highly modulated in order to re-equilibrate the electron transport chain in mutant lines.

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Revealing the structural bases for light utilization in diatoms

presented by Serena Flori

Serena Flori¹, Pierre-Henri Jouneau², Benoit Gallet³, Christine Moriscot³, Simona Eicke⁴, Samuel Zeeman⁴, Dimitris Petroutsos¹, Guy Schoehn³, Denis Falconet¹ and Giovanni Finazzi¹

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Diatoms have been dominating marine photosynthesis over the past 35 million years, largely contributing to the evolution of contemporary oceans. Like plants, their photosynthesis converts solar energy into reduced carbon. Unlike plants photosynthesis in diatoms occurs within chloroplasts originated by a secondary endosymbiosis event, where no differentiation in the photosynthetic membranes between appressed regions (Grana= rich in photosystem II) and non-appressed regions (Stroma lamellae= rich in photosystem I) was reported. This raises the question of optimum photosynthetic light utilization, because absence of spatial segregation of the PSs might trigger energy loss by direct transfer from PSII to PSI (spillover). Using *Phaeodactylum tricornutum* as a model organism we evaluated the occurrence of spillover in diatoms. Time resolved absorption spectroscopy revealed that the two photosystems do not share excitation energy suggesting the existence of a physical segregation between the photosystems. Biochemical and immunolocalization analysis confirmed this conclusion revealing a refined compartmentation of photosystems between the external and the innermost thylakoid membranes. Using a Focus Ion Beam - Scanning Electron Microscopy we were able to reconstitute the three-dimensional architecture of the chloroplast in *Phaeodactylum tricornutum* and to propose a structural model for the arrangement of photosynthetic complexes in this chloroplast. This model accounts for optimum partitioning of absorbed light between the photosystems, without restraining electron flow capacity, as required for optimum photosynthesis.

CURT1-mediated thylakoid membrane plasticity is required for efficient photosynthetic performance and plant fitness

presented by Mathias Pribil

Wenteng Xu, Mathias Labs, Anurag Sharma, Omar Alejandro Sandoval Ibáñez, Carolina Galgenmüller, Qiuping Liu, Shizue Matsubara, Stefan Jansson, Małgorzata Wessels, Dario Leister, Mathias Pribil
University of Copenhagen, Denmark

Thylakoids of land plant chloroplasts are composed of grana stacks and stroma lamellae, substructures that confer a characteristic three dimensional ultrastructure to the membrane system and support a lateral heterogeneity with respect to the distribution of photosynthetic complexes. Upon changes in environmental conditions thylakoids can undergo various structural rearrangements which account for the high plasticity of the membrane system. The relevance of the dynamic changes in these substructures in terms of regulating photosynthetic processes is poorly understood and little is known about the mechanisms underlying their formation. In *Arabidopsis thaliana*, the CURT1 protein family was shown to be involved in grana formation by facilitating membrane bending in the grana margins. The degree of membrane bending is thereby correlated with the amount of CURT1 being present in the thylakoid membrane. While a lack of CURT1 proteins (*curt1abcd*) leads to a general loss in thylakoid membrane curvature the accumulation of CURT1A (*oeCURT1A*) causes a hyper-membrane bending phenotype. Here, the effects of altered CURT1 levels on photosynthetic performance and plant fitness under variable light conditions will be addressed and the influence of changes in the assembly and stability of CURT1 complexes will be discussed. Based on these studies, an approach to implement a modular protein-anchor technology to target, spatially confine, and cluster pathways involving multiple enzymatic steps in defined thylakoid membrane domains will be presented.

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Session VI: Communities

Chaired by:

Prof. Alison Smith

University of Cambridge, United Kingdom

Martina Angeleri

University of Turku, Finland

It is now well established that most if not all microbes exist in stable communities with several other species. For communities that include photosynthetic organisms, there is an added dimension in terms of metabolite exchanges and signal molecules. In this session, the presenters will show examples of both synthetic and natural communities that enable the study of the mechanism important for community structures, and ways that could have application in biotechnology. The presentations will be on eukaryotic algae, prokaryotic algae and bacterial communities and among the methods presented will be metabolic modelling, genetic screening, and metabolic engineering.

Chemical ecology of microalgal-bacterial communities

Keynote Speaker: Prof. Jon Clardy

Jon Clardy¹, Mo Seyedsayamdst, Rebecca Case, Roberto Kolter, Einat Segev, Thomas Wyche, Yunji Davenport, Elena Kazamia

¹Harvard University, USA

Microscopic phytoplankton like *Emiliania huxleyi* and *Prochlorococcus marinus* typically have closely associated heterotrophic bacteria, but the molecular nature of the interaction between the two, if any, is not well understood. A few years ago, we began to study the small molecule exchanges underlying the relationship between *E. huxleyi* and the bacterium *Phaeobacter inhibens*. That study, which revealed that the interaction involved both mutualist and antagonistic phases with the same small molecules recombined in various ways, will be described. We are making new efforts to expand the original study to include a broader range of molecular signals as well as extend the same general strategy to *P. marinus* and some of its bacterial symbionts.

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Industrial Ecology: Exploiting Natural Cues in Algal Biotechnology

presented by Sebastiana Roccuzzo

Sebastiana Roccuzzo, Andrew P. Beckerman, Jagroop Pandhal

University of Sheffield, United Kingdom

Microalgae offer a promising way to biofuels production, animals feed or additives. Open Ponds are regarded as the most economically viable option for large scale, industrial cultivation systems; however, many hurdles still exist, including the energy requirements for harvesting cells and their openness to invasion by other species and organisms like grazers, which can potentially damage the entire cultivation. However, invasive organisms interact with algae, which we propose are possible to exploit for industrial applications. In this study, we focused on chemical cues (infochemicals) produced by the grazer Daphnia, reported to induce a defense mechanism of colony formation in microalgae to reduce their vulnerability against predation. Colony formation leads to sedimentation of algal cells and hence could provide a low energy and environmentally friendly harvesting method., with no chemical contamination of the biomass. We undertook a meta-analysis of 70 studies to assess the effects of Daphnia infochemicals on colony formation of Scenedesmus spp, determining the specificity of their interactions. Microalgae strains, grazers' identity, feeding regime and density and time of exposure were considered; however, the most significant effects are due to type of grazer. Concurrently, an experiment was run to evaluate the role and efficiency of Daphnia infochemicals on triggering flocculation of *S. subspicatus*. Estimated by optical density measurements, sedimentation increased by an order of magnitude from water cultured with living organisms as compared to control. Invasive grazers may actually present a controllable, low energy and cost effective harvesting tool.

Investigating and Engineering Algal-Bacterial Communities presented by Anthony Riseley

Anthony Riseley, Chris Howe

University of Cambridge, United Kingdom

Traditional algal biotechnology has focused on growing axenic cultures. This is in contrast to nature, where no organisms live in isolation. However, it is increasingly recognized that growth of axenic cultures is challenging and therefore there is renewed interest in manipulating multi-species interactions. A specific and regulated mutualistic interaction has been described between a Rhizobiales bacterium, *Mesorhizobium loti*, and *Lobomonas rostrata*, a freshwater green alga, where the bacterium supplies vitamin B12 in return for fixed carbon. We attempted to understand this interaction further at the genetic level by screening *M. loti* mutants generated by transposon mutagenesis. As part of this study we identified a non-Rhizobiales contaminant capable of symbiosis with *L. rostrata*, presumably by providing B12. This novel symbiosis has been studied and compared to the original *M. loti* – *L. rostrata* interaction. Furthermore, attempts have been made to introduce a third organism into the *M. loti* – *L. rostrata* co-culture to provide a nitrogen source. *Anabaena* sp. PCC 7120 was chosen as a candidate due to its ability to fix its own carbon and nitrogen. Nitrogen provision to the media will be carried out by introducing engineered strains of *Anabaena* capable of providing amino acids or ammonium. The presentation will give an update on the current state of these projects as well as future plans.

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Identification of bacteria capable of supplying algae with vitamin B12

presented by Ulrich Johan Kudahl

Ulrich Johan Kudahl, Alison G. Smith
University of Cambridge, United Kingdom

Cobalamin, or vitamin B12 (B12), a cobalt containing corrinoid ring, is an essential cofactor for several enzymes, such as methionine synthase (METH) and ribonucleotide reductase (RNR II). Eukaryotic algae are unable to synthesise B12 and approximately half of eukaryotic algae are dependent on an external supply of B12 for survival. B12 synthesis, which involves over 20 enzyme-catalysed steps, is only known to be present in a subset of bacteria. In this study we set out to identify bacterial species that are capable of B12 synthesis and are able to supply eukaryotic algae with B12. We combined curated protein sequences for the enzymes from the two B12 synthesis pathways with translated genomes from 4202 bacterial species to identify presence or absence of more than 50 enzymes based on sequence homology. Based on the presence or absence of these enzymes, we have categorised each bacterial species as either capable of B12 synthesis or not. We showed that 40% of bacterial species are predicted to synthesise B12. By grouping species into taxonomical orders we found the frequency of the B12 synthesis trait varies greatly between groups, with more than 95% of cyanobacteria being able to produce B12, while only 10% of lactobacillus has this trait. Using this approached we have identified a subset of bacteria, that could be suitable to for co-culturing with B12 dependent algae.

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Dynamics of the bacterial community associated with Phaeodactylum tricornutum cultures

presented by Fiona Wanjiku Moejes

Fiona Wanjiku Moejes^{1,2}, Ovidiu Popa², Julie Maguire¹, Oliver Ebenhöh²

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The pennate diatom *Phaeodactylum tricornutum* is a model organism able to synthesise a number of industrially relevant molecules. Realising the industrial potential of microalgal-derived products relies on keeping large-scale monocultures which are prone to contamination by other organisms. However, little is known about the identity and characteristics of the invading organisms. In nature, diatoms are not found as isolated entities but rather are active members of a complex ecosystem, which is poorly understood. Bacteria, which have co-existed with diatoms for more than 200 million years, form a crucial part of this ecosystem and have been shown to enhance the growth of diatoms. Increased understanding of the interactions could allow for the exploration of ‘synthetic ecology’ as a novel scaling up technique. To gain insight into the dynamics of the bacterial communities associated with diatoms, we translated the complexity of a natural system into a reproducible, systematic experimental approach where we investigated the microbiome of batch grown non-axenic cultures of *P. tricornutum* (CCAP 1052/1B) using barcoded 16S-V6-Next-Generation-Sequencing. Our results reveal that the bacterial community associated with *P. tricornutum* cultures changes over time. We identified four main families, Alteromonadaceae, Pseudoalteromonadaceae, Flavobacteriaceae and Pseudomonadaceae, as major players within the microbiome. From our results, we propose a network of putative interactions between *P. tricornutum* and each of the bacterial factions, thus providing a framework to understanding the dynamics of diatom-associated microbial communities. Further species-specific co-culture experiments coupled with a metabolic profiling approach are on-going. Preliminary results show increased growth rates and maximal cell densities when *P. tricornutum* is co-cultured with representative members of the four identified families.

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Modeling ecosystems of standalone organisms and consortia

presented by Antonella Succurro

Antonella Succurro, Oliver Ebenhöh
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Constraint based methods like Flux Balance Analysis (FBA) are powerful ways to investigate complex metabolic networks, including Genome Scale Models (GSM). However, by investigating flux distributions at the steady state, information on metabolite concentrations or reaction kinetics is lost and we miss the description of the interaction between the organism and the environment (the ecosystem). In the case of mutualistic consortia between algae and bacteria, organic carbons are known to be often exchanged for cofactors such as Vitamin B12. However, the effect of cofactors is not captured by standard FBA and dynamic FBA (dFBA) methods. We can partially recover these important aspects in a dFBA model where reaction kinetic functions of choice (typically Michaelis-Menten) set the flux limits, FBA determines the reaction rate and a Ordinary Differential Equations (ODE) system for external and internal metabolite concentrations is solved at each step. This type of model is validated with the Escherichia Coli metabolic network for single organism evolution in time and is extended to deal with multiple organisms consortia (E. Coli mutants). Another approach implemented within the same framework ignores the genome scale information and reduces the model to a set of ODE. We used it to describe hypothetical interactions between Phaeodactylum tricornutum and its associated microbial community to validate the growth dynamics observed in experiments. These two approaches can be effectively combined to reduce the complexity of the problem.

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Session VII: Omics

Chaired by:

Prof. Natalia Battchikova

University of Turku, Finland

Johan Kudahl

University of Cambridge, United Kingdom

“-omics” techniques (genomics, transcriptomics, proteomics and metabolomics etc.) aim to the collective characterization and quantification of a complete set of biological molecules. In the “-omics” section at ENCAP16 we are interested in innovative technical approaches used in “-omics” studies as well as in recent, interesting results obtained using one or a combination of omics approaches. Omics studies are producing basic knowledge that can be combined and used in biotechnological and system biology applications.

Daily life of cyanobacteria

Keynote Speaker: Jun.-Prof. Ilka Maria Axmann

Wiegard A, Schmelling N, Beck C, Hertel S, Lehmann R, Guerreiro AC, Maarten Altelaar AF, Heck AJ, Axmann IM¹

¹*Heinrich Heine University Düsseldorf, Germany*

Cellular clocks allow organisms to anticipate the environmental cycles of day and night by synchronizing their internal, circadian rhythms with the rising and setting of the sun. In cyanobacteria the clock consists of solely three proteins - KaiA, KaiB and KaiC - orchestrating gene expression. Complex formation between Kai proteins and, therefore, their stoichiometry is essential in maintaining robust circadian oscillations. Thus, it is puzzling that several cyanobacteria, e.g. Synechocystis, contain multiple kai-gene copies. Our global transcriptomic analyses of light-dark synchronised Synechocystis cultures indicate a rather light-driven than a circadian regulated pattern in global gene expression. We detected several small RNAs encoded at the kai gene loci but antisense to kai genes which might be involved or even interfere with circadian regulation. Besides several other studies, we have already shown how small RNAs can influence the temporal regulation of gene expression. Thus, regulation by antisense RNA might be a fundamental mechanism for the daily coordination in cyanobacteria. Although the expression of many gene transcripts fluctuates over day and night, these were less pronounced at the protein level. Therefore, abundance and constituency were probed of protein complexes present in cyanobacteria using size exclusion chromatography-based proteomics. Following complexes such as the RNA polymerase, the ribosome and complexes involved in photosynthesis, we observe that these complexes change not only in abundance but also in constituency, with associated proteins being either present or absent. We conclude that the dynamic assembly of protein complexes is also a key-player in the processes governing the daily rhythm.

