International Conference

Photosynthesis Research for Sustainability

in honor of Dr. George C. Papageorgiou

September 21–26 2015
Crete, Greece

ABSTRACTS AND PROGRAMME

International Conference

“Photosynthesis Research for Sustainability-2015”

in honor of Dr. George C. Papageorgiou

September 21–26, 2015
Crete, Greece

Abstracts and Programme

Crete – 2015

Abstracts and Programme of International Conference "Photosynthesis Research for Sustainability-2015: In honor of Dr. George C. Papageorgiou"

Eds. S. I. Allakhverdiev, K. Stamatakis, I. A. Naydov.
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The volume contains abstracts of the lectures and poster presentations at the International Conference "Photosynthesis Research for Sustainability: In honor of Dr. George C. Papageorgiou" to be held in September 21–26 2015 in Crete, Greece. The experimental and theoretical works covering a wide range of topics, from the primary processes of energy and electron transfer to the physiological aspects of photosynthesis will be discussed at the conference. Considerable attention will be given to discussion of the structural organization of photosynthetic reaction centers, and applied problems of photosynthesis – stress biology, artificial photosynthesis and fuel. The book will be of interest to researchers involved in the study of photosynthesis and other related fields.

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Additional information is available on our website:
http://photosynthesis2015.cellreg.org

WELCOME!

You are most welcome to the International conference "Photosynthesis Research for Sustainability-2015: in honor of Dr. George C. Papageorgiou" held in Greece.

This Meeting is a great occasion for discussions of previous, present, and future research on photosynthesis, from molecular to global, and to meet researchers of photosynthesis from around the world. This Meeting provides a forum for students, post-doctoral fellows and scientists from different countries to deepen their knowledge and understanding, widen professional contacts and create new opportunities, including establishing new collaborations. The topics of this conference range widely, including primary processes of photosynthesis, structure, function and biogenesis of the photosynthetic apparatus, photosystem I, and II, as well as water oxidation mechanism, artificial photosynthesis, regulation of photosynthesis and environmental stress, applied aspects of photosynthesis and emerging techniques for studying photosynthesis.

The multidisciplinary nature of this conference is obvious from the list of topics and presented lectures. In total, 120 lectures and posters will be presented.

Together with all of you, we look forward to a most interesting week with fascinating presentations and inspiring discussions within all aspects of photosynthesis research.

James Barber,
Kostas Stamatakis,
Suleyman Allakhverdiev.

SECTIONS

1. Primary Processes of Photosynthesis

2. Structure, Function and Biogenesis of the Photosynthetic Apparatus

3. Photosystem II and Water Oxidation Mechanism

4. Energy Transfer and Trapping in Photosystems

5. Photosystem I and Bacterial Photosynthesis

6. Carbon Fixation (C3 and C4) and Photorespiration

7. Artificial and Applied Aspects of Photosynthesis

8. Regulation of Photosynthesis and Environmental Stress

9. Systems Biology of Photosynthesis: Integration of Genomic, Proteomic, Metabolomic and Bioinformatic Studies

10. Photosynthesis Education

11. Emerging Techniques for Studying Photosynthesis

Schedule: Photosynthesis Research for Sustainability-2015

21 SEPTEMBER, 2015 (1ST DAY)

Arrival and Registration.

14:00 – Opening ceremony:
Special events in honor of Dr. George C. Papageorgiou

James Barber (UK), Govindjee (USA), Anastasios Melis (USA), Norio Murata (Japan), Eva-Mari Aro (Finland), Kimiyuki Satoh (Japan), Tingyun Kuang (China), Kostas Stamatakis (Greece)

Invited Plenary Lectures:

Chairpersons: Nathan Nelson (Israel), Bruce Barry (USA), Masahiko Ikeuchi (Japan)

15:00–15:50 (50 min)
James Barber (Imperial College London, London, UK)
Artificial Photosynthesis and Global Climate Change

15:50–16:40 (50 min)
Jian-Ren Shen (Photosynthesis Research Center, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan; Institute of Botany, Chinese Academy of Sciences, Beijing, China)
Mechanism of photosynthetic water oxidation based on atomic structure of photosystem II

16:40–17:00 Coffee break

Invited Plenary Lectures:

Chairpersons: Jian-Ren Shen (Japan), Eva-Mari Aro (Finland), Suleyman Allakhverdiev (Russia)

17:00–17:30 (30 min)
Peter J. Nixon (Department of Life Sciences, Sir Ernst Chain Building-Wolfson Laboratories, Imperial College London, London, UK)
Early steps of photosystem II assembly

17:30–18:00 (30 min)
Govindjee (University of Illinois at Urbana-Champaign, Urbana, IL, USA)
Adventures with the green *Chlamydomonas reinhardtii*: in honor of George C. Papageorgiou

19:00 – Welcome party

22 SEPTEMBER

Invited Plenary Lectures:

Chairpersons: James Barber (UK), Govindjee (USA), John F. Allen (UK)

9:00–9:30 (30 min)
Anastasios Melis (Plant and Microbial Biology, University of California, Berkeley, USA)
Photosynthesis for fuel and chemicals production

9:30–10:00 (30 min)
Eva-Mari Aro (Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland)
Photoprotection and Photodamage of Photosystems I and II – consequences on short-term thylakoid dynamics and long-term retrograde signaling

10:00–10:30 Coffee Break

Invited Plenary Lectures:

Chairpersons: Jian-Ren Shen (Japan), Gyozo Garab (Hungary), Tatsuya Tomo (Japan)

10:30–11:00 (30 min)
Norio Murata (National Institute for Basic Biology, Okazaki, Japan)
George Papageorgiou, glycinebetaine, and protection against photoinhibition

11:00–11:30 (30 min)
Nathan Nelson (Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel)
High-resolution structures of plant and cyanobacterial photosystem I. implications for sustainable hydrogen production

11:30–12:00 (30 min)
Xiaochun Qin, Michihiro Suga, Tingyun Kuang, Jian-Ren Shen (Key Laboratory of Photobiology, Institute of Botany, CAS, Beijing, China; Photosynthesis Research Center, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan)
Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex

12:00–14:00 Lunch

Invited Lectures:

Chairpersons: Toshiharu Shikanai (Japan), Peter J. Nixon (UK), Anjana Jajoo (India)

14:00–14:25 (25 min)
Arvi Freiberg (Institute of Physics, Tartu University, Estonia; Institute of Molecular and Cell Biology, Tartu University, Estonia)
Do we comprehend the *in vivo* fluorescence of photosynthetic pigments well enough?

14:25–14:50 (25 min)
Yuichiro Takahashi (Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan)
Functional and structural roles of Asn268 of D1 of photosystem II reaction center

14:50–15:15 (25 min)
Győző Garab (Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary)
The molecular architecture and functioning of LHCII *in vivo* and *in vitro*

15:15–15:40 Coffee Break

Invited Lectures:

Chairpersons: Anastasios Melis (USA), Seiji Akimoto (Japan), Alex Ivanov (Canada)

15:40–16:05 (25 min)
John F. Allen (Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London, UK)
Evolution of two light reactions. Cooperation and inter-dependence in photosynthesis, science, and society

16:05–16:25 (20 min)
Anjana Jajoo (School of Life Science, Devi Ahilya University, Indore, India)
Low pH induced changes in thylakoid membranes

16:25–16:45 (20 min)
Kiriakos Kotzabasis (Department of Biology, University of Crete, Voutes University Campus, GR-70013 Heraklion, Crete, Greece)
High yield H_2-production through a combinational system of photosynthetic electron flow and dichlorophenol biodegradation by green algae

16:45–17:15 Coffee Break

Invited Lectures:

Chairpersons: Vladimir Sukhov (Russia), Paula Mulo (Finland), Elena Tyutereva (Russia)

17:15–17:35 (20 min)
Natalya E. Belyaeva (Department of Biophysics, Biology Faculty of the M.V. Lomonosov Moscow State University, Moscow, Russia)
Analysis of charge fluxes in thylakoid based on the photosystem II electron transfer modeling

17:35–17:55 (20 min)
Kostas Stamatakis (Institute of Biosciences and Applications, NCSR “Demokritos”, Aghia Paraskevi Attikis, Greece)
Kleptoplasts: longevity in a new Ross Sea dinoflagellate host cell

18:00–21:00 Poster viewing/discussion (sections 1–11)

Chairpersons: Marian Brestic (Slovak Republic), Alex Ivanov (Canada), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan), Yiola Petropoulou (Greece), Hazem Kalaji (Poland), Ivelina Zaharieva (Germany), Takaya Tanabe (Japan)

23 SEPTEMBER (FREE TIME, TOURS)

TOURS:

A. Knossos-Museum of Heraklion-Rethymno
B. Elafonissi-Milia

24 SEPTEMBER

Invited Lectures:

Chairpersons: Yuichiro Takahashi (Japan), Kostas Stamatakis (Greece), Anatoly A. Tsygankov (Russia)

9:00–9:25 (25 min),
Barry D. Bruce (Departments of Microbiology & Biochemistry and Cellular and Molecular Biology University of Tennessee at Knoxville, USA)
i) Origin and evolution of PS I in cyanobacteria and chloroplasts;
ii) 'Travels of a transit peptide' or 'It takes two to translocate'

9:25–9:50 (25 min)
Masahiko Ikeuchi (Department of Life Sciences (Biology), University of Tokyo, Komaba, Meguro, Tokyo; Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Saitama, Japan)
Photosystem I assembly compensates the photodamage of photosystem I

9:50–10:15 (25 min)
Seiji Akimoto (Molecular Photoscience Research Center, Kobe University, Kobe, Japan)
Excitation relaxation dynamics and energy transfer in pigment-protein complexes containing keto-carotenoids

10:15–10:40 Coffee Break

Invited Lectures:

Chairpersons: Toshiharu Shikanai (Japan), Kiriakos Kotzabasis (Greece), Oxana Masyagina (Russia)

10:40–11:05 (25 min)
Tatsuya Tomo (Tokyo University of Science, Tokyo, Japan; PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan)
Characterization of unique photosystem I complexes and its application

11:05–11:30 (25 min)
Anatoly A. Tsygankov (Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)
Hydrogen production by marine microalgae under P-deprivation

11:30–11:55 (25 min)
Yuki Kato (Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan)
Redox potential of the secondary quinone electron acceptor Q_B in photosystem II as revealed by FTIR spectroelectrochemistry

11:55–12:20 (25 min)
Ryo Nagao (Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan)
Role of the hydrogen bond network around Y_z in photosynthetic water oxidation

12:20–14:00 Lunch

Invited Lectures:

Chairpersons: Esa Tyystjarvi (Finland), Yuki Kato (Japan),
Rajagopal Subramanyam (India)

14:00–14:25 (25 min)
Tsuyosho Endo (Graduate School of Biostudies, Kyoto University, Sakyo, Kyoto, Japan)
Chloroplastic NAD(P)H dehydrogenase complex in C_4 photosynthesis

14:25–14:50 (25 min)
Jörg Pieper (Institute of Physics, University of Tartu, Tartu, Estonia)
Protein structure and dynamics in photosystem II investigated by neutron scattering

14:50–15:10 (20 min)
Ivelina Zaharieva (Freie Universität Berlin, Arnimallee 14, Berlin, Germany)
Structural and functional parallels between the biological water oxidation site and a synthetic manganese-oxide catalyst

15:10–15:30 (20 min)
Rajagopal Subramanyam (Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)
Long and short term acclimatization and organization of photosynthetic apparatus under salt grown *Chlamydomonas reinhardtii*

15:30–16:00 Coffee Break

Invited Lectures:

Chairpersons: Arvi Freiberg (Estonia), Alexandrina Stirbet (USA),
George Grammatikopoulos (Greece)

16:00–16:20 (20 min)
Georgios Liakopoulos (Laboratory of Plant Physiology and Morphology, Department of Crop Science, Agricultural University of Athens, Athens, Greece)
Why anthocyanic leaves show lower risk of photoinhibition: Let the stomata speak

16:20–16:40 (20 min)
Merope Tsimilli-Michael (3, Ath. Phylactou str., Nicosia, CY-1100, Cyprus)
Sustainability of photosynthesis research

16:40–17:00 (20 min)
Suleyman I. Allakhverdiev (Controlled Photobiosynthesis Laboratory, Institute of Plant Physiology, RAS, Moscow, Russia; Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia; Department of Plant Physiology, M.V. Lomonosov Moscow State University, Moscow, Russia)
Nanostructured manganese oxide on silica aerogel toward water oxidation

17:00–20:00 Poster viewing/discussion (sections 1–11)

Chairpersons: Marian Brestic (Slovak Republic), Alex Ivanov (Canada),
Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan), Yiola Petropoulou (Greece),
Hazem Kalaji (Poland), Ivelina Zaharieva (Germany), Takaya Tanabe (Japan)

25 SEPTEMBER

Invited Lectures:

Chairpersons: Tsuyosho Endo (Japan), Jörg Pieper (Estonia), Ryo Nagao (Japan)

9:00–9:25 (25 min)
Toshiharu Shikanai (Graduate School of Science, Kyoto University, Kyoto, Japan)
Regulation of photosynthesis by cyclic and pseudocyclic electron flow

9:25–9:50 (25 min)
Esa Tyystjärvi (Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland)
Redox state of the plastoquinone pool depends both on wavelength distribution and intensity of incident light

9:50–10:15 (25 min)
Alexandrina Stirbet (204 Anne Burras Lane, Newport News, Virginia, USA)
Modeling the slow smt phase of chlorophyll *a* fluorescence induction in green alga *Chlamydomonas reinhardtii*

10:15–10:40 (25 min)
Iwane Suzuki (University of Tsukuba, Tsukuba, Japan)
Development of chimeric sensor to analyze function of histidine kinases in the cyanobacterium *Synechocystis* sp. PCC 6803

10:40–11:10 Coffee Break

Invited Lectures:

Chairpersons: Iwane Suzuki (Japan), Vasiliy Goltsev (Bulgaria), Georgios Liakopoulos (Greece)

11:10–11:35 (25 min)
Marc M. Nowaczyk (Plant Biochemistry, Ruhr University Bochum, Bochum, Germany)
Localization of auxiliary proteins on photosystem II by surface plasmon resonance spectroscopy and chemical cross-linking in combination with mass-spectrometry

11:35–12:00 (25 min)
Marek Zivcak (Department of Plant Physiology, Slovak Agricultural University, Nitra, Slovak Republic)
Physiological significance of photosystem I photoinhibition in wheat leaves

12:00–12:20 (20 min)
Eugene G. Maksimov (Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia)
The signaling state of orange carotenoid protein

12:20–14:00 Lunch

Invited Lectures:

Chairperson: Marc Nowaczyk (Germany), Eugene G. Maksimov (Russia), Merope Tsimilli-Michael (Greece)

14:00–14:20 (20 min)
Ginga Shimakawa (Graduate School of Agricultural Science, Kobe University, Nada-ku, Kobe, Japan)
Flavodiiron 2 and 4 proteins mediate an O_2-dependent alternative electron flow in *Synechocystis* sp. PCC 6803 under CO_2-limited conditions

14:20–14:40 (20 min)
Hazem M. Kalaji (Department of Plant Physiology, Warsaw University of Life Sciences WULS-SGGW, 159 Nowoursynowska Street, 02-776 Warsaw, Poland)
Which technique is better for studying photosynthetic apparatus? Modulated, prompt or delayed chlorophyll fluorescence

14:40–15:00 (20 min)
Georgia Zahariou (Institute of Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, NCSR "Demokritos", Athens, Greece)
Theoretical study of the EPR signal of the $S_3TyrZ^{\bullet}$ metalloradical intermediate state

15:00–15:20 (20 min)
Nikolaos E. Ioannidis (Department of Biology, University of Crete, Crete, Greece)
Polyamines in chemiosmosis: A cunning mechanism for the regulation of photosynthetic ATP synthesis during growth and stress

15:45 – Taking Photos (All together)

16:00 Special Events:

i) Young Talents (5 awards/prizes)
ii) Best posters (5 awards/prizes)

Committee: James Barber (UK), Jian-Ren Shen (Japan), Govindjee (USA), Eva-Mari Aro (Finland), Gyözö Garab (Hungary), Tatsuya Tomo (Japan), Barry Bruce (USA), Kostas Stamatakis (Greece), Suleyman Allakhverdiev (Russia)