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Functional capabilities of the microbiome associated with cultures of *Phaeodactylum tricornutum*

presented by Ovidiu Popa

Ovidiu Popa¹, Fiona Moejes^{1,2}, Julie Maguire², Oliver Ebenhöh¹

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The pennate diatom *Phaeodactylum tricornutum* is a model organism whose associated microbiome, as well as its impact on the diatom life cycle is still debated. Recently, we could convey the complexity of a natural system into a reproducible, systematic approach by investigating the microbiome of batch grown non-axenic cultures of laboratory strains of *P. tricornutum* (CCAP 1052/1B) using barcoded 16S-V6-Next Generation Sequencing. We identified four main families, Alteromonadaceae, Pseudoalteromonadaceae, Flavobacteriaceae and Pseudomonadaceae, which dominate the bacterial community alternatively, with respect to the growth phase of *P. tricornutum* and the culture medium. Thus, each member of the bacterial community is occupying a specific role in the diatom-bacteria interaction network. Functional classification of the community metagenome allows us to identify and characterize the metabolic potential of the accompanying microbiome. Based on the information from the marker genes 16S rRNA we predicted the metagenome functional content using the bioinformatic package PICRUSt. Our analysis revealed a significant difference between the two different cultivation media regarding their inferred metagenome profile. Further we observed a different contribution of the four dominant bacterial families to the functional content over the cultivation period. Our findings give insights into the functional capability of each community member within the interaction network of *P. tricornutum* and its microbiome.

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Phosphoproteins of photosynthetic apparatus in *Synechocystis* 6803

presented by Martina Angeleri

Martina Angeleri, Dorota Muth-Pawlak, Eva-Mari Aro and Natalia Battchikova

University of Turku, Finland

Reversible protein phosphorylation is a well characterized post-translational modification widespread in eukaryotic organisms from plant to human. The reversible “O-type” protein phosphorylation of Ser, Thr and Tyr residues is very extensive, and Phospho-Ser/Thr/Tyr-dependent regulation is involved in many physiological processes like the cell cycle maintenance, cell differentiation, stress response, coordination of cell division, etc. Recent findings show that bacteria are able to perform the “O-type” protein phosphorylation, and cyanobacterial phosphoproteins are engaged in many biological processes. We have performed a global phosphoproteomic study of the model unicellular cyanobacterium *Synechocystis* sp. PCC 6803 using a shotgun mass spectrometry approach combined with phosphopeptide enrichment. Our results showed the occurrence of about 200 phosphoproteins which participate in a broad range of physiological functions including signaling and energy pathways as well as central carbon and nitrogen metabolism. At present, we are setting up the SRM quantitation of phosphorylated forms of proteins involved in light harvesting, photosynthesis and alternative electron flows. Suitable transitions have already been identified for most of the selected targets, and functional analysis of phosphoproteins is on-going. The investigation will provide new insights into regulation and optimization of light harvesting, photoprotection and energy conversion mechanisms leading to novel suggestions into an intelligent design of cyanobacteria as renewable and CO₂-neutral bio-factories producing biofuel, H₂ and high-value chemical compounds.

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Metatranscriptomic analysis of an industrial microalgal culture presented by Witold Januszewski

Witold Januszewski¹, Christoph Schaal¹, Tiago Guerra², Björn Voß¹, Wolfgang R. Hess¹

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Microalgae are organisms with a broad range of existing industrial applications, such as production of food supplements, colorants, cosmetics. Moreover, methods of industrial hydrogen and ethanol production are under development. Metatranscriptomic analyses of industrial microalgal communities are capable of both increasing efficiency of these methods and identifying sources of undesired contamination in the photobioreactors. An experiment aimed at determining the influence of the industrial scale-up effect (moving the culture to increasingly larger vessels) on the metabolic and regulatory networks in Chlorella vulgaris has been set up on industrial premises. It involved two rounds of sampling from flasks, green walls, small, medium and large-scale photobioreactors. A total of 9 samples were chosen for transcriptome analysis based on total RNA sequencing. Optical density, temperature, light intensity and nitrate ion concentration have been tracked to complement the analysis. A total of 182,8 mln reads were subjected to a de novo metatranscriptomic assembly of quality-trimmed reads and differential expression analysis. Furthermore, the data was taxonomically and functionally classified. The functional classification of the data confirmed significant expression rates of genes responsible for the metabolism of proteins, RNA, DNA, phosphorus, iron, nitrogen, sulfur, potassium and aromatic compounds as well as secondary metabolism in the Chlorella. Regulation and cell-signaling genes, mostly cAMP signaling, stringent response and quorum sensing proteins were also expressed significantly. Still, different levels of scale-up have varied level of expression of these genes, which might lead to differences in metabolic or regulatory processes.

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Metagenomics for Industrial Photosynthesis

presented by José Flores-Uribe

José Flores-Uribe, Oded Beja

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Algae for human and animal consumption have been cultivated for centuries. Production of high-value biologically active compounds in microalgae has increased interest in its industrial cultivation. One of the challenges that microalgae production at industrial scale faces is the prevention and management of biological contamination of the cultures. Many of the products derived from microalgae grown in photobioreactors require high purity and the effort to carefully maintain monocultures is economically justified, even though sterile operation of microalgae cultivation systems is economically challenging and difficult to achieve. Identification of microorganisms contaminating industrial cultures relies on microscopic observations and culture-based assays; however, both methods generally underestimate diversity of the samples. Cultivation-independent methods for the characterization of microbial diversity take advantage of sequencing technologies and high-throughput analysis to understand the structure and genomic potential of microbial communities in several environments. In this study we describe the diversity of microbial community on industrial Chlorella vulgaris cultures at different stages of the scale-up process using clone libraries and amplicon sequencing of the 16S rRNA gene. The analysis of the system and the community dynamics will serve as productivity indicators and basis for improved microalgae production in PBRs.

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Global metabolic regulation of snow alga *Chlamydomonas nivalis* in response to nitrate or phosphate deprivation by metabolome profile analysis

presented by Dong Wei

Na Lu¹, Dong Wei¹, Jun-hui Chen¹, Feng Chen^{1,2}, Gu Chen¹

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Chlamydomonas nivalis, a model species of snow algae, was used to illustrate the metabolic regulation mechanism of microalgae under nutrient deprivation stress in the present work. The seed culture was inoculated to the medium without nitrate or phosphate to reveal the cell response to these stress by metabolome profile analysis using GC/TOF-MS. One hundred and seventy-one of identified metabolites were clustered in five groups by OPLS-DA model. Among them, thirty metabolites in nitrate-deprived group and thirty-nine metabolites in phosphate-deprived group were selected and identified as “responding biomarker” by metabolomic approach. The significant change in abundance of biomarkers indicated that the enhanced biosynthesis of carbohydrate and fatty acids coupled with the decreased biosynthesis of amino acids, N-compounds and organic acids in all stress groups. The up- or down-regulation of these biomarkers in metabolic network provided new insights into the global metabolic regulation and internal relations within amino acid and fatty acid synthesis, glycolysis, TCA and Calvin cycle in the snow alga under nitrate or phosphate deprivation stress.

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Session VIII: Engineering

Chaired by:

Dr Patrik Jones

Imperial College London, United Kingdom

Julie Zedler

University of Kent, United Kingdom

Rapid progress in molecular biology and genetics is continually changing the landscape of algal and plant research and potential biotechnological applications. The belief that engineering biological systems can become more like the engineering of any hardware has inspired researchers to create powerful genetic tools for real-world applications. From synthetic genetic circuits to the expression of industrially relevant proteins this session will reflect the true interdisciplinary nature of cell engineering covering a broad spectrum of recent advances in the engineering of various “green” host organisms.

Synthetic plant biology: The ultimate way to ‘go green’ - Light-driven production of structurally complex diterpenoids

Keynote Speaker: Prof. Birger Lindberg Møller

Birger Lindberg Møller¹, Irini Pateraki, Allison Maree Heskes, Johan Andersen-Ranberg, Agnieszka Zygodlo Nielsen and Poul Erik Jensen

¹ University of Copenhagen, Denmark

With 12,000+ known structures, diterpenoids are a prime example of bio-active natural products produced by plants. Many are used as highly valuable pharmaceuticals, fragrances, natural plant growth promoters, food ingredients such as flavors or as colorants and spices. Unfortunately, they are typically produced in minute amounts in plants and their structural complexity render them difficult to prepare from fossil resources using organic chemical synthesis. Terpenoid synthases, cytochrome P450s and acyl transferases are key multienzyme families involved in diterpenoid synthesis. Using mass spec based imaging of the target plant tissue, tracer studies, single cell-type based metabolomics and transcriptomics, functional characterization of gene candidates using transient expression in tobacco and LC-MS-NMR based structural identification, elucidation of even highly complex biosynthetic pathways is now possible within a short time frame. Terpenoid metabolism is modular right from assembly of the C5 building blocks to the final structurally complex diterpenoid. Using the approaches of synthetic biology for combinatorial biosynthesis, the functional modules may be assembled in new combinations to expand the landscape of diterpenoid structural diversity into new-to-nature structures. The entire pathway for forskolin was elucidated. Forskolin is a cyclic AMP booster approved for treatment of glaucoma but also used as a weight loss aid. The forskolin pathway is being used as test model system for large scale light driven production of high value diterpenoids following targeting of the pathway to the thylakoid membrane and using cyanobacteria or moss as photosynthetic production hosts grown in contained photo-bioreactors.

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Enhanced secretion of recombinant proteins from Chlamydomonas reinhardtii

presented by Erick M. Ramos-Martinez

Erick M. Ramos-Martinez, Yumiko Sakuragi

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The green algae Chlamydomonas reinhardtii is a model microalgae and a potential industrial biotechnology platform for production recombinant proteins. There are several advantages to using green algae for biomanufacturing including low cost of production, safety, metabolic diversity, and scalability. Current strategies for expression of nuclear genome encoded recombinant proteins involve the secretion of the recombinant product into the media, thus product can be easily recovered by separating the biomass from the culture media. These approaches, however, have significant disadvantages including low product yields, hence recombinant protein accumulation in the media needs to be significantly enhanced in order to be economically sustainable. To expand the genetic tools available for secretion of recombinant proteins from Chlamydomonas reinhardtii, we successfully demonstrated the ability of gametolysin signal peptide to secrete recombinant protein by fusing the yellow fluorescent protein Venus. In order to increase the yield of secreted Venus we tested a glycosylation technology has been shown to dramatically increase secreted protein yields from plant cell culture systems by expressing proteins as fusion with a HypRP tag comprised of tandem Ser-Pro repeats. We designed the two synthetic HypRP tags (10 and 20 Ser-Pro repeats) and fused them to C-terminus of Venus, expression analysis showed higher molecular weight for Venus-SP 10 and Venus-SP 20 compared with the untagged protein. Yields of the Venus-SPs were dramatically increased as compared to the Venus lacking the Hyp-glycomodules. The results suggest that glycosylation of recombinant proteins in microalgae facilitates their secretion resulting in higher accumulation in the media.

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Modelling and engineering *Anabaena* sp. PCC 7120 for bio-fertilizer of synthetic communities

presented by David Malatinszky

David Malatinszky¹, Ralf Steuer², Dennis Nürnberg¹, John Rowland¹, Paulina Bartasun¹, Patrik Jones¹

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Industrially cultivated photosynthetic organisms utilise atmospheric CO₂ as carbon source, but rely on externally supplied combined nitrogen sources. In nature, however, several species evolved nitrogen fixation to grow on atmospheric nitrogen under conditions (diazotrophic) when only N₂ was available. Among these organisms filamentous cyanobacteria form highly specialized heterocysts to protect nitrogenase from inactivation by oxygen. These cells provide neighbouring vegetative cells with assimilable nitrogen in return for a source of carbon and electrons. To understand the metabolite exchange between vegetative cells and heterocysts we created a two-cell genome-scale stoichiometric model of *Anabaena* sp. PCC 7120. The potential metabolite exchange between the two cell types was found to be highly flexible, with several metabolite sets providing higher growth rates than those metabolites suggested in literature. Using flux balance analysis of diazotrophically grown filaments we investigated the stoichiometric yield of nitrogen-containing compounds. Excretion of ammonia achieved the highest yield and metabolic engineering strategies to increase its intracellular pool were therefore designed. In *Anabaena* sp. PCC 7120, the glutamine synthetase GlnA is responsible for the rapid assimilation of ammonia produced by nitrogenase. The level of this protein is natively controlled by a small polypeptide, IF7A, which was therefore overexpressed. Furthermore, the amt cluster encoding three ammonium uptake transporters was knocked out to abolish recapture of ammonia lost via diffusion.

Using Tat proteins for pathway organization of light-driven biosynthesis of natural products

presented by Maria Henriques de Jesus

Maria Henriques de Jesus, Thiagarajan Gnanasekaran, Agnieszka Zygallo Nielsen, Birger Lindberg Møller and Poul Erik Jensen

University of Copenhagen, Denmark

Photosynthesis drives the production of ATP and NADPH, and also acts as a source of carbon for primary metabolism. NADPH is consequently used in the production of many natural bioactive compounds. Many of these compounds are synthesized by cytochrome P450 monooxygenases, found in the endoplasmic reticulum in plants, using electrons derived from NADPH. Recently, we have demonstrated that it is possible to break the evolutionary compartmentalization of energy generation and P450-catalyzed biosynthesis, by relocating an entire P450 dependent pathway to the chloroplast and driving the pathway by direct use of the reducing power generated by photosystem I in a light dependent manner [1]. We demonstrate the potential to transfer pathways for structurally complex high value compounds by directly tapping into the reducing power generated by photosynthesis. Current work is directed towards the optimization of substrate channeling in order to improve product formation and reduce the formation of side products. Using the dhurrin pathway as a proof of concept, we attempt to increase the effective concentration of pathway intermediates by the co-localization of enzymes in the thylakoid membrane. This is achieved by fusing the relevant enzymes to components of the Twin-arginine translocation pathway – TatB and TatC. TatB and TatC are membrane anchored proteins that inherently self-assemble, which allows us to recruit enzymes into close spatial proximity. We show improved substrate channeling, reduced formation of side products, and consequently increased yields in tobacco. Parallel work is currently being carried out in the cyanobacterium *Synechocystis* sp. PCC6803.

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Using synthetic biology to engineer medicinal algae

presented by Payam Mehrshahi

Payam Mehrshahi, Alison Smith
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An aging and ever-growing world population demand for food, fuel and pharmaceuticals is fast outstripping sustainable supply. Researchers have demonstrated that adoption of industrial biotechnology platforms for synthesis of high-value and high-demand products provides a means to improve the sustainable supply bottleneck. While large-scale bacterial and yeast platforms are mature industries, there are biological constraints on the range of molecules that can be synthesised by these organisms. Photosynthetic algal platforms have been shown capable of production of diverse and complex compounds, including vitamins such as carotenoids (vitamin A), oils (such as triacylglycerol) and pharmaceuticals (cancer-targeting peptides). However, despite these success stories, low yields reported for many target products is a major obstacle to up-scaling and industrial viability of algal platforms.

In this project we are exploiting the green alga, *Chlamydomonas reinhardtii*, as a chassis organism for scalable and high-throughput production of anti-cancer medicinal compounds. We are taking a synthetic biology driven approach, following the doctrine of design, build, test to overcome the scalability constraints associated with first-generation engineered algal platforms. Specifically, our research is defining bottleneck steps in the biosynthesis of precursor isoprenoids, which form the backbone of the mono- and diterpenoids with medicinal properties. Using functionally defined standardised parts, these bottleneck steps are being lifted, while efficient and controlled expression of multi-heterologous gene cassettes, coupled with specific subcellular targeting, is allowing the synthetic engineering of complex pathways for production of compounds of interest.

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BioSC Session: Innovative Algae Research for a Bio-based Economy

Chaired by:

Prof. Roberto Bassi

University of Verona, Italy

Fiona Wanjiku Moejes

Daithi O'Murchu Marine Research Station, Ireland

Heinrich Heine University Düsseldorf, Germany



This session is co-organized by the **Bioeconomy Science Center (BioSC)**.

This session aims to address issues associated with the scaling up of algae cultures from lab-scale to large outdoor ponds and bioreactors. It will introduce innovative techniques to optimise large-scale algal cultures, including novel production techniques, and the optimisation of bioreactor production. The session will also look at the use of molecular biology techniques to gain insight into the enhancement of high-value compound production as well as fatty acids for biofuel applications in algae.

Direct more CO₂ towards oil through development of new oleaginous microalgae strains

Keynote Speaker: Dr Laurent Fourage

Séverine Collin¹, Frédéric Laeuffer², Jean-Michel Brusson³ and Laurent Fourage¹

TOTAL ¹*New Energies Division;* ²*Refinery and Chemistry;* ³*Scientific Direction*

TOTAL – An international oil and gas company based in France - is developing new energies that can partner oil and gas: solar today and, tomorrow, biomass. Total is involved in a variety of partnerships and R&D projects to develop biomass conversion pathways, including phototrophic microalgae. Today, several technological barriers prevent the use of phototrophic microalgae for the production of low-cost biomolecules, for fuels and chemicals. Thus, long-term focused R&D is still required before considering scale-up and therefore TOTAL New Energies is pursuing exploratory R&D projects on microalgae to assess the long-term feasibility of low-cost production. These initiatives focus on two main areas: improving microalgae strain performances and developing low-cost, energy-efficient, robust and sustainable process. For instance, as optimizing the properties of eukaryotic oleaginous microalgae is crucial to lower production costs, TOTAL, through participative collaboration, has developed the 1st tool-box for gene deletion in 2 eukaryotic microalgae species. TOTAL is now focusing on metabolic engineering, using a panel of developed tools, to assess their performances at lab scale and reduce large scale production costs. In addition, TOTAL is assessing potential impacts of large scale cultivation on the environment, resources (water, fertilizer, CO₂, land area) and how to master them.

Oil is on the agenda: metabolism in *P. tricornutum*

presented by Kirstin Feussner

Kirstin Feussner¹, Jennifer Popko¹, Till Ischbeck¹, Richard Haslam², Johnathan Napier², Inna Khozin-Goldberg³, Cornelia Herrfurth¹, Ivo Feussner¹

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Diatoms are of special interest because they are a major component of phytoplankton communities and believed to be responsible for 1/4 of the global productivity. *Phaeodactylum tricornutum* serves as model organism for studying the molecular physiology of diatoms, because its genome has been sequenced and a transformation system is available. Moreover, it produces high amounts of the very long chain-polyunsaturated fatty acid (VLC-PUFA) eicosapentaenoic acid (EPA, 20:5 Δ 5,8,11,14,17). Its fatty acid (FA) profile harbors beside EPA, mainly palmitic acid (16:0) and palmitoleic acid (16:1 Δ 9) whereas C18 FAs can barely be detected. This unique FA profile makes *Phaeodactylum* very well suited for food and feed applications since it is rich in EPA as well as for the production of feed stocks for the chemical industry out of the mid chain-FA fraction. During stationary phase or nitrogen starvation diatoms accumulate triacylglycerols (TAG) in lipid droplets. However, till now it is neither known which metabolic pathways and membrane lipids serve as precursors for the TAG formation nor from which cellular membrane these TAG species derive. To contribute to this open question and to increase TAG production, lipidomic and metabolomic analysis of the exponential and stationary growth phase were performed. As expected, the amount of neutral lipids increased during stationary phase and predominantly 16:0 and 16:1 accumulated in the TAG fraction. Additionally, the amounts of different glyco- and phospholipids were analyzed as well as central and specialized metabolites. The obtained data provide now a deeper insight into the lipidome and metabolome of *P. tricornutum* and will help to understand the metabolic pathways of lipid production and storage in diatoms.

Different intensity of photosynthetic light energy enhanced biodiesel production on marine algae diatoms

presented by Natanamurugaraj Govindan

Natanamurugaraj Govindan, Mashitah.M Yusoff, Mohd Hasbi Ab. Rahim, Gaanty Pragas Maniam
University Malaysia Pahang, Malaysia

Investigate the effect of light intensity on the growth rate, chlorophyll a and lipid content in the selected algae diatom *Amphora copulata* and *Gyrosigma* sp. from the Balok Coast, East Coastal region of Peninsular Malaysia. Microalgae were isolated and cultured in f/2 medium under controlled conditions. The diatoms were cultured under different light intensities, which are 6, 10, 16 and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at the temperature $25 \pm 2^\circ\text{C}$ with a regimen of 16 hours and 8 hours light: dark cycles. Growth rate, chlorophyll a, lipid content and fatty acid compositions of the diatoms were studied. The growth rates of *Amphora* sp. and *Gyrosigma* sp. both recorded the highest point for the highest light intensity (40 $\mu\text{mol m}^{-2}\text{s}^{-1}$) which is 0.2860 d^{-1} and 0.3067 d^{-1} respectively. The highest dry cell weight resulted using the highest light intensity as well where both documented 0.1354 g and 0.1683 g. At the highest light intensity, the chlorophyll a readings were 0.1779 ± 0.0059 and 0.1744 ± 0.0133 . The micro algal oils extracted from the freeze dried microalgae sample using the solvent extraction method and converted it into biodiesel through transesterification process. The purity of biodiesel samples were analyzed by using Gas Chromatography (GC). The productivity of micro algal oils was higher than other conventional crops. Microalgae had been highlighted as a good source for biodiesel production. The strength of light energy is believed to influence the growth of microalgae, chlorophyll a, micro algal biomass and its lipid content for biodiesel production purposes.

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Mass balance analysis of carbon and nitrogen in industrial scale mixotrophic microalgae cultures

presented by L. Tiago Guerra

L. Tiago Guerra, Ana Barros, Manuel Simões, Edgar Santos, Diana Fonseca, Joana Silva, Luís Costa
A4F Algae for Future, Portugal

Large-scale cultivation of *Chlorella vulgaris* is of great interest given the extent of products and potential applications that can derive from its biomass. From an industrial point of view it is necessary to consistently obtain high productivities at the lowest production costs. The mass balance of critical nutrients such as carbon and nitrogen is therefore necessary to quantify its recovery and consumption yields and efficiency of the biomass production system. The mass balance of *C. vulgaris* mixotrophic growth throughout scale-up from 10 m³ to 100 m³ on acetate and urea as carbon and nitrogen sources resulted on recovery factors of 0.99±0.08 and 0.99±0.25, respectively. Under these conditions *C. vulgaris* growth rate was highest at the 10 m³ scale and decreased progressively with the increase in scale. Global carbon and nitrogen yields of 0.76 mol_{C-X} mol_C⁻¹ and 0.72 mol_{N-X} mol_N⁻¹ were obtained throughout the cultivation. The mass balance determination indicates the incorporation of both acetate and urea carbon atoms into the biomass. Therefore, external inorganic carbon from CO₂ was concluded to have little influence on microalgae growth in the conditions studied apart from pH control. The observation of ammonium in the medium reveals a fast and unregulated transformation of Urea to ammonium that needs to be constantly monitored as both urea and ammonium were found to be effectively and simultaneously used by *C. vulgaris* cells.

Improving polyunsaturated fatty acid (PUFA) and triacylglyceride (TAG) accumulation in *Phaeodactylum tricornutum*

presented by Nodumo Zulu

Nodumo Zulu, Jennifer Popko, Ivo Feussner

University of Göttingen, Germany

Although microalgal products such as polyunsaturated fatty acids (PUFAs) have received a great deal of attention, there are still challenges impeding on their commercial production. PUFAs occur mostly in membrane lipids; thus making their extraction expensive. It is imperative that they are deposited in triacylglycerides (TAGs) because they are cheaper to handle during downstream processing. This project was conducted with the aim of enhancing TAG accumulation in *P. tricornutum* (Pt4, a strain growing with low salt concentrations) and to increase PUFA levels in the TAGs. To reach this goal, the TAG-forming enzyme acyl-CoA:diacylglycerol acyltransferase from yeast (*ScDGA1*) and the stabilizing lipid droplet protein oleosin from *Arabidopsis thaliana* (*AtOleo3*) were expressed in Pt4. The impact of these proteins on biomass production, TAG accumulation and composition was investigated. Biomass productivities for the improved Pt4 and wild type were calculated to be 3.47×10^6 and 4.4×10^6 cell ml $^{-1}$ day $^{-1}$, respectively. The improved Pt4 has a TAG productivity of $3.6 \mu\text{g mg}^{-1} \text{ day}^{-1}$, which is 4 folds higher than that of the wild type ($0.8 \mu\text{g mg}^{-1} \text{ day}^{-1}$). Total lipid productivities for improved Pt4 and wildtype were calculated to be 4.9 and $1.9 \mu\text{g mg}^{-1} \text{ day}^{-1}$, respectively. Furthermore, it can be concluded from the data that the simultaneous expression of *ScDGA1* and *AtOleo3* has a significant impact on TAG accumulation in comparison to their individual expression (p value < 0.05). However, PUFA levels in TAGs were not significantly increased; this could imply that the *ScDGA1* enzyme has a low specificity towards the PUFAs, which is in contrast to what has been reported. To unravel this, the distribution and composition of DAG and TAG molecular species and the available acyl-CoA pool will be looked into. The improved Pt4 will be cultivated in photobioreactors at 8L scale using the industrial fertilizer for growth.

Forward genetics toward the light-use efficiency improvement of *Nannochloropsis gaditana*

presented by Giorgio Perin

Perin G., Bellan A., Segalla A., Meneghesso A., Alboresi A. and Morosinotto T.

University of Padova, Italy

When microalgae are cultivated in a photobioreactor or pond, the high cells densities cause an inhomogeneous light distribution. Consequently, cells at the peripheral layers absorb most of the radiation, saturate their photosynthetic ability and dissipate most of the harvested energy. On the other side, limited energy reaches the cells in inner layers to support their metabolism. This fact causes a strong reduction of the overall solar light to biomass conversion efficiency, limiting productivity and therefore the development of economically competitive industrial processes based on microalgae biomass. Strains with a tuned composition of the photosynthetic apparatus have the potential of enhancing overall productivity by improving light distribution and energy conversion mechanisms. To meet these needs, we generated a collection of *Nannochloropsis gaditana* random mutants and selected strains for alterations in their photosynthetic properties. We used two major phenotypes as selection criteria, the reduction in Chl content and in the ability to activate heat dissipation mechanisms. When tested in industrial-simulating growth conditions, both phenotypes indeed proved to be effective in enhancing biomass productivity. Their theoretical advantage is achieved thanks to a higher photosynthetic efficiency with a consequent higher electron transportation rate. Physiological, biochemical and genome-wide analysis were carried out to understand the molecular origin of these phenotypes, highlighting a minor remodeling of the photosynthetic apparatus. This work represents a proof of concept that genetic engineering efforts needs to be directed toward a fine tuning of photosynthesis, since expensive photosynthetic alterations would most likely negatively affect productivity.

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Intracellular Spectral Recompositioning of Light: A Novel Strategy for Improving Photosynthetic Efficiency of Diatoms

presented by Weiqi Fu

Weiqi Fu^{1,2}, Amphun Chaiboonchoe¹, Mehar Sultana¹, Kourosh Salehi-Ashtiani¹

¹*New York University Abu Dhabi, United Arab Emirates;*² *University of Iceland, Iceland*

In order to concurrently address the global resource scarcity and impending climate change, the use of photosynthetic diatoms can provide an exciting solution for sustainable production of biofuels and bioactive compounds. However, photosynthetic efficiency in diatoms needs to be optimized to reduce the energy costs for sustainable production. We have developed and implemented a strategy herein referred to as Intracellular Spectral Recompositioning of light (or ISR), which can increase the quantum yield of light if the otherwise wasted portion of blue light is shifted to green, which diatoms have evolved to harvest through accessory pigments. We demonstrate that ISR can be employed chemically or biogenically to improve photosynthesis in the diatom *Phaeodactylum tricornutum* in photobioreactors. Genetically engineering diatom cells with enhanced green fluorescent protein (EGFP) achieved desired spectrum recombination with high photo-stability. These engineered strains exhibited higher efficiency in photosynthesis than the wild type by 30%. This increase can be attributed to an increase of quantum yields in photosystem II in diatom cells under light stress conditions. Long-term cultivation experiments demonstrated the stability of EGFP transformants and the robustness of GFP expression upon the presence of nitrate. Pond simulator experiments also observed EGFP transformants could outperform their wild type counterpart. In addition, genome-scale transcriptome and gene set enrichment analysis of the obtained strains were presented in comparison to the wild type to characterize these strains at the system level. The ISR approach is expected to be broadly applicable toward improving energy efficiency for cultivation of microalgae at industrial production scales.

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Session X: Industrial Cultivation

Chaired by:

Dr Vitor Verdelho

A4F Algae for Future, Portugal

Artur Włodarczyk

University of Copenhagen, Denmark

This session is a follow-up of the “Innovative Algae Research for a Bio-based Economy” session. It combines metabolic engineering approach together with generation of computational models for increasing the productivity – both growth and product yields. It will mostly focus on scaling up of the microalgal cultures to the industrial levels and emphasize major bottlenecks related to the topic. Furthermore it will bring up the contamination of microalgae and diatom cultures issue and also identification and possible exploitation of the multi-species interactions for the industrial applications.