Closing ceremony

James Barber (UK); Govindjee (USA), Eva-Mari Aro (Finland), Gyözö Garab (Hungary), Nathan Nelson (Israel), Norio Murata (Japan), Kimiyuki Satoh (Japan),Tingyun Kuang (China), Kostas Stamatakis (Greece), Suleyman Allakhverdiev (Russia)

19:00 Banquet

26 SEPTEMBER (DEPARTURE)

List of posters

Section 1: Primary Processes of Photosynthesis

S1.5 Detection of both monovinyl chlorophyll *b* and divinyl chlorophyll *b* in a picoplankton *Prochlorococcus sp.* NIES-2086
Hirohisa Komatsu, Masanobu Kawachi, Mayumi Sato, Tadashi Watanabe, Yutaka Hanawa, Yoshihiro Shiraiwa, Masami Kobayashi

S1.6 The LIR1 protein regulates membrane tethering of ferredoxin-$NADP^+$ oxidoreductase (FNR)
Paula Mulo, Chao Yang, Hongtao Hu, Hongyan Ren, Yuzhu Kong, Hongwei Lin, Lingling Wang, Yi He, Xiaomeng Ding, Magda Grabsztunowicz, Yu Liu, Zhongchang Wu, Yunrong Wu, Chuanzao Mao, Ping Wu, Xiaorong Mo

S1.7 The F_0 level of chlorophyll *a* fluorescence induction: Does it reflect a standard and reproducible physiological state?
George C. Papageorgiou, Kostas Stamatakis, Govindjee

S1.8 Slow phase signal enhancement method using convolution for chlorophyll fluorescence
Takaya Tanabe, Tomohiro Tsunoda, Takeshi Hiyama, Mitsuo Fukuda

S1.9 Carotenoid composition determines the structural and functional properties of the phycobilisomes and photosystems
Sindhujaa Vajravel, Tamas Zakar, Ildikó Domonkos, Josef Komenda, Mihály Kis, László Kovács, Herbert van Amerongen, Zoltán Gombos, Tünde Tóth

Section 2: Structure, Function and Biogenesis of the Photosynthetic Apparatus

S2.6 Narrow-band red and blue light affect chloroplast ATP-synthase structure and function in barley seedlings
Olga Avercheva, Daria Gorshkova, Elizaveta Bassarskaya, Galina Kochetova, Tatiana Zhigalova, Eugene Lysenko

S2.7 The soluble carbonic anhydrase in thylakoids of higher plants
Tatyana Fedorchuk, Natalia Rudenko, Lyudmila Ignatova, Boris Ivanov

S2.8 Heterologous expression of genes for thermophilic phycocyanin in the mesophilic cyanobacteria *Synechococcus elongates* PCC 7942
Shiori Funatsu, Wattana Chetkul, Fumihiro Itoh, Wipawan Siangdung, Supapon Cheevadhanarak, Yoshihiro Shiraiwa, Kalyanee Paithoonrangsarid, Iwane Suzuki

S2.9 Interactions of photosynthetic core complexes with light harvesting antenna proteins in centric diatom *Cyclotella meneghiniana*
Zdenko Gardian, David Bina, Frantisek Vacha, Radek Litvin

S2.10 The cyanobacterial PsbP orthologue assists the assembly of photosystem II
Jana Knoppová, Jiangfeng Yu, Peter J. Nixon, Josef Komenda

S2.11 Post-translational modifications of ferredoxin-NADP$^+$ oxidoreductase in *Arabidopsis* chloroplast
Nina Lehtimäki, Minna M. Koskela, Käthe M. Dahlström, Eveliina Pakula, Minna Lintala, Martin Schloz, Michael Hippler, Guy T. Hanke, Anne Rokka, Natalia Battchikova, Tiina A. Salminen, Paula Mulo

S2.12 Application of the TwinStrep-tag/Streptactin system for the analysis of Photosystem II assembly intermediates from *T. elongatus*
Pasqual Liauw, Marc M. Nowaczyk

S2.13 Architecture of light harvesting apparatus of eustigmatophyte algae
Radek Litvin, David Bina, Miroslava Herbstova, Zdenko Gardian

S2.14 The antarctic psychrophile, *Chlamydomonas* sp. UWO241, preferentially phosphorylates a psi-cytochrome b6/f supercomplex
Beth Szyszka-Mroz, Paula Pittock, Alexander G. Ivanov, Gilles Lajoie, Norman P. A. Hüner

S2.15 Specific Lhcb4 and Lhcb5 phosphorylation sites are absent in the psychrophilic state transition variant, *Chlamydomonas* sp. UWO241
Beth Szyszka-Mroz, Marc Possmayer, Denis P. Maxwell, Norman P. A. Hüner

S2.16 *In vitro* enzymatic assay for 13^2-demethoxycarbonylation in chlorosomal bacteriochlorophyll biosynthesis
Misato Teramura, Jiro Harada, Tadashi Mizoguchi, Hitoshi Tamiaki

S2.17 Truncated chlorophyll *b*-less antenna of *chlorina* f2 3613 barley mutant can provide light-tolerance and wild type level productivity
Elena V. Tyutereva, Wolfram G. Brenner, Alexandra N. Ivanova, Katharina Pawlowski, Olga V. Voitsekhovskaja

Section 3: Photosystem II and Water Oxidation Mechanism

S3.7 Trapping $Tyr_Z^\bullet$ during $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions of the water oxidizing complex of Photosystem II
Maria Chrysina, Georgia Zahariou, Nikolaos Ioannidis, Yiannis Sanakis, Vasili Petrouleas

S3.8 PsbO protein isoforms in angiosperms: parallel subfunctionalisation revealed by phylogenetic analysis and mapping of sequence variability onto protein structure
Miloš Duchoslav, Lukáš Fischer

S3.9 Temperature dependence of photoinhibition
Heta Mattila, Sujata Mishra, Vesa Havurinne, Kumud B. Mishra, Esa Tyystjärvi

S3.10 FTIR evidence for proton release into the bulk upon photooxidation of tyrosine D in photosystem II
Shin Nakamura, Takumi Noguchi

S3.11 High-field EPR characterization of the redox centers of photosystem II
Nadia Seibel, Eiri Heyno, Wolfgang Lubitz, Matthias Rögner, Marc M. Nowaczyk, Nicholas Cox

S3.12 Tracking structural, energetic and kinetic properties of cyanobacterial Photosystem II variants
Zhiyong Liang, Rebecca Christiana, Holger Dau, Yvonne Zilliges

Section 5: Photosystem I and Bacterial Photosynthesis

S5.8 A comparative study of *Rhodobacter sphaeroides* mutants without peripheral light harvesting antenna
Zinaida Eltsova, Anatoly A. Tsygankov

S5.9 Ferredoxin-binding modulates the redox reaction rates between $NADP^+$/H and ferredoxin-$NAD(P)^+$ reductase from the green sulfur bacterium *Chlorobaculum tepidum*
Daisuke Seo, Ken Okado, Takeshi Sakurai

S5.10 Analysis of energy transfer system in chlorophyll *f* containing cyanobacterium
Toshiyuki Shinoda, Seiji Akimoto, Daisuke Nii, Hisataka Ohta, Min Chen, Suleyman I. Allakhverdiev, Tatsuya Tomo

Section 7: Artificial and Applied aspects of Photosynthesis

S7.6 Heterologous production of monoterpene hydrocarbons in cyanobacteria (*Synechocystis*)
Cinzia Formighieri, Anastasios Melis

S7.7 The study of genetic diversity and determination of the heritability in promising lines of bread wheat in the Moghan – Iran
Khanzadeh Hassan, Zeynab Teadadi Ajirlou

S7.8 Probing the photosynthetic efficiency of green microalgae used for bioremediation and valorization of anaerobic digestion effluents
Eleni Koutra, George Grammatikopoulos, Michael Kornaros

S7.9 Evaluation of antibacterial activity of a novel anionic hyperbranched dendritic polymer and its effect on photosynthesis
Katerina Panagiotaki, Zili Sideratou, Kostas Stamatakis