Characterisation and optimisation of mixotrophic growth in Phaeodactylum tricornutum

presented by Valeria Villanova

V Villanova¹, AE Fortunato², M Conte³, T Obata⁴, D Singh⁵, A Falciatore², E Marechal³, Allisdair Fernie⁴, Mark Poolman⁵, J Pagliardini², A Le Monnier¹, G Finazzi³, D Petrotousou³

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Diatoms are photosynthetic organisms with a strong influence on the global biogeochemistry. Among diatoms, the pennate *Phaeodactylum tricornutum* is considered a potential candidate for biotech applications, via TAGs production for biofuel. However, under strict phototrophic regime, its productivity is not compatible with industrial standards. On the other hand, while *P. tricornutum* is not an heterotroph, it can use a carbon source in the light via mixotrophy. The simultaneous use of photosynthesis and respiration largely increases biomass productivity and reduce the energy cost for its industrial exploitation. Previous works have shown that *Phaeodactylum* is capable to grow in mixotrophy in presence of different carbon sources such as glycerol, acetate, glucose and fructose. So far, glycerol is the best candidate for enhancing biomass and lipid productivity. However, there are little information of how the glycerol is used by this alga. During my PhD I have studied the glycerol metabolism and the mechanisms of mixotrophy combining metabolomic, transcriptomic, lipidomic and physiology approaches. All together, these analyses have elucidated the main pathway used by the glycerol to support growth and lipid production. Moreover, the development of a new growth medium have largely improved both uptake of inorganic (HCO_3^-) and organic (glycerol) carbon to boost productivity. In these optimized conditions, mixotrophic cells increased the biomass concentration of about 78% comparing to phototrophic control.

Modelling metabolism of the diatom *Phaeodactylum tricornutum*

presented by Dipali Singh

Dipali Singh¹, Mark Poolman¹, David Fell¹, Ross Carlson²

¹Oxford Brookes University, United Kingdom; ²Montana State University, U.S.A.

Diatoms are photoautotrophic unicellular algae and are among the most abundant, productive and environmentally adaptable marine phytoplankton. In contrast to higher plants, diatoms possess different localisation and regulation of the Calvin cycle enzymes and the oxidative pentose phosphate pathway. Recently, Entner-Doudoroff and phosphoketolase pathways, commonly found in prokaryotes, have been reported to be present in the model diatom *Phaeodactylum tricornutum*. The major storage molecules of diatoms are triacylglycerols and polysaccharides, and this capability has raised new possibilities to increase algal oil production as an alternative source of energy. The goal of this project is to investigate the metabolism of *P.tricornutum* using a genome scale metabolic model (GSM), which describes the metabolic interactions in a given organism based on the reaction network predicted from enzymes encoded by the genome. This investigation is an effort to better understand the biochemistry of diatoms, particularly lipid synthesis, in order to make them an economical algal strain for biofuel production. A GSM of the *P.tricornutum* has been constructed and is analysed using Flux Balance Analysis (FBA). It is capable of producing all major biomass components under a range of realistic environmental conditions. Here we present FBA results from a mixotrophic condition where the source of energy is light and organic carbon, glycerol. It shows that acetyl-CoA, the precursor for lipid synthesis, can be produced through the phosphoketolase pathway and/or via pyruvate dehydrogenase, which not only reflects the metabolic potential of this organism but also highlights the importance of the presence of the phosphoketolase pathway.

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Expression of an active plant cytochrome P450 in the chloroplast of the green alga *Chlamydomonas reinhardtii* and cultivation of transgenic strains in an industrial setting

presented by Doris Gangl

Doris Gangl¹, Julie A. Z. Zedler¹, Artur Włodarczyk², Tiago Guerra³, Edgar Santos³, Vitor V. Verdelho³, Poul Erik Jensen², Saul Purton⁴ and Colin Robinson¹

¹University of Kent, United Kingdom; ²University of Copenhagen, Denmark; ³A4F Algae for Future, Portugal; ⁴University College London, United Kingdom

Chlamydomonas reinhardtii has great potential as a cell factory for the production of recombinant proteins, but mainstream adoption has been hindered by a scarcity of genetic tools and a need to identify products that can be generated in a cost-effective manner. A promising strategy is to use algal chloroplasts as a site for synthesis of high value bioactive compounds such as terpenoids, requiring metabolic building blocks that occur naturally in the chloroplasts. However, synthesis of these complex plant metabolites requires the introduction of membrane-associated enzymes including cytochrome P450 enzymes (P450s). We show that a gene encoding the model P450 CYP79A1 can be expressed in the *C. reinhardtii* chloroplast in an active form using a simple, low-cost transformation system. The gene is stably expressed and the enzyme is efficiently targeted into chloroplast membranes by means of its endogenous N-terminal anchor domain. We further show that two transgenic strains expressing CYP79A1 and the bifunctional diterpene synthase TPS4 can be successfully cultivated at pilot scale in industrial bioreactors. To date only a single study has reported growth data for *C. reinhardtii* grown at pilot scale and the growth of cell wall-deficient strains has not been reported at all. The transgenic strains were grown for 7 days under mixotrophic conditions in a tris-acetate-phosphate medium. They reached dry cell weights of 0.3 g/L within 3-4 days with stable expression levels of the recombinant proteins during the whole upscaling process. The strains proved to be generally robust, despite the cell wall-deficient phenotype, but grew poorly under phototrophic conditions. The data indicate that cell wall-deficient strains may be highly amenable for transformation and suitable for commercial-scale operations under mixotrophic growth regimes.

Chlamydomonas reinhardtii as a platform for sustainable diterpene production

presented by Julie A. Z. Zedler

Julie A. Z. Zedler¹, Doris Gangl¹, Tiago Guerra², Kamil Bakowski³, Trine Bundgaard Andersen³, Edgar Santos², Vitor Verdelho², Poul Erik Jensen³, Björn Hamberger⁴, Saul Purton⁵, Colin Robinson¹

¹University of Kent, United Kingdom; ²A4F Algae for Future, Portugal; ³University of Copenhagen, Denmark; ⁴Michigan State University, USA; ⁵University College London, United Kingdom

Microalgae are emerging as an alternative, sustainable biotechnological platform. Most studies focus on the production of recombinant proteins such as oral vaccines, but the synthesis of other high value compounds in microalgae by genetic engineering is also being explored. This proof-of-concept study investigates the suitability of microalgae for the production of high value diterpenes using the model green alga Chlamydomonas reinhardtii. Many terpenes are too complex for chemical synthesis and are currently extracted from plants, their natural source, at low yields. In microalgae, diterpene catalysis can be integrated with the natural metabolism providing an attractive alternative to current methods. We show that the bifunctional diterpene synthase TPS4 can be expressed recombinantly in the chloroplast at stable levels. Our data also indicate that the enzyme is active, producing the diterpene cis-abienol. In a further study we also investigated the suitability of growing the transgenic strain at a 100L pilot scale. These results are promising for further development of diterpene production in microalgae.

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Posters - Session I

Tuesday, 26th April 2016

17:00 - 18:30

Posters Number 1 to 15

1 Microalgal growth modelling and nutrients uptake in response to light and temperature

presented by Ana L. Gonçalves

Ana L. Gonçalves, José C.M. Pires, Manuel Simões
University of Porto, Portugal

Cultivation of microalgae and cyanobacteria has been intensified in the last decades, due to the numerous applications described for these microorganisms. However, the high process costs associated to biomass production systems reduce the economic feasibility of microalgal/cyanobacterial cultivation. To improve biomass productivities and reduce the costs associated to the optimization of culture parameters, a better understanding of the effects of light and temperature on growth kinetics is required. In this study, the effects of incident light and temperature on growth and nutrients uptake was assessed using *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Additionally, a mathematical model relating specific growth rates with these variables was developed. Both kinetic growth parameters and nutrients removal have shown a similar response to light and temperature: increasing light supply, higher specific growth rates, biomass productivities and nutrients removal efficiencies were achieved. Among the studied temperatures, all microorganisms presented higher biomass productivities and nutrients removal efficiencies at 25 °C. Regarding the results from the mathematical model, optimal temperature for the studied microorganisms was approximately 25.0 ± 1.3 °C. On the other hand, optimal light irradiances varied with the light:dark ratio; for longer light periods a decrease in optimal irradiance values was observed.

2 Microbial consortia: a way forward for the commercialisation of microalgae for industrial biotechnology?

presented by Christian J A Ridley

Christian J A Ridley, Alison G Smith
University of Cambridge, United Kingdom

Algal biotechnology has so far struggled to achieve economically and energetically viable algal cultivation. There are many reasons for this, including the loss of yields at scale and contamination by adventitious organisms. In recent years, it has become apparent that algae are dependent on bacteria for the provision of nutrients, and algal-bacterial symbioses are common in nature (Croft et al., 2005). By understanding and exploiting these interactions, it may be possible to ameliorate some of the issues facing algal biotechnology. In this presentation, I will describe some of the ways that I have enhanced algal culture productivity and stability through the use of microbial consortia. A model algal-bacterial consortium was used between the green alga *Lobomonas rostrata* and the bacterium *Mesorhizobium loti*. Unfortunately, this co-culture exhibited a lower growth rate than axenic *L. rostrata*. Nonetheless, I characterised the effects of contaminating bacteria on *L. rostrata* and *L. rostrata* + *M. loti*, and using two of these bacteria I was able to boost the growth rate of the consortium to be the same as axenic *L. rostrata*. I also determined that the invasion of contaminating bacteria into algal cultures was reduced in *L. rostrata* + *M. loti* cultures compared to axenic *L. rostrata*. Using another consortium (*Phaeodactylum tricornutum* + natural bacterial assemblages), I showed that the presence of bacteria enhanced lipid (TAG) productivity by up to 100%. In conclusion, microbial consortia can be significantly more productive and stable than axenic algal cultures, and should be explored more in an industrial context.

3 Valorization of the aqueous phase from hydrothermally treated remnant biomass of *Dunaliella salina*

presented by Kristin Pirwitz

K Pirwitz¹, L Rihko-Struckmann¹, K Sundmacher^{1,2}

¹*Max-Planck-Institut Magdeburg, Germany*; ²*Otto-von-Guericke-University Magdeburg, Germany*

The green microalga *Dunaliella salina* is an industrially exploited organism for natural β-carotene production. After the extraction of the pigment up to 90% biomass remains unexploited in the process. Valorization of this remnant biomass can improve the overall process economics significantly. The potential of hydrothermal liquefaction (HTL) to exploit the residual biomass as source of valuable by-products was assessed. Initially, the macromolecular and elemental biomass composition was determined to identify possible liquefaction products. To test the economically most feasible biomass treatment, moderate temperatures between 100-200°C were investigated. As the HTL experiments resulted mainly in the conversion of biomass into water-soluble components, aqueous phase products were identified. The analyses indicated that 80% of the applied biomass was converted into glucose. The recovered glucose was successfully used as carbon source to cultivate biotechnologically relevant microorganism, namely *Chlorella vulgaris*, *Escherichia coli* and *Saccharomyces cerevisiae*. One of the main challenges of HTL is the considerable energy consumption due to the high operation temperatures and pressures used in the process. Therefore, energy consumption and operating costs for the applied liquefaction condition were calculated based on a process model. The cost analysis confirmed the beneficial effect of mild liquefaction on the overall process economics.

4 A single *Arabidopsis* gene encodes two differentially targeted geranylgeranyl diphosphate synthase isoforms essential for the production of photosynthesis-related plastidial and extraplastidial isoprenoids

presented by M. Águila Ruiz-Sola

M. Águila RUIZ-SOLA¹, M. Victoria Barja, Alex GRAF, Ralf WELSCH, Wilhelm GRUISSEM

¹ETH Zürich, Switzerland

Isoprenoids are essential secondary metabolites for plant function and development. A wide diversity of isoprenoids is produced in plastids, where many of them play important roles in photosynthesis. Most groups of plastidial isoprenoids and some synthesized elsewhere in the plant cell derive from geranylgeranyl diphosphate (GGPP), which is generated from universal isoprenoid precursors by the enzyme GGPP synthase (GGPPS). In agreement with the requirement of GGPP in multiple subcellular sites, plants usually have gene families encoding GGPPS enzymes targeted to different cell compartments. In *Arabidopsis thaliana*, 5 genes appear to encode GGPPS isoforms localized in plastids, the endoplasmic reticulum, and mitochondria. However, we have seen that single loss-of-function of GGPPS11, the most abundant isoform in vegetative tissues, is sufficient to cause lethality. Our work demonstrates that the absence of a strong transcription initiation site in the GGPPS11 gene results in the production of transcripts of different lengths. The longer transcripts encode an isoform with a functional plastid import sequence that produces GGPP for the major groups of photosynthesis-related plastidial isoprenoids. The shorter transcripts produce a short isoform lacking this N-terminal domain that retains GGPPS activity, localizes in the cytosol, and is essential for embryo development. Finally, we have shown that GGPPS11 plastidial protein can physically interact with the enzymes that transform GGPP into the first committed intermediates of the downstream pathways to possibly mediate channeling of GGPP precursors towards the production of different isoprenoid end-products.

5 Ethanol pathway optimization in *Synechocystis* sp. PCC 6803

presented by Paulina Bartasun

Paulina Bartasun, Patrik Jones

Imperial College London, United Kingdom

Depletion and rising cost of fossil fuels generate interest in alternative methods for production of hydrocarbon chemicals. Genetic modification of photosynthetic microorganisms can redirect metabolic pathways towards valuable end-products. Cyanobacteria represent an interesting host with promising potential for sustainable light-driven biotechnology. In the DEMA (Direct Ethanol from MicroAlgae) project we use a model cyanobacterium, *Synechocystis* sp. PCC 6803 for biosynthesis of ethanol. Introduction of a non-native, synthetic pathway is likely to cause disturbance in the host metabolism and may lead to accumulation of a toxic intermediate, low flux through the pathway or protein burden in cells, all resulting in low product yield. To improve the performance of the DEMA ethanol producing strains we are investigating the balance of the target product pathway through the modulation of enzymes expression (pyruvate decarboxylase - pdc and aldehyde dehydrogenase - adh) at the translation level. We have created a library of 25 strains and investigated the influence of five RBS sequences placed upstream of pdc and adh on the ethanol production. SRM Mass spectrometry (LC-MS/MS) was used to provide a relative measure of the quantity of each enzyme in the pathway. The RBS library approach enabled us to obtain variation in gene expression and ethanol production in *Synechocystis* sp. PCC 6803. This encouraged us to apply this strategy for balancing more complex pathways involving not only pdc and adh enzymes but also enzymes influencing cell catabolism and the rate of photosynthesis.