S7.10 Potassium deficiency, a “smart” cellular switch for sustained high yield photosynthetic hydrogen production by green algae
Aikaterini Papazi, Armida-Irene Gjindali, Elizabeth Kastanaki, Konstantinos Assimakopoulos, Konstantinos Stamatakis, Kiriakos Kotzabasis

S7.11 Sucrose production: *Synechococcus* sp. PCC 7942, an ideal candidate
Dimitris Vayenos, Kostas Stamatakis

Section 8: Regulation of Photosynthesis and Environmental Stress

S8.13 Spectrally resolved fluorescent signatures of light treated *Synechocystis* PCC 6803
Alonso M. Acuña, Joris J. Snellenburg, Michal Gwizdala, Bart van Oort, Rienk van Grondelle, Ivo H. M. van Stokkum

S8.14 The effect of ionising radiation on pigment production, photochemical efficiency, ptotein level and generation of reactive oxygen species in plants
Saftar Suleymanov, Konul Qasimova, Irada Huseynova, Jalal Aliyev

S8.15 Drought-induced changes in photosynthetic apparatus and antioxidant components of wheat (*Triticum durum* Desf.) varieties
Irada Huseynova, Samira Rustamova, Saftar Suleymanov, Durna Aliyeva, Jalal Aliyev

S8.16 Impact of soil water deficit on morphophysiological parameters of durum (*Triticum durum* Desf.) and bread (*Triticum aestivum* L.) wheat genotypes
Tofig Allahverdiyev

S8.17 Photochemistry of Photosystem II: Is it a reliable tool for selecting cotton (*Gossypium hirsutum* L.) with improved flooding tolerance?
Habib-ur-Rehman Athar, Altaf Hussain, Javed Iqbal, Muhammad Iqbal, Zafar Ullah Zafar, Saghir Ahmad, M. Ashraf

S8.18 Adjustment of photosynthetic electron transport in wheat under drought stress
Marian Brestic, Marek Zivcak, Kristyna Kunderlikova, Oksana Sytar, Katarína Olšovská, Pavol Slamka, Suleyman I. Allakhverdiev

S8.19 Early response of photosynthetic apparatus efficiency to nitrogen deficiency in radish plants
Magdalena D. Cetner, Katarzyna Kowalczyk, Wojciech Borucki, Aritra R. Choudhury, Tanmay Tanna, Hazem M. Kalaji

S8.20 Photosynthesis in juveniles and adults of three mediterranean species with different life forms during leaf developmental period
Christos Chondrogiannis, George Grammatikopoulos

S8.21 Chlorophyll catabolism under light deficiency in *Datura suaveolens*
Nina Djapic

S8.22 Effect of silver nanoparticules on *Arthrospira platensis*
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S8.23 Diurnal changes in photosynthetic enzyme activities and their regulation in some C4 species of Chenopodiaceae family
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S8.24 Photosynthetic activity in degradation of xenobiotics, a possible connection with organochlorine formation?
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S8.26 An alternative approximation of the ABS/CS parameter when the JIP-test is applied in the field
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S8.28 Alternating functions of terminal oxidases under light and dark conditions in *Synechocystis* sp. PCC 6803
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S8.29 Photosystem I rather than photosystem II is selectively inhibited under short term salinity stress in two halophyte species *Sulla carnosa* and *Atriplex halimus*
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S8.30 Hydrogen peroxide signaling – implications for photosynthetic cold stress tolerance in *Zea mays* L.
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S8.33 Function of prenyllipids during photo-oxidative stress in plants
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S8.35 Screening 16 plant species by *in vivo* reflectance spectroscopy indicate a common photosynthetic pigment profile for green fruits, as a response to their distinct internal microenvironment
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S8.38 Photosynthetic activity of *Larix* trees grown on permafrost soils
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S8.39 Photosynthesis and transpiration changes after wounding and perception of herbivore elicitors in *Nicotiana attenuata*: role of stomata regulators abscisic acid, OPDA and cytokinins
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S8.40 Cell and chloroplast size related to long-term acclimation of barley to combined drought and low nitrogen
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S8.41 Effect of progressive drought on mesophyll conductance to CO_2 flow in photosynthetizing leaves of wheat at different ploidy levels
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S8.42 Effect of nitrate stress on photosynthetic electron transport in *Chlorella saccharophila* grown under high light
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S8.43 Foliar anthocyanin accumulation leads to adjustments in photosystem and chlorophyll ratios, compatible to the shade acclimation syndrome
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S8.44 New insights into short-term light acclimation in plants – the role of high molecular mass protein complexes
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S8.45 Electron flow from PSII to PSI under high light is regulated by PGR5 but not by PsbS
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S8.46 Initial disorder in structure and functions of Photosystem II in radish plants under magnesium deficiency
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S8.47 Elevated temperatures facilitate rapid light-dependent accumulation of zeaxanthin in *Picea abies* needles but not in *Arabidopsis thaliana* leaves
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S8.48 Non-photochemical fluorecence quenching in the pigment apparatus of cyanobacteria
Igor Stadnichuk, Dmitrii Zlenko, Pavel Krasilnikov

S8.49 Electrical signals as potential mechanism of photosynthesis regulation in higher plants
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S8.50 Natural variation in tocochromanols content in *Arabidopsis thaliana* accessions – the effect of temperature and light intensity
Renata Szymańska, Michał Gabruk, Iwona Habina, Jerzy Kruk

S8.51 Role of phosphatidylglycerol in cyanobacterial cells
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S8.52 The effect of lanthanides on photosynthesis and cell proliferation
Milada Vitova, Katrin Kaineder, Dana Mezricky

Section 9: Systems Biology of Photosynthesis:

S9.1 Proteome analysis of enriched heterocysts from three hydrogenase mutants from *Anabena* sp. PCC 7120
Eftychia Manarolaki, Antigoni Nikolaki, Dimitrios Dedoglou, Aikaterini Kourpa, Georgios Tsiotis

S9.2 *In silico* modelling of photosynthetic electron transport
László Sass, Zsuzsanna Deák, Éva Kiss, Imre Vass

Section 10: Photosynthesis Education

S10.2 Is chlorophyll *e* 15^1-OH-lactone chlorophyll *a* or chlorophyllide *a*?
Yuhta Sorimachi, Masataka Nakazato, Hideaki Miyashita, Masami Kobayashi

Section 11: Emerging Techniques for Studying Photosynthesis

S11.3 Compartment Markers for Plant Science
Zakir Hossain, Joanna Porankiewicz-Asplund, Christopher M. Brown

S11.4 Critical assessment of protein cross-linking – a modified model of the interaction between photosystem II and Psb27
Kai U. Cormann, Madeline Puschmann, Marc M. Nowaczyk

LECTURES

Lecture S7.1

Artificial Photosynthesis and Global Climate Change

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Global energy consumption is projected to increase, even in the face of substantial declines in energy intensity, at least two-fold by midcentury relative to the present because of population and economic growth. This demand could be met, in principle, from fossil energy resources, particularly shell gas and coal. However, the cumulative nature of CO_2 emissions in the atmosphere demands that holding atmospheric CO_2 levels to even twice their preanthropogenic values by midcentury to avoid devastating irreversible climate change will require invention, development, and deployment of schemes for carbon-neutral energy production on a scale commensurate with, or larger than, the entire present-day energy supply from all sources combined. Among renewable energy resources, nuclear fusion energy or solar energy are by far the largest exploitable resource. However in both cases technological breakthroughs are required with nuclear fusion being very difficult. On the other hand, one hour of sunlight falling on our planet is equivalent to all of the energy consumed by humans in an entire year. If solar energy is to be a major primary energy source, it must be stored and dispatched on demand to the end user. An especially attractive approach is to store solar-converted energy in the form of chemical bonds as occurs in natural photosynthesis. However a technology is needed which has a year-round average efficiency significantly higher than current plants or algae, to reduce land-area requirements and to be independent of food production. Therefore the scientific challenge is to construct an "artificial leaf" able to efficiently capture and convert solar energy and then store the energy in the form of chemical bonds, producing oxygen from water and a reduced fuel such as hydrogen, methane, methanol, or other hydrocarbon species. The "artificial leaf" must be robust and constructed of common materials and with effort there is no reason why such technology cannot be created for future prosperity, sustainability and harmony of the human race. In my lecture I will give an overview of the activities and recent successes in constructing catalytic systems which may form the basis of the artificial leaf technology against the background of global energy supply and demand.

Lecture S3.1

Mechanism of photosynthetic water oxidation based on atomic structure of photosystem II

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Photosystem II (PSII) catalyzes light-induced water-splitting leading to the evolution of molecular oxygen indispensible for oxygenic life on the earth. We have solved the crystal structure of PSII from a thermophilic cyanobacterium *Thermosynechococcus vulcanus* at a resolution of 1.9 Å (1) using synchrotron radiation (SR) X-rays, which revealed a clear picture of Mn_4CaO_5-cluster, the catalytic center for water-splitting. Some of the inter-atomic distances within the metal cluster, however, were shown to be slightly longer than those obtained by EXAFS and theoretical studies, presumably due to radiation damage caused by the SR X-rays. In order to avoid possible radiation damage and eliminate the uncertainties in the inter-atomic distances, we used femtosecond X-ray pulses from an X-ray free electron laser (XFEL) facility SACLA, Japan, to solve the structure of PSII. In order to obtain a high resolution structure, we used large PSII crystals, and adopted an approach where every point of the crystal was shot by 1 XFEL pulse, and every two XFEL pulses were separated at least by 50 μm on the crystals. This approach required a huge number of large, isomorphous PSII crystals, but allowed us to collect damage-free, high resolution diffraction data, enabling us to solve the PSII structure at 1.95 Å resolution (2). This structure showed that most of the Mn-Mn and Mn-ligand distances were 0.1–0.3 Å shorter than those observed in the previous SR structure. However, the bond distances of O5, a unique oxo-bridged oxygen, with two of its nearby Mn ions Mn1 and Mn4, are still unusually longer (2.3 and 2.7 Å, respectively) compared with typical Mn-O bond distances. This unusual property implied that this oxo-bridge binds weakly to its nearby metal ions, and may participate in the O-O bond formation during O_2 release. I will discuss the possible mechanisms for photosynthetic water-splitting.

This work is a collaboration of many colleagues, and I thank them for their contributions.

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Lecture S2.1

Early steps of photosystem II assembly

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The oxygen-evolving photosystem II (PSII) complex found in the thylakoid membrane system plays a vital role in the growth of plants, algae and cyanobacteria. We are interested in understanding at the molecular level how the complex is assembled and then repaired following irreversible damage by light. Our studies on the cyanobacterium *Synechocystis* sp. PCC 6803, done in close collaboration with Dr. Josef Komenda and colleagues, indicate that PSII is assembled from smaller pre-assembled pigment-protein modules and involves a number of accessory factors not present in the final dimeric PSII complex [1]. In this talk I will discuss the implications arising from our recent discovery of a new pigment complex (the Ycf39-Hlip complex) involved in the assembly of the PSII reaction centre complex [2] and describe recent results on the structure and function of Ycf48, a conserved accessory factor also involved at an early stage in PSII assembly [3].

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Lecture S1.1

Adventures with the green alga *Chlamydomonas reinhardtii*: in honor of George C. Papageorgiou

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Our adventures with *Chlamydomonas* (*C.*) *reinhardtii* began in 1984, when we studied the influence of [CO2] on chlorophyll (Chl) *a* fluorescence induction. This was followed, in the 1990s, by preparation of pure Photosystem II (PSII) particles; the role of bicarbonate on the acceptor side of PSII, using newly constructed mutants; role of a specific histidine on the donor side of PSII; and construction of a new homology model of PSII. In the 2000s: acidocalcisome was discovered (2001); the first FLIM (Fluorescence Lifetime Imaging Microscopy) measurements on Non-photochemical quenching mutants were made (2007); and the role of *state change* in the slow S to M Chl *a* fluorescence rise was inferred through the use of a state-less mutant (2015). Here, we shall present our most recent work on two mutants (IM and KO) of *C. reinhardtii* with improved biomass production when grown under low light and under mixotrophic conditions (Zhou et al. (2015) *Algal Research* **11**: 134–147), and on a fluorescence lifetime imaging technique, used in conjunction with a quantitative image analysis method, to study photosynthetic responses to various light conditions at single cell level (Chen & Govindjee (2015), *J Biomed Opt*, submitted). With our FLIM technique, we have observed a complex and a delicate balance between the de-excitation pathways of the excited state of chlorophyll *a* in *C. reinhardtii* at the single cell level. We plan to investigate how these changes differ between the wild type and the IM and KO mutants.

Lecture S7.2

Photosynthesis for fuel and chemicals production

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The concept of a direct Photosynthesis-to-Fuels approach [1] entails application of a single organism acting both as photocatalyst and processor, absorbing sunlight, photosynthesizing, and generating useful bio-products [2]. Reduction to practice of this concept is offered by the photosynthetic production of hemiterpene (C_5H_8) and monoterpene ($C_{10}H_{16}$) hydrocarbons by green microalgae and cyanobacteria, useful compounds that are generated from sunlight, carbon dioxide, and water. The work describes chemical and metabolic engineering approaches, whereby photosynthesis in microalgae and cyanobacteria is diverted toward terpene hydrocarbon synthesis and release. Green microalgae, e.g. *Chlamydomonas reinhardtii*, and cyanobacteria, e.g. *Synechocystis* sp., are model microorganisms amenable to transformation for bioenergetic and metabolic flux manipulation, leading to photosynthetic isoprene [1–3] or β-phellandrene [4, 5] hydrocarbons as paradigm volatile products. An important feature of this approach is the spontaneous product separation from the biomass, and from the liquid culture, simplifying product sequestration, harvesting, and quantification, thus enabling scale-up for process commercialization. The work will further discuss current challenges, including a fundamental problem that impacts the renewable generation of fuels and chemicals via synthetic biology. At issue is the regulation of endogenous cellular carbon partitioning between different biosynthetic pathways, over which the living cell exerts stringent control. The latter applies to the terpenoid biosynthetic pathway, where flux is restricted to only about 5% of photosynthetically fixed carbon. Experimental approaches to up-regulate carbon flux through the terpenoid biosynthetic pathway, enhancing product-to-biomass carbon partitioning ratios in the cell and correspondingly enhancing the yield of hydrocarbons production will be discussed.

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Lecture S2.2

Photoprotection and Photodamage of Photosystems I and II – consequences on short-term thylakoid dynamics and long-term retrograde signaling

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Photodamage and repair of PSII have been studied extensively already for decades and a number of auxiliary proteins assisting the assembly and repair of PSII have been identified (1). Search for proteins involved in regulation of intersystem electron transfer and that of PSI have attracted less attention and relatively few such proteins are known. This is partially due to the fact that while PSII is damaged upon high light illumination, the damage of PSI is easily monitored only at fluctuating light, more precisely upon increase in light intensity after a low light period (e.g. the passing of clouds) and being strongly affected by regulation of the intersystem electron transfer (2) PSI damage occurs at the level of Fe-S clusters and has been shown to be protected by the PGR5 protein and photoinhibitory down regulation of PSII but not by the PSBS protein (3, 4). Thus, the maintenance of the trans-thylakoid proton gradient and concomitant slow-down of intersystem electron flow to PSI at the Cyt b_6f complex, are key factors for sustenance of PSI upon naturally fluctuating light conditions.

Likewise, the reversible and opposite phosphorylation of the PSII core and LHCII proteins by the STN7 and STN8 kinases and respective phosphatases upon fluctuations in light intensity plays a crucial role in photoprotection by allowing dynamic interactions between PSII-LHCII with PSI, and thus allowing PSI to participate in dissipation of excess energy (5).

The above mentioned regulatory mechanisms, and most likely a number of other still undiscovered regulatory mechanisms, collectively guide a fluent acclimation of the photosynthetic apparatus to changing environmental and metabolic cues. In long-term, if the protection capacity is exceeded, chloroplasts send retrograde signals to the nucleus for changing the growth and metabolism to correspond the new environmental condition (6, 7). Such signaling pathways and their interaction with hormonal signaling will be likewise discussed.