6 Regulation of ascorbate biosynthesis in the green alga Chlamydomonas reinhardtii

presented by Szilvia Z. Tóth

André Vidal Meireles¹, Juliane Neupert², Valéria Nagy¹, László Kovács¹, Laura Zsigmond¹, Laise Rosado de Souza², Alisdair R. Fernie², Ralph Bock², Szilvia Z. Tóth¹

¹ Biological Research Centre (Hungarian Academy of Sciences), Hungary; ² Max-Planck-Institut Potsdam-Golm, Germany

Ascorbate (Asc, vitamin C) is of vital importance in plants because it is a scavenger of reactive oxygen species (ROS), plays essential roles in development, signaling, hormone biosynthesis, and regulation of gene expression. The genome of Chlamydomonas reinhardtii encodes all the enzymes of the L-galactose Asc biosynthesis pathway described for higher plants and the VTC2 gene encoding GDP-L-galactose phosphorylase, like its plant homologues, is highly regulated (Urzica et al., 2012). In order to provide experimental proof for the operation of the L-galactose pathway and to gain more information on the regulation of Asc biosynthesis in C. reinhardtii, we have targeted the VTC2 gene using artificial microRNA. Our VTC2 transformants have about 10% Asc relative to the control strains showing that GDP-L-galactose phosphorylase plays a pivotal role in Asc biosynthesis. The VTC2 transformants also grow more slowly, have lower chlorophyll content, smaller light-harvesting antenna and they are also more susceptible to high light and H₂O₂ treatments than the control strains. We have also established that i) the expression of the VTC2 gene is induced by H₂O₂ and singlet oxygen; ii) the photosynthetic electron transport per se is not required for Asc biosynthesis, except that it is the main source of ROS; iii) in contrast to higher plants, there is no feedback regulation of Asc and dehydroascorbate on the L-galactose pathway; iv) there is no indication for a circadian rhythm of Asc biosynthesis in C. reinhardtii, but it is strongly upregulated upon dark-to-light transitions.

7 Developing an energy-efficient artificial light source for macroalgae

presented by Tonia Schmitz

Tonia Schmitz¹, Eckhard Kraft¹, Thomas Pabst¹, Karin Bieske², Dennis Schlehuber³, Annette Somborn-Schulz³

¹ Bauhaus University Weimar, Germany; ² Technical University Ilmenau, Germany; ³ Fraunhofer UMSICHT, Germany

In times of overburdened coastal ecosystems and declining fossil energy resources, energetically optimized photobioreactorsystems for macroalgae cultivation can help to meet the growing demand for algae-based feedstocks in an ecologically sustainable way. Moreover, implemented as a closed production process, algae cultivation can be independent of major growth limiting factors in natural environments such as varying temperatures, nutrient availability, the quality of seawater or the intensity of sunlight. Current research shows that especially the provision of adequate light leaves major energy saving and biomass growth enhancing potentials. Aiming at creating light conditions adapted in intensity and spectral distribution to the needs of different eucaryotic macroalgae, a prototypal light source using energy efficient LED-technology has been designed. Based on literature and experimental research, photosynthetically active pigments in representatives of chlorophyta (*Ulva lactuca*), rodophyta (*Palmaria palmata*) and phaeophyta (*Fucus vesiculosus*) have been ascertained to chose appropriate wavelengths for their cultivation. Summarizing, the prototypal light source is to be equipped with LEDs primarily emitting light in the wavelengths of 430 and 660nm for chlorophyll a, 450, 465 and 620 nm for chlorophyll b, 560 and 620 nm for chlorophyll c as well as 560 and 620nm for carotenoids. Awaiting the results of cultivation experiments under the designed LED-prototype (to be finalised in summer 2016), the results from preliminary growth experiments with different algae species under conventional artificial light sources are being presented and discussed.

8 Nutrient recovery from raw blackwater using green microalgae Chlorella vulgaris

presented by Aleksandra Krivograd Klemenčič

Griessler Bulc T.¹, Žitnik M.¹, Segovia Bifarini M.A.², Krivograd Klemenčič A.¹

¹ University of Ljubljana, Slovenia; ² Università La Sapienza, Roma, Italy

Lab-scale experiment consisted from four different tests of blackwater (BW) treatment (BW dilutions of 10%, 20%, 30% and 50%) by using a strain of green microalgae Chlorella vulgaris was conducted in order to evaluate the impact of BW concentrations on C. vulgaris growth and its capacity of nutrients recovery. Tests were performed in batch-mode by using 2 L Erlenmeyer flasks at temperature of 25 ± 2 °C, and irradiance of 5000-6000 LUX in 18/6 h light/dark intervals. COD, TN, N-NH⁴⁺, P-PO₄³⁻, chlorophyll-a, dry weight and ash free weight of C. vulgaris inoculum and BW were measured every 24 hours for 14 days. The BW was sampled in a pilot BW separation system with low water consumption vacuum toilets (JETSTM). The concentrations of BW influenced a) the algae growth and b) the process of nutrient recovery. Lower BW concentrations lead to lower C. vulgaris growth yields and lower nutrient recovery. N was the nutrient preferred by C. vulgaris (removal efficiencies >70%), while the removal efficiencies of P were lower (<50%). This study demonstrates that C. vulgaris can recover N and P from raw BW, while gaining valuable algal biomass. The kinetics of microalgae growth depends on the dilution of BW, which correlates with the colour of BW and light availability for microalgae. More diluted BW represents less extreme conditions for microalgae, nevertheless biomass growth was higher in more concentrated BW. This work was performed within national ARRS J2-5462 project and by support of Koto Ltd. and AlGen Ltd.

9 Potential of biodiesel production from native Finnish microalgae strains

presented by Anita Santana-Sánchez

Anita Santana-Sánchez, Fiona Lynch and Yagut Allahverdiyeva

University of Turku, Finland

The imminent depletion of fossil fuels and their significant contribution to environmental pollution have increased the need for new alternatives which are clean, sustainable and renewable. Microalgae (cyanobacteria and green algae) have been proposed as a promising biodiesel feedstock, predicted to outperform traditional energy crops on an area basis. An exceptional adaptability to changes in environmental conditions may be an advantage towards a more efficient and inexpensive production of biodiesel. Drastic seasonal variations in temperature and daylight availability are characteristic for Nordic climates. The screening of Nordic native microalgae strains to find well-adapted organisms may reduce the energy input in the cultivation process. We have assessed eight native Finnish microalgae strains, using synthetic wastewater as a low-cost nutrient source, for their lipid characteristics. A one-step *in situ* transesterification method was employed to maximize the coverage of lipids detected in the whole microalgal biomass. These lipids were converted to Fatty Acid Methyl Esters (FAME) and identified by GC/MS analysis. The native Finnish green alga UHCC0027 stood out as the best candidate for application as a biodiesel feedstock, having highest neutral lipid content and promising fatty acid composition (C16:0, 15.3%; C18:1, 17.5%; C18:2, 10.5%; C18:3, 17%).

10 Isolation and characterization of mutants deficient in four steps of the phylloquinone biosynthesis pathway in Chlamydomonas reinhardtii

presented by Barbara Emonds-Alt

Barbara Emonds-Alta, Claire Remacle and Pierre Cardol

University of Liege, Belgium

In photosystem I (PSI), phylloquinone participates to electron transfer as secondary electron acceptor (A1). The phylloquinone biosynthesis pathway, previously characterized by reverse genetic in Synechocystis sp. PCC 6803, involves 8 enzymatic steps from chorismate. In the green alga Chlamydomonas reinhardtii, characterization of phylloquinone biosynthesis was still partial and only one mutant deficient for MEND was characterized. In the present work, we found MENA-H homologs in C. reinhardtii genomic database. In particular, MENF, MEND, MENC, and MENH catalytic domains are present in a single ORF (named PHYLLO by similarity to gene organisation in Arabidopsis). We then took advantage of the fact that a double reduction of plastoquinone (PQ) in PQH2 occurs in anoxia into the A1 site in the mend mutant, interrupting photosynthetic electron transfer, to isolate new phylloquinone-deficient strains. UPLC-MS analysis confirmed the absence of phylloquinone in four new mutants impaired in MENA, MENB, MENC (PHYLLO) and MENE. Despite this loss, men mutants are still able to grow in low light but are high light-sensitive. In low light, the level of active PSII in men mutants is identical to that of the wild-type, but the level of active PSI is reduced by 30-40% as assayed by spectroscopic measurements. This decrease is more pronounced when cells are exposed to high light intensities during 4 hours. The level of active PSI is ~10% of wild-type cells and the electron photosynthetic transfer is reduced accordingly. Reorganization of the photosynthetic apparatus following lack of phylloquinone in men mutants is discussed.

11 Scale-up of a Production Process for Starch-rich Algal Biomass

presented by Claudia Holdmann

C. Holdmann¹, U. Schmid-Staiger², G. Brinitzer³, K. Frick², C. Hering², T. Hirth⁴

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First a screening in shaking flasks was conducted and Chlorella sorokiniana SAG 211-8k was chosen for the scale-up due to its high growth rate and high starch accumulating capacity under nutrient depleted conditions. A two-stage process was developed: First biomass is produced and in the second step starch accumulation is induced by nitrogen limitation. Chlorella sorokiniana was cultivated indoors in 6 liter flat panel airlift (FPA) reactors with LED illumination and outdoors with natural sunlight in 28 and 180 liter FPA reactors. For starch accumulation during the indoor cultivation the light per cell ratio was kept constant by increasing light intensity according to biomass increase. The algae were able to accumulate up to 50% (w/w) starch within two days. At outdoor conditions, the available sunlight varies during the day. To test different relative light availabilities, the biomass concentration in the reactor was varied in parallel experiments. This resulted in a starch content of 50% (w/w), but with low starting biomass concentrations in the reactor and seven days of cultivation. The process was transferred to a pilot plant which consists of 40 flat panel airlift reactors (each 180 liter). In 20 of these reactors 10 kilogram algae with a starch content of 43% (w/w) were produced during 7 to 12 days of nitrogen limitation. In summary the starch production process was successfully scaled-up from shaking flasks over 6 liter lab reactors and 28 liter outdoor reactors to a pilot process in 180 liter FPA reactors within 1.5 years.

12 Beyond green - harvesting and dissipating light energy in the heterokont alga *Nannochloropsis oceanica*

presented by Dagmar Lyska

Dagmar Lyska, Matthew D. Brooks, Krishna K. Niyogi
UC Berkeley, USA

Understanding the fundamental processes of photosynthesis and its regulation has become more and more important with growing energy and agricultural demands and the need to enhance feedstock performance and yields. Until recently, research on photosynthesis has been focused largely on cyanobacteria, green algae and land plants. However, heterokont microalgae, such as *Nannochloropsis*, are emerging as potential feedstocks for biofuels due to their high photoautotrophic biomass and lipid accumulation rates. The photosynthetic properties of *Nannochloropsis* are similar to, but also significantly different, from green algae, e.g. in terms of light harvesting or dissipation of excess light energy (Non-Photochemical Quenching, NPQ). We want to understand these mechanisms and their regulation in *Nannochloropsis* and therefore designed molecular tools for the genetic modification of this non-model organism. Using a non-lethal, easy-to-screen mutant phenotype, we have established protocols for targeted gene knockout by homologous recombination and by the CRISPR/Cas9 system for the *Nannochloropsis oceanica* strain CCMP1779. Furthermore, we have created a library of random insertional mutants with altered NPQ capacities and/ or kinetics and identified disrupted genes using PCR-based methods. Combining the forward and reverse genetics approaches with biochemical and spectroscopic analyses, we are now able to address the significance of the xanthophyll cycle-dependent vs. the LHCX(LHCSR)-dependent component of NPQ and the molecular basis of light harvesting in *Nannochloropsis*. (This work was funded by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy, FWP number 449B.)

13 Metaproteomics provides insight into algal-bacterial interactions

presented by David A. Russo

David A. Russo, Narciso Couto, Andrew Beckerman, Jagroop Pandhal
University of Sheffield, United Kingdom

The concept of microalgae as a biomanufacturing platform has rapidly expanded in the last decade. Current algal cultivation systems utilise uni-algal populations and are prone to contamination and sudden crashes. Therefore, cultivation practices have to be improved. One approach that has been suggested is to harness the extensive ecological knowledge of microbial systems and apply this to algal cultivation. Therefore, utilising ecological principles to study natural communities that thrive under similar characteristics as those used in industrial cultivation can provide valuable insight. Here, we applied a metaproteomic approach to investigate the interactions between algal and bacterial communities, over time, in freshwater microcosms. 1048 proteins were identified and quantified by their exponentially modified protein abundance index. Bacteroidetes express extracellular hydrolases and Ton-B dependent receptors to degrade and transport high molecular weight compounds captured while attached to the phycosphere. Alpha- and Beta-proteobacteria were found to capture different substrates from algal exudate (carbohydrates and amino acids, respectively) suggesting resource partitioning to avoid direct competition. This study provides insight into freshwater algal-bacterial interactions and creates new avenues of research for the improvement of algal cultivation.

14 Innovative microalgae research for obtaining aviation turbine biofuel

presented by Emil Stepan

Emil Stepan¹, Sanda Velea¹, Olimpiu Blajan², Cristina Enascuta¹, Gabriel Vasilievici¹, Emilia Oprescu¹, Elena Radu¹, Adrian Radu¹

¹ICECHIM, Romania; ²Research Institute of Organic Auxiliary Products, Romania

The use of biomass as energy source will provide an excellent opportunity for mitigation of greenhouse gas emission and reducing global warming through the substitution of conventional fossil-based energy sources. Among the various biomass candidates for biofuel production, microalgae are being considered as a more viable feedstock, because they are producing 15–300 times more oil than traditional crops on an area basis, requires very little nutrients supply for growth, present higher level of oil accumulation, and can grow in fresh water or marine environments. Our research was focused on an integrated multi-stage system for obtaining aviation turbine biofuel, based on production and valorisation of microalgae biomass. The first stage refers to mixotrophic microalgae culture on nutrient media supplemented with glycerin as carbon source, obtained as by-product in the later process stages, and stressed to increase the lipid productivity. In the 2nd stage, microalgae biomass was harvested and extracted to produce algae oil. In the 3rd stage, algae oil was subjected to a complex process comprising catalytic hydrogenation, dehydration, hydrocracking and isomerization resulting a mixture of iso/n alkanes, suitable for uses such as aviation turbine biofuel.