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Lecture S8.1

George Papageorgiou, glycinebetaine, and protection against photoinhibition

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George Papageorgiou and I started our collaboration at the National Institute for Basic Biology in 1989. He brought an idea that the quaternary ammonium ions should be specifically effective on the oxygen-evolving complex (OEC). We tried choline chloride and glycinebetaine (hereafter, GB), both containing the quaternary ammonium ions, and found that GB had an unusually strong effect in stabilizing the OEC. Prasanna Mohanty and Hidenori Hayashi joined to work on this topic and we characterized the nature of the GB-induced stabilizing effect [1]. This success encouraged us to start the transformation of plants and cyanobacteria by introducing the GB-synthesizing system. We used the *codA* gene for choline oxidase from a bacterium, which converts choline to GB. Resultant transgenic plants, which contained relatively low levels of GB, revealed high ability of protection against various kinds of abiotic stress. Moreover, it protected photosystem II against photoinhibition particularly under abiotic stress conditions by stimulating the synthesis of the D1 protein, leading to the repair of photosystem II, but did not affect the light-induced inactivation (photodamage) of photosystem II [2].

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Lecture S5.1

High-resolution structures of plant and cyanobacterial Photosystem I – implications for sustainable hydrogen production

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Plant Photosystem I (PSI) is one of the most intricate membrane complexes in Nature. It is comprised of two complexes, a reaction center and light-harvesting LHCI. We developed a method for obtaining better mass spectroscopy data from membrane complexes. Using the corrected amino acid sequences an improved plant PSI structure was obtained. An atomic-level structural model of higher plant PSI at 2.8 Å resolution has been constructed based on new crystal form. The crystal belongs to $P2_12_12_1$ symmetry space group, with one protein complex in each asymmetric unit. The structure includes 16 subunits and more than 200 prosthetic groups, the majority of which are light harvesting pigments. The model reveals detailed interactions, providing mechanisms for excitation energy transfer and its modulation in one of Nature's most efficient photochemical machine.

An operon encoding PSI was identified in cyanobacterial marine viruses. We generated a PSI that mimics the salient features of the viral complex containing PsaJ-F fusion subunit. The mutant is promiscuous for its electron donors and can accept electrons from respiratory cytochromes. We solved the structure of the PsaJ-F fusion mutant as well as a monomeric PSI at 2.8 Å resolution, with subunit composition similar to the viral PSI. The novel structures provided for the first time a detailed description of the reaction center and antenna system from mesophilic cyanobacteria, including red chlorophylls and cofactors of the electron transport chain. Our finding extends the understanding of PSI structure, function and evolution and suggests a unique function for the viral PSI. Based on the observation of a promiscuous PSI that is able to accept electrons from respiratory cytochrome c we designed a bioreactor for hydrogen production. To achieve this goal we will engineer two changes into the two photosystems of the cells. One will allow us to inactivate PSII at will, at elevated temperatures and another will direct the cellular redox pool toward H_2 production. Achieving both of these targets will constitute a significant advance in biohydrogen production since these problems are generic to the process and not dependent on the organism in which the studies will be performed.

Lecture S5.2

Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex

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Photosystem I (PSI) performs light-induced, trans-membrane electron transport, leading to the reduction of $NADP^+$ to NADPH that is required for the conversion of CO_2 into sugars. In higher plants, the PSI core is surrounded by a large light-harvesting complex I (LHCI) that captures sunlight and transfers the excitation energy to the core with an extremely high efficiency. The structure of the PSI-LHCI has been solved previously up to 3.3 Å resolution [1], revealing the subunit organization and arrangement of a number of cofactors. However, this resolution was not enough to determine the precise structure of PSI-LHCI, especially its subfraction LHCI. We have succeeded in improving the crystal quality of the PSI-LHCI super-complex from pea, and analyzed its structure at 2.8 Å resolution [2]. Our structure showed that the PSI-LHCI super-complex consisted of 16 subunits (PsaA-L, Lhca1-4), and revealed the detailed arrangement of 205 cofactors (143 Chl *a*, 12 Chl *b*, 26 β-car, 5 Lut, 4 Vio, 10 lipids, 3 Fe_4S_4 clusters, 2 phylloquinones). Based on this structure, we proposed 4 plausible pathways for the energy transfer from LHCI to the PSI core.

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Lecture S1.2

Do we comprehend the *in vivo* fluorescence of photosynthetic pigments well enough?

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Fluorescence has been widely used as a sensitive probe of photosynthetic activity [1]. For a proper interpretation all aspects of solar energy conversion to the photosynthetic material excitation and back to the emitted fluorescence photons should be understood. Here we present a discovery of a weak up-converted fluorescence related to the bacteriochlorophyll a pigments in various light-harvesting pigment–protein complexes as well as in the functional membranes (chromatophores) of photosynthetic purple bacteria under relatively low-power continuous-wave infrared laser excitation, far outside all the optically allowed singlet absorption bands of the chlorophyll and carotenoid chromophores. The fluorescence increases linearly with the excitation power, clearly distinguishing it from the previously observed two-photon excited fluorescence upon femtosecond pulse excitation. We have critically analysed three possible mechanisms for arising of this fluorescence: (i) one-photon absorption mediated by thermally activated vibrations in the ground state, (ii) two-photon absorption, and (iii) delayed fluorescence. The first interpretation appears to be disqualified by the inverse thermal dependence of the emission intensity, while the second explanation, by the linear dependence between the excitation and emission intensities, leaving the delayed fluorescence involving intermediate long-lived triplet states either the bacteriochlorophyll or carotenoid pigments as the only viable option. Two possible mechanism of the delayed fluorescence will be discussed, (i) two-step triplet state mediated excitation and (ii) triplet–triplet annihilation, which both show linear excitation intensity dependence under appropriate choice of model parameters [1].

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Lecture S3.2

Functional and structural roles of Asn268 of D1 of photosystem II reaction center

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Hydrogen bond networks around the oxygen-evolving Mn_4CaO_5 cluster has been identified from the crystal structure of photosystem II [1]. Asn298 of PsbA (D1) is involved in one of hydrogen bond networks starting from Mn_4CaO_5 cluster to Ala411 of CP43, Asn322 of D1, and Tyr137 of PsbV via amino acid residues of D1 and several water molecules. The Asn298 is hydrogen bonded to His190 of D1 but little is known on its function in PSII. We have generated 19 chloroplast transformants in which the residue has been substituted by each of the other 19 amino acid residues [2]. The chloroplast mutant, Fud7, which has a deletion of most part of *psbA* gene, was used as a host strain for introducing site-directed mutations into the *psbA* gene encoding D1 [3]. All mutants showed significantly impaired or no photosynthetic growth, and had significantly reduced or no oxygen-evolving activity. However, the light-induced electron transfer reaction from DPC to DCIP was almost normal on a PSII basis. We have furthermore estimated manganese contents with an atomic absorption spectrometry and analyzed S-state transitions of Mn_4CaO_5 cluster by thermoluminescence measurements. Many transformants had a defect in S-state transtion; the transition from S_1 to S_3 occurred normally but the transition from S_3 to S_0 was significantly impaired by the mutations. The effect of the amino acid substitutions on the function and structure of Mn_4CaO_5 cluster will be discussed.

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Lecture S2.3

The molecular architecture and functioning of LHCII *in vivo* and *in vitro*

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In addition to its primary function, to capture sunlight and utilize its energy, light-harvesting complex II (LHCII) plays important roles in the self-assembly and stabilization of the granal ultrastructure of thylakoid membranes as well as in light-adaptation and photoprotection of green plants. In order to understand these diverse functions, it is essential to have detailed and accurate information about the molecular interactions within and between the complexes as well as about the architecture of protein complexes and their macroarrays. High-resolution structure of LHCII is known from X-ray crystallography (Liu et al. 2004 Nature 428:287). In general, it is assumed that the crystal structures can be imposed both on the native state(s) of LHCII *in vivo* and on its *in vitro* forms. However, reports using ultrafast and steady state spectroscopy techniques have shown that this assumption must be used with great caution and more investigations are required to understand the spectroscopic data in terms of the molecular architecture of the complexes. In order to elucidate this question we have started systematic investigations on stacked and washed thylakoids and LHCII-enriched membranes, as well as on detergent-solubilized trimers, proteolimposomes, lamellar aggregates and microcrystalline samples. By using circular dichroism (CD) spectroscopy on isotropic and anisotropic samples (ACD, cf. Miloslavina et al. 2012 Photosynth Res 111:29) as well as linear dichroism and time-resolved fluorescence, we have identified the CD signatures of detergent-induced perturbations of the complexes, and identified well discernible effects of lipid-protein and protein-protein interactions on *in vitro* samples, which were compared to *in vivo* states (Akhtar et al. 2015 J Biol Chem 290:4537). Ultrafast coherent two-dimensional spectroscopy also revealed significant differences in energy transfer dynamics of trimers and aggregates (Enriquez et al. 2015 J Chem Phys 142:212432).

Lecture S2.4

Evolution of two light reactions. Cooperation and inter-dependency in photosynthesis, science, and society

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The "Z-scheme" describes the electrical connection, in series, of two pigment systems each of which contains its own photochemical reaction centre and attendant light-harvesting antenna. The two photosystems are complementary, and inter-dependent: photosystem I and photosystem II cooperate in the conversion of absorbed excitation energy into electrochemical potential. Competition between the photosystems leads to inefficiency, and, if uncorrected, to their inactivation. In state transitions, absorbed excitation energy becomes optimally distributed between the two photosystems. The sensor that initiates redistribution is a departure from equal rates of electron transport into and out of the intermediary electron carrier, plastoquinone. The response is a redox-regulated reversible protein phosphorylation that serves to re-allocate a mobile antenna to the otherwise rate-limiting photosystem. In photosystem stoichiometry adjustment the rates of transcription of the genes for apoproteins of the two photochemical reaction centres are adjusted in response to the same sensor of imbalance that initiates state transitions. In eukaryotes, reaction centre genes are universally retained in chloroplast DNA where they are placed under redox regulatory control by components that have been inherited with little modification from the cyanobacterial endosymbionts from which chloroplasts evolved. While biochemistry and biophysics are satisfyingly immune to the naturalistic fallacy of inferring what ought to be from what is, it is clear that excitation energy, supplied in parallel to the two light-harvesting antennae, becomes equitably re-distributed by both post-translational and transcriptional mechanisms. Neither photosystem is allowed to remain in excess of the other in its capacity to contribute to overall quantum yield. By analogy, I shall argue that the yield of research – new knowledge and understanding – is increased by optimal distribution of resources between laboratories that cooperate by sharing their findings. In contrast to this view, a current trend is to sever cooperative links, placing researchers in competition with each other. At the boundary of existing knowledge there is neither competition nor scope for restriction on freedom of enquiry. All scientific progress is initiated by unique and unpredictable events.

Lecture S2.5

Low pH induced changes in thylakoid membranes

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The increase in 77K fluorescence of PSI and quenching of PSII fluorescence upon exposure of isolated thylakoid to low pH is not caused by state transitions as evident from the observation that similar change was observed also in the stn7 kinase mutants. On the contrary, the pH induced change in the PSI/PSII ratio was found to be absent in npq4 mutants, providing evidence that PsbS dependent NPQ is involved in regulating energy distribution between the two photosystems. Zeaxanthin is likely not involved in qE changes induced by pH. No major change by different pH treatments occurred in the thylakoid membrane protein composition of WT or any of the mutants. As to the 77K fluorescence excitation spectra, the change in pH was shown to cause similar changes in the absorption cross section of both the photosystems. This indicates that pH does not affect the attachment of the LHC system with the photosystems as such, but simply enhances the spillover of energy between the two photosystems. Low pH may lead to isolation of LHCII system from PSII and PSI and concomitantly enhanced direct interaction between PSII and PSI (spillover). An enhanced cyclic electron flow around PSI also supports this contention.

Lecture S7.3

High yield H_2-production through a combinational system of photosynthetic electron flow and dichlorophenol biodegradarion by green algae

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Hydrogen (H_2) is a highly promising energy source with important social and economic implications. The ability of green algae to produce photosynthetic hydrogen under anaerobic conditions has been known for years. However, until today the yield of production has been very low, limiting an industrial scale use. In this contribution, we present a combinational biological system where the biodegradation procedure of one *meta*-substituted dichlorophenols (*m*-dcps – chemical pollutants in industrial waste-water) is the key element for maintaining continuous and high rate H_2-production of the green alga *Scenedesmus obliquus*. In particular, we report that reduced *m*-dcps, according to their redox potential, take place as electron donors to the photosynthetic electron flow, close to the plastoquinone pool (PQ). In parallel, they block the activity of photosystem II and the release of O_2, leading to the establishment of oxygen-depleted conditions in a closed system. Additionally, the first step of *m*-dcps biodegradation seems to be the *m*-dcps reduction that supports a continuous circuit between oxidized and reduced *m*-dcps, which continuously promotes strong electron flow to PQ-pool, and in turn to ferredoxin. As a result, photosynthetic hydrogen production is induced strongly and continuously by the hydrogenase activation, because of the establishment of oxygen-depleted conditions. The present contribution brings out the possibility of green microalgae to operate as "smart bioenergetic machines" for a continuous H_2-production, through an electron bypass between *m*-dcps biodegradation pathway and the photosynthetic procedure. The regulation of these multistage and highly evolved redox pathways leads to high yields of photosynthetic hydrogen production and paves the way for an efficient application to industrial scale use, utilizing simple energy sources and one *meta*-substituted dichlorophenol as regulating elements.

Lecture S1.3

Analysis of charge fluxes in thylakoid based on the Photosystem II electron transfer modeling

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The electron/proton transfer (ET/PT) processes regulated by the thylakoid electrical ($\Delta\Psi$) and ΔpH (pH_{Stroma}–pH_{Lumen}) components should be taken into account when chlorophyll fluorescence (FL) is analyzed [1]. The data quantification was developed due to our modeling [2–6]. In the general Thylakoid Model (TM) [2] the FL induction (FI) data obtained at different light intensities were simulated qualitatively. The photosystem (PS) II model that is incorporated in TM was developed as a separate block to fit the FL yield measured after 10 ns flash [3, 5, 6] as well as upon continuous light [4]. Our extended PSII model [3–6] took into consideration dissipative losses in antenna and in the reaction center and contained analytical descriptions for the light induced $\Delta\Psi(t)$, $pH_L(t)/pH_S(t)$ and the dynamical rate constants.

The present study uses the extended PSII model to fit FI curves up to the time interval of 2 s. The results provide the basis to use the Multimeric Thylakoid Model (MTM) which contains the new PSII model block. The MTM parameters were fitted in parallel to data measured on pea leaves in the time interval up to 20 s: FI curve and $P700^+$ oxidation–reduction signal. Two waves of these induction signals were described quantitatively for dynamics of $\Delta\Psi(t)$, $pH_L(t)/pH_S(t)$ which were found in physiologically relevant ranges. Parameters of both proton and counterions fluxes were defined.

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Lecture S8.2

Kleptoplasts: longevity in a new Ross Sea dinoflagellate host cell

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Kleptoplasty is a remarkable type of symbiotic association, involving the ingestion of algae by non-photosynthetic cells and the transformation of their chloroplasts into functional "kleptoplasts" by the host cell. Kleptoplasty, has been described for several heterotrophic dinoflagellates. A novel and abundant dinoflagellate group, related to the icthyotoxic genera *Karenia* and *Karlodinium*, was discovered by Gast et al. (2006) in the Ross Sea, in Antarctica. These novel Ross Sea dinoflagellates (RSD) are closely related to the free-living unicellular photosynthetic haptophyte *Phaeocystis antarctica*, a species that often dominates phytoplankton blooms in the Ross Sea. In the present research we studied the similarities and the differences in light harvesting and photosystem functionality of chloroplasts in *P. antarctica* and the kleptoplasts in RSD cells.

Both cell types emit Chl *a* fluorescence which is centered at 689 nm (F689). A second derivative analysis of the F689 band revealed two individual emissions centered at 683 nm and 689 nm (F683, F689). F683 was identified to originate from Photosystem II (PS II) and F689 from Photosystem I (PS I). Compared to the algal chloroplast, the Chl *a* fluorescence ratio F683/F689 in the RSD kleptoplast was found to be diminished, suggesting a diminished role for Photosystem II (PS II) and an enhanced role for Photosystem I (PS I) in the kleptoplast.