15 Genome-scale metabolic reconstruction and analysis of tomato metabolism

presented by Huili Yuan

Huili Yuan¹, C. Y. Maurice Cheung², Mark G.Poolman³, Peter A.J. Hilbers¹, Natal A.W. van Riel¹

¹Eindhoven University of Technology, The Netherlands; ²Yale-NUS College Singapore; ³Oxford Brookes University, United Kingdom

Genome-scale metabolic modelling has been proven useful for investigating the feasible metabolic states of an organism. With the genome information available on databases, we built iHY3410, a genome-scale model of tomato metabolism, we then applied this metabolic network to simulate tomato leaf metabolism under normal and drought conditions. To mimics the effect of drought, we introduced the condition-specific growth rate that accounts for biomass accumulation in drought conditions. Simultaneously, we set the flux ratio of carboxylation to oxygenation of RubisCO (V_c/V_o) to a value of 1 to represent drought stress. The in silico investigation of the metabolic characteristics for photorespiration and other relevant metabolic processes under drought stress suggested that the flux distributions through the mevalonate (MVA) pathway under drought were distinct from that under normal conditions. In addition, we improved on previous studies of reaction essentiality analysis for leaf metabolism by including potential alternative routes for compensating reaction knockouts. iHY3410 allowed us to simulate the metabolic behavior under various environmental conditions, including normal and drought conditions using flux balance analysis. The model predicted that the metabolic flux through the MVA pathway under drought conditions significantly differed from that under normal conditions, which gave hints to possible adaptive metabolic responses to drought stresses.

Posters - Session II

Wednesday, 27th April 2016

17:30 - 19:00

Posters Number 16 to 30

16 The influence of mixotrophic cultivation on regulations of autotrophic and heterotrophic growth in Arthrospira platensis KMMCC CY-007 as a model of cyanobacteria

presented by Kisok Kim

Kisok Kim, Jaeho Choi, Yosep Ji, Hajun Park, Hyungki Do, Changki Hyun, Bongju Lee, Wilhelm Holzapfel
Handong Global University, South Korea

Investigations on the transition from autotrophic to heterotrophic growth, or vice-versa (from heterotrophic to autotrophic growth) in the cyanobacteria, we studied the influence of mixotrophic growth conditions on inorganic carbon uptake system (NDH-I4 complex) and organic carbon uptake system (sugar ABC transporter), comparing with RuBisCO and cytochrome C oxydase on basis of mRNA expression levels in Arthrospira platensis KMMCC CY-007 at 30°C with continuous light or continuous dark exposure. Growth rate, ATP, elemental analyses and glucose consumption were examined over 48 h under continuous light exposure or dark exposure with non-carbon supply (L and D), only 1% of CO₂ supply (CL and CD), only 0.5 g of glucose (GL and GD) and 1% of CO₂ with 0.5 g glucose supply (GCL and GCD). NDH-I4 complex and RuBisCO mRNA expression levels showed an “increasing-decreasing” pattern; after 12 h and 36 h the relative RNA fold increase was higher than after 24 h and 48 h, respectively. While sugar ABC transporter and Cytochrome C oxydase mRNA expression levels showed the reverse “decreasing-increasing” pattern.

17 Co-evolution of assembly of photosynthetic complexes and ribosome pausing

presented by Lars B. Scharff

Piotr Gawroński, Dario Leister and Lars B. Scharff

University of Copenhagen, Denmark

The core parts of the photosynthetic complexes in higher plants are made of proteins encoded in plastids. We used ribosomal profiling data from chloroplast transcripts to analyse, which features influence the speed of translation elongation. Our results demonstrate that not a single feature as reported in the literature for other biological systems, but multiple features are responsible to slow elongation locally. mRNA structure, internal Shine-Dalgarno sequences and positively charged amino acids in the nascent peptide chain cause ribosomal pausing, whereas rare codons have no significant influence. The distribution of the pause sites is not random. Our analysis indicates their importance for co-translational transmembrane protein integration and folding as well as the integration of co-factors as the FeS clusters and the Mn cluster into the photosystems. The features responsible for ribosome pausing are conserved from higher plants to green algae demonstrating the importance of the regulation of the speed of translation elongation for the assembly of photosynthetic complexes.

18 Characterization of different ferredoxin modules in a light-driven P450 fusion enzyme

presented by Marcos Hamborg Vinde

Marcos Hamborg Vinde, Silas Busck Mellor, Agnieszka Zygadlo Nielsen, Poul Erik Jensen

University of Copenhagen, Denmark

The cytochrome P450 (P450) superfamily is one of the largest enzyme families in nature, members of which catalyze complex oxidation reactions necessary in the synthesis of a large number of specialized metabolites. Many of these metabolites are bio-active high-value products. The activity of most eukaryotic P450s depends on the delivery of electrons from an ER-located P450 oxidoreductase (POR), which uses NADPH as the electron donor. Natural low synthesis of many specialized metabolites in plants makes it interesting to develop more efficient production systems for these metabolites. Using photosynthetic organisms as hosts for such production systems is practical, since they are a sustainable production system, and known to accommodate heterologously expressed plant P450s. A light-driven P450 system was previously developed in our group where eukaryotic cytochrome P450 enzymes can tap into the electron flow of the photosynthetic light-reaction. In this system P450s are relocated into photosynthetic membranes of plants or cyanobacteria. Inside the chloroplast, the P450 receives electrons from photosystem I (PSI) via the iron-sulfur protein ferredoxin. However, ferredoxin serves to deliver electrons to many other redox processes. The major sink for photosynthetic reducing power - the regeneration of NADP⁺ to NADPH catalyzed by ferredoxin-NADP⁺ reductase (FNR) - thus competes strongly for electrons delivered by ferredoxin, which reduces light-driven activity for P450s. To increase the amount of electrons partitioned towards the P450 we have designed a fusion construct linking it directly to ferredoxin, and will test different ferredoxin variants and functional homologues - like the FMN domain of POR - to find redox partners with reduced affinity for FNR. Preliminary data suggest that it is possible to modulate specific electron partitioning towards the light-driven P450 enzymes and make them less susceptible to competition from FNR.

19 Design of communities with mutualistic dependency on assimilable nitrogen exchange to enhance the productivity of photobiotechnological processes

presented by Marine VALTON

Marine VALTON and Patrik R. JONES

Imperial College London, United Kingdom

Cyanobacteria can potentially be used as a host for biotechnology if they can be engineered to channel the majority of their metabolic flux into commercially attractive chemical products. In the FP7 DEMA project the model cyanobacterium *Synechocystis* sp. PCC 6803 is engineered for the biosynthesis of ethanol, an established biofuel. Towards the goal of enabling commercial production we are attempting to improve the productivity of the intended biotechnological process by constructing an ethanol-producing synthetic consortium that is dependent on nitrogen fixation by a diazotroph. The reciprocal exchange of nutrients between different species, a key concept of ecology, is indeed recognized to enhance the stability of populations¹ and the supply of assimilable nitrogen to algal cultivation media represents a significant cost and supplies an essential nutrient to many potential contaminants. We are therefore interested in investigating the impact of co-cultivation strategies on the robustness of the production system as a whole in relation to the possible impact of undesirable contaminants.

20 Cyanobacteria-induced phytotoxic effects and oxidative stress in several nutritionally important plants

presented by Mehboob Ahmed

Mehboob Ahmed and Anum Fatima

University of the Punjab, Pakistan

Freshwater cyanobacteria release a wide variety of secondary or bioactive metabolites including toxins, vitamins and growth hormones in surrounding environment. One of these, the cyanotoxins are the secondary metabolites that are harmful for living organisms. Irrigation water contaminated with cyanotoxins can reduce crop quality and yield. The main purpose of this research was to evaluate the negative effects of cyanotoxins on various nutritionally important plants in Pakistan. Seeds of nutritionally important plants were inoculated with filamentous strains of cyanobacteria (with cells and culture supernatant) and allowed to germinate. After germination seedlings were analyzed physically by examining various growth parameters. Oxidative stress was determined in terms of peroxidase content, total phenolic content and flavonoid content. Stress conditions i.e. phosphate and nitrate limitation were implied to induce toxin production. Exposure of tested cyanobacterial strains caused reduction in growth parameters i.e. shoot length (2.1%-83.6%), root length (3.03%-82.9%), number of leaves (2%-90%), number of lateral roots (1.2-100%) and stem diameter (24.6%-66%). In addition, oxidative stress was evident from high peroxidase (up to 1236.2%), flavonoid (up to 2479.2%) and phenol content (up to 1090%). Moreover browning of roots, growth reduction and roots constriction manifested morphological damage to the plant by cyanotoxins. Stress conditions i.e. phosphate and nitrate limitations enhanced toxicity of cyanobacterial strains. Water contaminated with toxic cyanobacteria used for irrigation purposes have negative consequences on plants ultimately decreasing crop yield. In conclusion toxins producing cyanobacteria should be considered as plant repressing factor besides other known factors.

21 The effect of light pulse intervals on PAM measurements in various microalgae

presented by Philipp Norf

Philipp Norf, Anna Matuszyńska, Oliver Ebenhöh
Heinrich Heine University Düsseldorf, Germany

Pulse amplitude modulation (PAM) is a commonly used noninvasive method to measure chlorophyll fluorescence from which various photosynthetic parameters like quantum yields can be derived. At the end of a period of prolonged darkness a high intensity pulse of white light is applied, followed by a short period of low intensity actinic light ($40 \mu\text{E}$ to $100 \mu\text{E}$) and then darkness again. During this low light and darkness phase one or several additional pulses of light are applied. These light pulses saturate the photosystem II by blocking the photochemistry and allowing for specific fluorescence trace interpretation. Despite the wide use of this method (e. g. Lavaud et al. 2012, Quaas et al. 2015) there seems to be no consent on a standardized protocol in the literature. Beside experimental approaches, there are also mathematical models of the photoelectron transport chain (PETC) available (e. g. Ebenhöh et al. 2014) allowing for in silico simulations of PAM measurements. Here I used a dual approach featuring theoretical work on the PETC model and experimental work using a flatbed bioreactor (Photon Systems Instruments, FMT150) to investigate if the time intervals between the saturating pulses of light have some effect on and will interfere with the measurements. This was done for the green alga *Chlamydomonas reinhardtii* and the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*.

22 Protein signatures involved in the mechanism of oxidative stress tolerance in cyanobacteria

presented by Piyoosh Kumar Babel

Piyoosh Kumar Babele and Ashok Kumar
Banaras Hindu University Varanasi U.P. India

Effects of Ultraviolet-B radiation (2 Wm^{-2}) on the proteome and transcriptome was studied in the model nitrogen fixing cyanobacterium *Anabaena* PCC 7120. The impact of UV-B stress on growth and survival was initially tested which showed more than 50% survival after 3h thus selected for further exposure. Results of fluorescence microscopy and spectrofluorometry proved that UV-B radiation induces oxidative stress by excessive production of ROS which in turn result in decreased photosynthetic activity as well as affect several other metabolic processes. For the proteome analysis 2-DE and MALDI-TOFMS-MS were employed and various proteins were characterized and identified. Twenty four protein spots of *Anabaena* showing more than 2-fold higher and/or lower level of expression were selected for MALDI-TOF MS analysis. On the basis of physiological functions the identified proteins are classified in eight functional groups. Among these proteins chaperones/stabilizing proteins, reductases and antioxidants, transcription and cellular processes, cofactor and secondary metabolite synthesis proteins, and hypothetical proteins (HPs) belonged to upregulated group of proteins while proteins involved in amino acid biosynthesis, carbohydrate/ photosynthesis and energy metabolic proteins and translational proteins are grouped as downregulated. Transcriptomic analysis employing qRT-PCR also justifies their up and down regulation at RNA level under stress condition thus provided a comprehensive expression analysis of hypothetical genes and it appears that their up regulation may play important role in the stress acclimatization strategy. Several bioinformatic and computational tools were employed for functional analysis of HPs. These findings indicate that such proteins may play an important role in successful acclimation to stress conditions. Their functional characterization will certainly describe new mechanisms involved in this complex mitigation process. Findings of this study will be of importance to understand the regulation of cellular processes in order to achieve successful metabolic engineering and/or cultivation of cyanobacteria for production of biomass and biofuels.

23 Increased efficiency of integrated biomass – biofuels – energy systems by reuse of resulted CO₂ through algal photosynthesis

presented by Sanda Velea

Sanda Velea, Emil Stepan, Olimpiu Blajan

ICECHIM, Romania

We have developed an original integrated “closed” process for a complex exploitation of selected biomass wastes, that combines an anaerobic co-digestion of organic wastes with the microalgae cultivation for purification of the biogas, to remove carbon dioxide, using algal culture immobilized on polymeric substrates and recycling algae biomass residue to digester, as well as further production of bio-products, derived from algal biomass such as: lipids, used for biodiesel production, osmoprotectants, phytohormones used as ammonia based eco-fertilizer. The sequence of individual technological steps linked in one unique complex technology for the integrated biomass-biogas-energy system are as follows: - Anaerobic co-digestion of organic waste with algae biomass residue; - Conditioning (purification) of biogas before pre-combustion step, by chemical scrubbing to remove hydrogen sulfide and further CO₂ capture process in algal photosynthesis process, to increase the methane content in biogas; - Integration of a photobioreactor prototype with biofilms to produce algal biomass before pre-combustion step in co-generation unit; - Valorization of algal biomass as source of lipids, used as biofuels and horticultural oils, and for other bioactive compounds such as osmoprotectants and plant hormones, useful as innovative inputs (eco-fertilizers) for plant cultivation technologies; Our original and innovative contributions are focused on the main weak points on the systems of cultivation of microalgae in photobioreactors, namely photobiofilm formation on transparent walls of photobioreactor, which leads to light decreasing and energy consumption and carbon footprint increasing.

24 Creating model-driven algal cultures in autonomously experimenting appliances

presented by Simon Schliesky

Simon Schliesky, Philipp Norf, Rainer Machné, Oliver Ebenhöh

Heinrich Heine University Düsseldorf, Germany

Finding suitable mathematical models to explain biochemical mechanisms in algae has come into focus, recently. A common approach is to derive model parameters from a controlled experiment and generate hypotheses for new experiments by investigating these parameters. This project is presenting a complementary approach. Adjusting algal cultures to match a given model outcome by altering the experimental conditions. Thus, automatically creating model-derived experiments instead of experiment-derived models. Here, we present the principle ideas behind unsupervised model-driven experiment design and compare the capabilities of these two complementary approaches. There is no off-the-shelf solution for such a setup, therefore cost, reusability, and extensibility of self-made appliances are the main concerns. Fortunately, the recent spread of 3D-printing, laser sintering, and laser cutting applications, in combination with the availability of high power microcontrollers allows for highly customized, modular, yet low-cost experimental setups. Hence, an implementation design and the major challenges of automation will be illustrated.

25 Effects of time and nutrients concentration in the red seaweed *Kappaphycus alvarezii* cultivated in vitro with pulse-fed

presented by Thallis Felipe Boa Ventura

Thallis Felipe Boa Ventura, Vitor de Almeida Pontinha, Ticiane Rover, Leila Hayashi, Mathias Pchara

Federal University of Santa Catarina, Brazil

Kappaphycus alvarezii is one of the most important sources of carrageenan, a commercial polysaccharide widely used in food, pharmaceutical and cosmetics industry. Usually, this species is cultivated with von Stosch solution (VS) in laboratory conditions; however, the constant presence of nutrients in seawater make cultures susceptible to contaminations. Therefore, nutrients pulse-fed is recommended to guarantee the seaweed growing, avoiding contamination. The present work aims to find the best pulse-fed treatment for *K. alvarezii* cultivated in vitro, considering time and nutrients concentration. For that, 0.5 g thalli were acclimated for 10 days in the dark, with aeration and divided in three concentrations of VS solution: 50% (VS50), 100% (VS100) and 200% (VS200) in sterilized seawater. As control, seawater with no VS solution was used. Two pulse periods were tested for each concentration and control: 24 hours and seven days. All treatments and control were made in triplicates, and seedlings were cultivated for 35 days. Weekly, water and flasks were changed and seaweeds were weighted. Thalli grown in the 7 day-pulse in VS100 have significantly higher grow rate in comparison of other treatments and control. No significant differences among the 24 hours' treatments and control were observed. The use of the 7 days pulse-fed with 100% VS solution after 10 days in the dark can be a used for the maintenance of *K. alvarezii* in vitro preventing further contamination, and minimizing the use of nutrients without prejudice in growth rates.

26 Deletion of metabolic electron sinks in *Synechocystis* enhances biophotovoltaic power output

presented by Toby P. Call

Toby P. Call, David Lea-Smith, Paolo Bombelli, Chris J. Howe

University of Cambridge, United Kingdom

We are investigating the effect of removing genes involved in photosynthesis and downstream metabolism on biophotovoltaic (BPV) electrical power output from the photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803. The precise mechanisms of extracellular electron export are as yet unknown in *Synechocystis*, but by removing target genes we hope to demonstrate increased electron flux to the anode. This project builds on previous work in our lab enhancing BPV power output from *Synechocystis* by knocking out the photosynthetic and respiratory terminal oxidases. Unmarked genomic knockouts were generated in the wild type and the triple terminal oxidase knockout backgrounds, and the light response measured in BPV devices. We observe changes in chronoamperometric current response to light and the power output. Biophotovoltaics uses self assembling and repairing light harvesting units in cells to convert sunlight to electrical power, and could play a part in a carbon free renewable energies future.

27 Ascorbate accumulation during sulphur deprivation and its effects on photosystem II activity and H₂ production of the green alga Chlamydomonas reinhardtii

presented by Valéria Nagy

Valéria Nagy¹, André Vidal-Meireles¹, Roland Tengölics², Gábor Rákely², Győző Garab¹, László Kovács¹, Szilvia Z. Tóth¹

¹Biological Research Centre (Hungarian Academy of Sciences), Hungary; ²University of Szeged, Hungary

Chlamydomonas reinhardtii is capable of producing significant amounts of H₂ by its hydrogenase enzyme located at the acceptor side of photosystem I. In nature, H₂ production serves as a safety valve during the induction of photosynthesis in anoxia and it prevents the over-reduction of the photosynthetic electron transport chain. The released H₂ could be exploited as renewable energy, but industrial application is strongly limited by the O₂-sensitivity of the hydrogenase enzyme. Sustained H₂ production was observed upon sulphur deprivation (Melis et al., 2000), which triggers a complex metabolic response resulting in the induction of various stress-related genes, downregulation of photosystem II (PSII), establishment of anaerobiosis and expression of active hydrogenase. It is generally thought that the inactivation of PSII results from an imbalanced photoinhibition and repair of the PsbA protein due to the lack of sulphur. Here we show that upon sulphur deprivation the ascorbate content in C. reinhardtii increases about 100-fold, reaching the mM range; at this concentration ascorbate inactivates the Mn-cluster of PSII and afterwards it can donate electrons to tyrosin Z+ at a slow rate. This stage is followed by donor-side induced photoinhibition, leading to the loss of charge separation activity in PSII and reaction center degradation. The time point at which maximum ascorbate concentration is reached in the cell is critical for the establishment of anaerobiosis and initiation of H₂ production. We also show that ascorbate influenced H₂ evolution via altering the photosynthetic electron transport rather than hydrogenase activity and starch degradation.

28 Species dependent biosorption selectivity of rare earth elements by algae

presented by Wojciech Jurkowski

Wojciech Jurkowski¹, Marcus Heilmann², Rainer Buchholz², Thomas Brück¹, Anna Maria Becker²

¹Technische Universität München, Germany; ²Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Algae poses a high potential as selective sorbents for heavy metal ions. The sorption of rare earth metals in particular has also been investigated with selected species. A common procedure to determine the sorption capacity of a sorbent is the measurement of adsorbate concentration decrease in solution after equilibration with the adsorbent. We developed quick spectroscopic methods to obtain concentrations of REEs. This allows for a screening of many species for their selective biosorption of lanthanides in solutions containing also other metal ions. It could be shown, that while the brown algae are praised for their capacity for Ln-ions ($Q_{Ln} = 0,6\text{--}0,8 \text{ mmol/g}$), they lacks the selectivity found in green algae ($S_{Eu,Cu} = 2\text{--}5$).

29 Improving the efficiency of paddlewheels in microalgae production

presented by Ed Musgrove

Ed Musgrove¹, S Heaven, G. Muller

¹ University of Southampton, United Kingdom

A large proportion of industrial algae are cultivated in raceways with the use of paddlewheels. Very little experimental work has been conducted on paddlewheel efficiency. Where research has been done there are conflicting results with a wide range of efficiencies being reported from 10 to 183% mainly due to the experimental procedure followed varying. Increasing the efficiency of paddlewheels will ultimately increase the surplus energy produced by algal biofuels. A test rig was constructed to investigate the effect different parameters and an innovative insert have on the paddlewheel performance. A novel approach was designed in order to reliably calculate the shaft power of the wheel. Additionally a weir was employed in order to calculate the hydraulic power and increase the head difference across the wheel imitating longer raceways. The results indicate that the number of blades, rpm and immersion depth have a great effect on the efficiency, with optimum values of up to 70% being recorded with higher blade numbers and lower rpm. For example if a 260 m long raceway with a fluid depth of 0.225 m is analysed then reducing the wheel speed from 7 to 4 rpm while increasing the blade number from 8 to 12 then the efficiency of the wheel rises from 39 to 66%. If the wheel is run continuously it would lead to an energetic saving of over 1000 kWh per year. This work indicates that there is scope to increase the efficiency of the wheel and generate more surplus energy.

30 Design Starch: Stochastic modelling of starch granule biogenesis

presented by Adélaïde Raguin

Adélaïde Raguin¹, Oliver Ebenhöh¹, Barbara Pfister², Samuel Zeeman², Michael Rugen³, Robert Field³

¹ Heinrich Heine University Düsseldorf, Germany; ² ETH Zürich, Switzerland; ³ John Innes Centre, Norwich, UK

Starch is a natural product produced by most land plants and algae with remarkable physico-chemical properties. Starch is composed of two polymers of glucose: amylose, a predominantly linear polymer of α -1,4 linked glucose units, and amylopectin, which also contains α -1,6 linkages (branch points) resulting in a tree-like structure. The simple constituents of starch (one type of monomer and two types of linkages) is contrasted by its complex and highly ordered structure, in which crystalline and amorphous layers alternate in a defined and regular fashion. Despite decades of intense research, it is still not understood how precisely starch granule biogenesis initiates and progresses. A relatively small number of enzymes are involved, but it is unclear how their activities are coordinated in order to ultimately control the structure and properties of starch. The objective of our project is to gain a profound understanding of the regulation and control of the biophysical and biochemical processes involved in the formation of the complex polymeric structure that is the starch granule. In parallel with the biochemical and biological investigations, we develop a numerical approach based on a stochastic model. Using Gillespie algorithms we first investigate the chain length specificity of elongation enzymes and the reversibility of the elongation process. Then, we would also simulate a larger scale model where elongation, branching and debranching are taken into account, and study the crystalline properties of the emerging tree-like structure based on the arrangement of tightly packed clusters of amylopectin double helices.

Posters - Session III

Thursday, 28th April 2016

18:00 - 19:00

Posters Number 31 to 43

31 The dawn of a symbiosis? - Investigating the evolution of vitamin B12 auxotrophy in *Chlamydomonas reinhardtii* and how this may lead to a mutualistic relationship with *Mesorhizobium loti*

presented by Freddy Bunbury

Freddy Bunbury, Alison Smith
University of Cambridge, United Kingdom

Roughly half of all algal species depend upon exogenous provision of cobalamin (B12) for their survival. The complex distribution of B12 auxotrophy among algal lineages may suggest that the fitness cost or benefit of this trait is both weak and highly dependent upon environmental conditions. The parsimonious aspect of survival of the fittest ensures that when it is less metabolically expensive to take up a resource than it is to synthesise it or go without it, then auxotrophs are favoured. However, auxotrophs live a less autonomous lifestyle: their fitness is determined in part by the growth of producers of their required nutrient, and can hence be much more variable. A strain of B12-dependent *Chlamydomonas reinhardtii* (*metE*) previously arose and was selected for under high concentrations of B12 by experimental evolution. Here I show that at low concentrations of B12 growth of *metE* is arrested and cell diameter increases. Growth can be restored by co-culturing *metE* with B12-producing bacteria including *Mesorhizobium loti*. A system of ordinary differential equations was developed to model the interaction between *M. loti* and *metE* via production of B12 and organic carbon respectively. This model accurately predicts the growth of both species under normal conditions, however perturbing the interaction by addition of nutrients to the co-culture elicits an unpredicted response, potentially indicative of *metE* being able to reduce its contribution of organic carbon to the interaction when B12 is not limiting.

32 ChloroKB: an interactive visualization knowledge base and a curated resource for the exploration of the chloroplast metabolism

presented by Gilles Curien

Gilles Curien, Pauline Gloaguen, Sylvain Bournais, Christophe Bruley, Marianne Tardif, Myriam Ferro, Yves Vandenbrouck and Norbert Rolland

CNRS CEA Grenoble, France

The chloroplast metabolic network produces a high number of metabolites of industrial interest. Success of metabolic engineering relies on kinetic modelling of metabolic systems. Unfortunately, current knowledge of the plastidial metabolism is still dispersed in the scientific literature and databases are not designed for kinetic modelling. We thus started to build a knowledge base named ChloroKB focused on *Arabidopsis thaliana* chloroplast metabolism to structure qualitative and quantitative data currently available. The knowledge base contains a user-friendly interface allowing rapid visualization of the molecular actors. This corresponds to a snapshot of the entire chloroplast metabolism in relation with other cell compartments and that can be used as a reference state for dynamic modelling. We built a series of metabolic maps (1000 proteins, 100 processes) using the software CellDesigner. These maps have then been integrated into a web interface providing direct links with biological and bibliographical databanks and enabling the access to semi-quantitative data on protein abundance. Each component of a given map is linked to its description page and every map is connected with others to follow a metabolite from one metabolism to another. These maps are useful for deep curation and for sharing knowledge as well. Graphical data representation and visualization functionalities allow to directly pinpoint the metabolic steps that still need to be characterized at the protein level and provide a better understanding of the cross-talk between different metabolisms and subcellular compartments. A first release of this knowledge base is expected by the beginning of 2016.

33 Algae delivering waste phosphorus to soil and crops

presented by Ines S. Hotopp

Ines S. Hotopp, Oliver Ebenhöh

Heinrich Heine University Düsseldorf, Germany

Phosphorus (P) is a finite non-renewable resource, a major nutrient for plants, and a foundation of modern agriculture. Nevertheless, the efficiency of P usage today hardly reaches 20% with the rest ending in wastewater or being carried away by runoff from fields to rivers and oceans. In our work, we investigate the potential to close the cycle from waste back to agriculture by exploiting the capability of microalgae to accumulate large P quantities. This potential of algae for a ‘luxury P uptake’ will be combined with the benefit of delayed release of P from the algal biomass applied as a fertilizer to soil. The increased accumulation of phosphorus at non-surplus conditions of external phosphorus in certain algae strains has yet to be understood completely. By now, it is not known how, where and in which form the phosphorus is stored. An assumption is that the storage of surplus P occurs in form of polyphosphates in the cell. Also, different light conditions affect the concentrations of different algal P-pools, and these in turn have a strong impact on metabolic activity, such as the flux of the Calvin-Benson-Cycle. We use models to understand the underlying dynamics of the conversion of different P-pools in soil, the transport and utilization of P within the plant, and the distribution and usage of P-forms in the algal cell under different environmental P conditions.

34 Phylogenetic analyses of iron and manganese superoxide dismutases in Chromera velia and Vitrella brassicaformis: Do chromerid plastid SODs represent a monophyletic lineage?

presented by Heather J. Esson

Heather J. Esson¹ and Miroslav Oborník^{1,2}

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Chromera velia and Vitrella brassicaformis are unicellular algal symbionts of corals. Known informally as the “chromerids,” their plastids are derived from a red algal ancestor and are the closest extant relatives of the relict, non-photosynthetic plastid (apicoplast) present in some apicomplexans. Chromerid plastids therefore present a unique opportunity to understand the loss of photosynthesis en route to a parasitic lifestyle. Biochemical analysis of Photosystem I (PSI) in Chromera has revealed the presence of two strongly associated, distinct superoxide dismutases (SODs) – enzymes that catalyse the transformation of harmful superoxide radicals to hydrogen peroxide. To investigate the evolutionary history of these proteins, we retrieved eleven SOD protein sequences from the genomes of Chromera and Vitrella. Based on analyses with SignalP and TransitP, two iron (Fe) SODs in Chromera and two in Vitrella possess bipartite plastid targeting sequences. Depending on the method used, phylogenetic analyses of manganese (Mn) and FeSODs place the chromerids in a clade largely consisting of apicomplexan, stramenopile and other alveolates, or in two distinct clades composed largely of green plastids, cyanobacteria and photosynthetic alveolates; and alveolates and bacteria, respectively. One or both Chromera FeSODs form a clade with an *Oxyrrhis marina* FeSOD possessing a bipartite transit sequence. Targeted Vitrella FeSODs are monophyletic and group either with one Chromera FeSOD or with cyanobacteria, green plastids and photosynthetic alveolates, but not with non-targeted Vitrella FeSODs. While statistical support is low or non-existent, our results suggest that chromerid plastid FeSODs have an unexpectedly complex evolutionary history.

35 Production and safety evaluation of Ag85B expressed in the chloroplast of *Chlamydomonas reinhardtii* as a subunit vaccine for Tuberculosis

presented by José Luis Castrejón Flores

Daniel Guzmán-Zapata; Alma Lorena Almaraz Delgado, Noé Valentín Duran Figueroa, Jesús Agustín Badillo Corona, José Luis Castrejón Flores

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Tuberculosis (TB) is a disease with great impact worldwide. TB is caused by *Mycobacterium tuberculosis*, an intracellular bacillus from the genus mycobacteria, which is highly virulent. The only method of prevention available is the BCG vaccine, but it provides variable protection ranging from 0 to 80%. Thus, the development of subunit vaccines have been suggested as a most effective and safer vaccines. Ag85B is a membrane-associated antigen of *M. bovis*, which according to robust evidence, constitutes a potential alternative for vaccination against TB. *Chlamydomonas reinhardtii* (CR) is an attractive organism for protein production, because it is recognized as a Safe GRASS organism compared with others platforms of expression such as bacteria or virus. Moreover, the cost for growing it is relatively low. Therefore, we aimed to demonstrate that it is possible to produce the antigen protein, Ag85b, in CR and demonstrated that it is safe to use as an administered vehicle. Our results showed that it is possible to obtain transformed algae that constantly express the antigen. Moreover, the administration of the total soluble protein by intraperitoneal administration resulted moderate toxic, although other routes of administration need to be tested. In conclusion, our results showed that CR express proteins of therapeutic with reasonable safety margin. Ongoing experiments ought to demonstrate that the administration of the transformed algae results in an immunogenic response.

36 Co-evolution of assembly of photosynthetic complexes and ribosome pausing

presented by Lars B. Scharff

Piotr Gawroński, Dario Leister and Lars B. Scharff

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The core parts of the photosynthetic complexes in higher plants are made of proteins encoded in plastids. We used ribosomal profiling data from chloroplast transcripts to analyse, which features influence the speed of translation elongation. Our results demonstrate that not a single feature as reported in the literature for other biological systems, but multiple features are responsible to slow elongation locally. mRNA structure, internal Shine-Dalgarno sequences and positively charged amino acids in the nascent peptide chain cause ribosomal pausing, whereas rare codons have no significant influence. The distribution of the pause sites is not random. Our analysis indicates their importance for co-translational transmembrane protein integration and folding as well as the integration of co-factors as the FeS clusters and the Mn cluster into the photosystems. The features responsible for ribosome pausing are conserved from higher plants to green algae demonstrating the importance of the regulation of the speed of translation elongation for the assembly of photosynthetic complexes.

37 Sub- and supercritical water extraction of valuable substances from microalgae

presented by Lin Du

Lin Du, Andrea Kruse

University of Hohenheim, Germany

The research is based on the properties of water, specifically, lower dielectric value and density at high temperature and pressure, which are comparable with polar solvents. Sub- and supercritical water (critical point 374°, 22.1MPa) is promisingly a feasible green solvent for algal extracts production. After pretreatment, the extraction will be conducted in a self-assembled flow reactor where temperature, pressure and flow rate are controlled. Thus, the selectivity of extraction can be examined at different conditions for different substances, for instance, fatty acids, amino acids and phenols. Extracts will be analyzed to obtain the efficiency and to understand chemical reactions of valuable substance from microalgae. With life cycle assessment, the extraction process of various compounds will be analyzed both from economical and environmental view. The extraction system will be optimized to get high efficiency and low input maintaining the functional properties of extracts.

38 Antioxidant activity of metal-stressed Chlamydomonas and Scenedesmus microalgae

presented by Maryam Ameri

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We are interested in characterizing the antioxidant responses of microalgae to toxic metals, mainly by determining the enzymatic activity of antioxidant enzyme activities in Chlamydomonas reinhardtii and Scenedesmus spp. With this purpose we tested several extraction procedures to obtain good quality protein preparations to identify different isoforms of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). Firstly, two different extraction buffers (Tris-HCl and MOPS), and two cell disruption means (mortar grinding in liquid-N2 and ultrasonic vibration) were compared. We found that the best protein recovery was obtained using MOPS (30 mM and pH 7.3), and two freeze-thawing cycles with mortar disruption, as observed by the colorimetric Bradford's protein quantification method. Secondly, the enzymatic activities were assayed in gel, once proteins were separated by non-denaturing polyacrylamide gel electrophoresis (ND-PAGE). Prior to enzymatic activity staining, the protein concentration and integrity of the prepared extracts was confirmed by Coomassie-blue staining after SDS-denaturing polyacrylamide gel electrophoresis (SDS-PAGE). The best polyacrylamide concentration in ND-PAGE was 8% for CAT, and 10% for POX and SOD activities. It was found critical the use of fresh extracts to get an optimal SOD activity, and the extracts could be kept stored at -80°C for longer periods to analyze POX and CAT. The three enzymes appeared to exhibit various isoforms, as revealed by the presence in the gels of different bands with enzyme activity. It was necessary to use an adequate amount of extract protein to get a good resolution of the bands. Our latest experiments with Chlamydomonas and Scenedesmus treated with AlCl₃ (10 and 100μM), and the changes observed in the studied antioxidant enzymatic activities will be presented.

39 Development of high efficiency cyanobacterial cell factories: Isolation and taxonomic analysis of new group of high light, high salinity Cyanobacterium sp. from the Red Sea

presented by Noor Azlin Mokhtar

Noor Azlin Mokhtar, Suhaiza Ahmad Jamhor, John Archer

King Abdullah University of Science and Technology, Saudi Arabia

The climate, geography and industrial infrastructure of the Arabian Peninsula make it one of the preeminent locations for algal biotechnology. However, current industrial and model cyanobacteria cannot grow efficiently in the high salinity, oligotrophic and high insolation conditions of the Red Sea , there is a need to discover new robust, high biomass accumulating marine strains capable of growing in Red Sea-derived media for algal biotechnology applications in Saudi Arabia. We have developed a Red Sea cyanobacterial isolation, characterization pipeline targeting physiologically robust high salinity tolerant, high biomass-accumulating strains. Here we report the isolation and characterization of a new clade of marine Cyanobacterium from the Red Sea that grows at 22°C – 50°C, 41 PSU, 200 μM photons m⁻² s⁻¹ on Red Sea media. Isolates grow as single or binary cells; single cells are ovate 2μm wide, 2 - 3 μm long; in fast growing cultures on Red Sea media pairs of binary cells 2μm wide 4 - 6μm long are observed. Cells are blue-green. Cell ultrastructure is clearly visible under phase contrast light microscopy revealing a mucilaginous outer layer surrounding a multigranular cell interior. Phylogenetic analysis based on full-length 16S rRNA and ITS regions from independently isolated Red Sea strains place them unequivocally within the Cyanobacterium clade, closely related to each and forming a distinct Red Sea sub-clade.

40 Micronanobubbles (MNBs) and LEDs for Enhanced Microalgae Biomass Production

presented by Philippe Mozzanega

P. Mozzanega, B. Sherwood, T.C. Arnot, W.N. Wang, R.J. Scott

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The production of microalgae biomass commercially can be hindered by various factors. The clever provision of light and CO₂ can greatly participate in drastically increasing the product, be it the biomass or a (high) value-added product extracted from the biomass (e.g. astaxanthin, omega-3 oils, neutraceuticals etc.) Here, we demonstrate how the integration of flashing LEDs lighting and CO₂ (micro)nanobubbles (MNBs) in the production set up can help boost performance and reduce costs. CO₂ MNBs were characterised and their light guiding effect demonstrated: MNB-media scattered 200% more light through the microalgae culture, with implications in PBR design and culture densities. LED lighting (red at 685 nm, blue at 460 nm) was also explored, with flashing LEDs (20% ON duty cycle at 100 Hz, mean light intensity $I_0=272\mu\text{E}$) conferring an 11% gain in dry weight over continuous lighting. MNBs are expected to provide greater capabilities of CO₂ mass transfer against conventional macro bubble sparging, coupled with a longer longevity in solution (reduced associated cost of dissipated CO₂). A negative zeta potential creates a negative charge barrier around the cells preventing aggregation. MNBs transport nutrients directly to the microalgae in concentrated packages. MNBs in a PBR will distribute the provided light more efficiently and overcome the effect of mutual shading. A flashing light supply through LEDs will enhance microalgae biomass production while reducing energy consumption by > 80% (against conventional white lighting). The flashing light supplies enough energy to optimize light-dependent photosynthetic reactions (Z- Scheme), also limiting the effects of photo-inhibition which have adverse effects on growth.

41 Systematic metabolic characterization of *Botryococcus braunii* CCAP 807/2 (race A) reveals distinct growth, hydrocarbon and EPS-production phase

presented by Swapnil Chaudhari

Swapnil Chaudhari, Olga Blifernez-Klassen, Viktor Klassen and Olaf Kruse

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Botryococcus braunii is a colony forming green microalga which produces large amount of hydrocarbons, 30-86% of dry weight. Apart from producing hydrocarbons some of the strains of *braunii* are known for their ability to synthesize high value exo-polysaccharides. The ability to produce hydrocarbons and exo-polysaccharides in large quantities attribute to slow growth rate of this alga and is largely dependent on the physiological state of the cell. Thus, present study focuses on systematic metabolic characterization of race A strain CCAP 807/2, to address some of the challenges associated with its slow growth and product formation. The results obtained in the present study suggest that there are distinct growth and product formation phases for *braunii* depending on total chlorophyll. Furthermore, results show an increase in the abundance of hydrocarbon and role of oleic acid as a precursor for C21-C31 alkadienes and trines in race A of *B.braunii*. These results provide important insight of growth behavior, carbohydrates, hydrocarbons, lipids (FAME) and metabolic profiles during defined periods of cultivation. Additionally, we discuss about the kinetics of hydrocarbons and exo-polysaccharide formation and quantification during different growth stages. The results from this study will serve as a basis for an integrated OMICS data set which would be applied for strain development and biofuel production.

42 Computational analysis of hydrodynamics and light distribution in photo-bioreactors for algae biomass production

presented by Varun Loomba

Varun Loomba, Eric von Lieres, Gregor Huber

Forschungszentrum Jülich, Germany

Microalgae can be directly used in health food or as bio-filters for waste water treatment. They also have numerous commercial applications in cosmetics, aquaculture and chemical industry as a source of highly valuable molecules, e.g., polyunsaturated fatty acids. Moreover, they are increasingly recognized as a promising source for biodiesel production. To realize the full potential of microalgae, optimal operating conditions for their cultivation in photo-bioreactors (PBR) need to be identified in order to maximize productivity, lipid content, and efficiency of photosynthesis. The most important parameters affecting PBR performance are reactor shape, light intensity distribution, algae growth and other metabolic properties. The presented study aims at optimizing these parameters using Computational Fluid Dynamics (CFD) simulations with the COMSOL Multiphysics software. Specifically, flat panel photo-bioreactors with turbulent mixing due to air sparging and one-sided lighting are studied. First, flow profiles of both liquid and gas phases are computed using the Euler-Euler approach for analyzing the air sparging and detecting potential dead zones. Then, light intensity distributions are calculated inside different PBR types, based on absorption and light scattering by algae and gas bubbles. Subsequently, the paths of individual algae are traced, and the environmental conditions they are exposed to are recorded over time, in particular aeration and light intensity. Results of the above described simulation stages will be presented and discussed.

43 A quantitative evaluation of ethylene production in the recombinant cyanobacterium *Synechocystis* sp. PCC 6803 harboring the ethylene-forming enzyme

presented by Zuzana Benediktyová

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Ethylene is one of the most important petrochemical utilized in manufacturing. However, its production is coupled with a substantial energy requirement (gigajoules per 1 ton of ethylene), vast carbon emission (up to 2 tons of CO₂ per 1 ton of ethylene) and co-production of extensive amount of toxic compounds when produced from traditional resources, fossil fuels. This arguments make appealing the concept of ethylene renewable production, harvesting solar energy via photosynthesis coupled to conversion of CO₂ into chemical feedstocks and fuel. In recent years, great progress has been achieved in constructing stable ethylene-producing cyanobacteria. We compared two recombinant strains of *Synechocystis* sp. PCC 6803: 2x Sy-efe and pDF-trc-EFE. For quantitative real-time monitoring of ethylene evolution, a novel instrumentation setup was introduced. A flat-panel photo-bioreactor coupled to a membrane-inlet mass spectrometer allowed to detect ethylene during quasi-continuous culture and evaluate a number of illumination levels. Our results showed that ethylene was produced under a wide range of light intensities with an optimum at modest irradiances. Carbon partitioning was estimated using a stoichiometric model of cyanobacterial metabolism. That made possible to relate ethylene evolution to other cellular light-driven processes including growth and photosynthesis. This analysis allowed to optimize conditions for ethylene production in highly controlled setup.

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08:15 – 09:00	Registration	Foyer Area
09:00 – 09:20	Opening Session	Casablanca
09:20 – 12:30	Session I: Photosynthetic electron flow	Casablanca
10:40 – 11:10	Coffee break	Foyer Area
12:30 – 14:00	Lunch Break and Networking	Gueliz Restaurant
14:00 – 16:50	Session II: Modelling Metabolism	Casablanca
15:40 – 16:10	Coffee break	Foyer Area
17:00 – 18:30	Industrial Exhibition + Poster Session I	Foyer Area
19:00 – late	Welcome BBQ	Café Del Mar

Wednesday 27th April

09:00 – 11:00	Session III: Light Acclimation	Casablanca
11:00 – 11:30	Coffee break	Foyer Area
12:00 – 13:00	Lunch Break and Networking	Gueliz Restaurant
11:30 – 14:40	Session IV: CO ₂	Casablanca
12:50 – 14:00	Lunch Break and Networking	Gueliz Restaurant
14:40 – 17:30	Session V: Chloroplast Structure	Casablanca
15:40 – 16:10	Coffee break	Foyer Area
17:30 - 19:00	Industrial Exhibition + Poster Session II	Foyer Area

Thursday 28th April

09:00 – 11:50	Session VI: Communities	Casablanca
10:40 – 11:10	Coffee break	Foyer Area
11:50 – 15:20	Session VII: Omics	Casablanca
12:50 – 14:00	Lunch Break and Networking	Gueliz Restaurant
15:20 – 15:50	Coffee break	Foyer Area
15:50 – 17:50	Session VIII: Engineering	Casablanca
18:00 - 19:00	Industrial Exhibition + Poster Session III	Foyer Area
20:00 - late	Conference Dinner	Razzett L-Antik

(Buses will depart from San Antonio Hotel & Spa at 19:45)

Friday 29th April

09:00 – 12:20	BioSC Session IX: Innovative algae research for a bio-based economy	Casablanca
10:30 – 11:00	Coffee break	Foyer Area
12:20 – 13:30	Lunch Break and Networking	Gueliz Restaurant
13:30 – 15:00	Session X: Industrial Cultivation	Casablanca
15:00 – 15:10	Closing Session	Casablanca
15:10 – 15:30	Coffee break	Foyer Area
15:30 – 18:00	Afternoon Career Session: A Discussion Panel: what's next for young researchers?	Casablanca