In addition, we show for the first time the Chl *a* fluorescence of the RSD kleptoplasts to be sensitized by the ultraviolet light (320–390 nm) – absorbing mycosporine-like aminoacids (MAAs) they contain. This may implicate a light harvesting function for MAAs, whose primary role is to serve as UV light screens. Light harvesting by MAAs may make the kleptoplast less dependent on nucleus-encoded and cytosol-synthesized chlorophyll and carotenoid containing light harvesting proteins. In addition, the diminished role of PS II in the kleptoplast may diminish the presence of reactive oxygen species. We suggest that these two factors contribute to the remarkable longevity of the RSD kleptoplast.

Lecture S5.3

Origin and evolution of PSI in Cyanobacteria and Chloroplasts

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Photosystem I (PSI) is a reaction center associated with oxygenic photosynthesis. Unlike the monomeric reaction centers in green and purple bacteria, PSI forms trimeric complexes in most cyanobacteria. This oligomer has 3-fold rotational symmetry and is stabilized via interactions between adjacent PsaL subunits. However, upon endosymbiosis the organization of PSI in plants/algae became exclusively monomeric. In this study, we discovered a tetrameric form of PSI in the thermophilic cyanobacterium *Chroococcidiopsis* sp TS-821. In TS-821, PSI forms only tetrameric and dimeric species. To investigate why TS-821 forms tetramers instead of trimers, we cloned and analyzed its psaL gene. Interestingly, this gene product contains a short insert between the second and third predicted transmembrane helices. Based on both psaL and 16S rRNA sequence analysis TS-821 is quite closely related to the heterocyst-forming cyanobacteria. We have now extended this biochemical analysis of the PSI tetramer to ~40 different cyanobacteria. This tetrameric symmetry is quite widespread in heterocyst-forming cyanobacteria. This close relationship is discussed in light of chloroplast evolution, and we propose that PSI evolved stepwise from a trimeric form to tetrameric oligomer en route to becoming monomeric in plants/algae.

Lecture S5.4

"Travels of a Transit Peptide" or "It Takes Two to Translocate"

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With the recent expansion of plant genomes it is clear that a significant amount of the genome is predicted to encode pre-proteins destined for one or more of the plastids that are ubiquitous to plant cells. Different bioinformatics tools are used to detect N-terminal extensions (collectively known as transit peptides) allowing a rapid prediction of a putative plastid proteome. These transit peptides are N-terminal extensions of ~50 amino acids in length that are cleaved during or shortly following membrane translocation via the stromal processing protease. The transit peptide is responsible for both the targeting and translocation of the pre-protein across the dual membranes via translocons (Toc and Tic) in the plastid envelope. Although considerable progress has been made to identify the components of the Toc and Tic translocons, we still lack even a low-resolution structure of these multisubunit complexes. Although we have known of this targeting activity of transit peptides for nearly 40 years, we are still struggling to decode and extract the information encoded within these short peptides. For the past 25 years, my lab has worked to decode these sequences by a combination of biochemical, biophysical, cellular, and *in vivo* assays. Although still lacking in some molecular details we now propose a versatile, bimodal model that involves a "trapping" state via one or more Toc receptor(s) on the cytoplasmic surface and a "pulling" state with an interaction with one or more ATP-dependent molecular motors on the stromal surface of the inner membrane. Due to multiplicity of the components that can fulfill these two distinct interactions, we can easily explain the high sequence diversity of transit peptides. Current work seeks to determine the design constraints for such a bimodal mechanism of targeting and translocation.

Lecture S5.5

Photosystem I assembly compensates the photodamage of photosystem I

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Oxygenic phototrophs are prepared for photosystem II photodamage under high light by enhanced turnover of D1 protein. On the other hand, photosystem I is relatively stable against high light but is sensitive photooxidative photodamage especially under fluctuating light or high light at low temperature. During these conditions, transient accumulation of reducing equivalents at the acceptor side of photosystem I may be the target of photodamage and protecting electron sinks such as cyclic electron flow and Mehler reaction to oxygen via flavodiiron proteins have been extensively studied. However, turnover or reassembly of photosystem I has been scarcely studied, partly because the photodamage of photosystem I is crucial for plant survival. Here, we generated a mutant for biosynthesis of phylloquinone in *Synechocystis* and *Thermosynechococcus*. These mutants are highly sensitive to irradiation of normal light. We found that overexpression of photosystem I assembly factor suppressed this sensitivity of the mutant to the normal growth light. Moreover, the recovery from the high light photoinhibition was enhanced by the overexpression of the factor. These results demonstrate that the photosystem I assembly is also essential for photosynthesis under certain photoinhibitory conditions.

Lecture S1.4

Excitation relaxation dynamics and energy transfer in pigment-protein complexes containing keto-carotenoids

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Light-harvesting complexes found in some marine algae contain specific carotenoids and chlorophylls: siphonaxanthin and chlorophylls *a* and *b* in siphonaxanthin-chlorophyll *a*/*b*-protein (SCP) complexes of green algae, fucoxanthin and chlorophylls *a* and *c* in fucoxanthin-chlorophyll *a*/*c*-protein (FCP) complexes of diatoms and brown algae, and peridinin and chlorophylls *a* and *c* in peridinin-chlorophyll *a*/*c*-protein (iPCP) complexes of photosynthetic dinoflagellates. As peripheral antenna complexes, photosynthetic dinoflagellates possesses peridinin-chlorophyll *a*-protein (sPCP) complexes The keto-carotenoids, such as siphonaxanthin, fucoxanthin, and peridinin exhibit common conjugation structures consisting of eight C=C double bonds and one C=O bond, which brings a characteristic absorption band in the green region. This is ecologically advantageous under the green-light-rich water condition. We have reported energy transfer processes in SCP and FCP; carotenoid-to-chlorophyll energy transfer occurred via the carotenoid S1 state with high efficiency in both complexes. In the present study, excitation relaxation dynamics in PCP isolated from the photosynthetic dinoflagellates, *Symbiodinium* sp., was investigated by time-resolved fluorescence spectroscopies. We will discuss the energy transfer processes in PCP.

Lecture S5.6

Characterization of unique photosystem I complexes and its application

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Photosynthetic energy transfer and electron transfer reactions are highly quantum yield. Especially, Photosystem I (PS I) produces highly reducing power to obtain hydrogen energy. Therefore, to clarify the mechanism of PS I is important for acquiring sustainable clean energy. The crystal structure of the PS I isolated from the cyanobacterium and higher plant has been determined. However, there is a number of diversity in photosynthetic organisms. In this study, we isolated PS I complexes from *Symbiodinium* and unique cyanobacterium. The polypeptide compositions were different against general PS I complexes in *Symbiodinium* and unique cyanobacteria. *Symbiodinium* are sensitive to anomalous changes in their thermal environment. High temperatures cause photosynthetic damage to *Symbiodinium*, which can initiate coral bleaching. Isolated PS I also showed characteristic carotenoid composition. These changes might be responsible for the reason of sensitive to thermal environment to *Symbiodinium*.

We also tried to establish photosystem based energy conversion system. We modified the amino acid sequence of PS I, then we bind modified PS I and new material. We observed electron transfer from PS I to new material under photo-irradiation.

We will discuss the new properties of PS I complexes.

Lecture S5.7

Hydrogen production by marine microalgae under P-deprivation

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Energy is vital to human population. Unfortunately, dependence on the fossil fuel as our primary energy source contributes to global changes of climate. Molecular hydrogen is environmentally friendly fuel since the product of its utilization is only the water. However, the hydrogen production should be produced without concomitant carbon dioxide evolution. Currently, hydrogen might be produced without environmental pollution via electrolysis powered by electricity from photovoltaics. Biological light dependent production of hydrogen using sun light is an exciting idea for technology development.

Fresh water microalgae, for example, *Clamydomonas reinhardtii*, are capable to sustained H_2 photoproduction under sulfur-deprived conditions. Sulfur-deprivation gradually inactivates photosystem II (PSII) activity in cells which results in culture transition to the anaerobic conditions. During sulfur deprivation photoheterotrophic algal culture passes through several physiological phases: an aerobic, oxygen consumption, an anaerobic, a hydrogen production and a termination phase. One of drawbacks of fresh water microalgae is the demand in fresh water. Marine microalgae use sea water which is much cheaper and biotechnological processes based on these microalgae should simpler and cheaper in operation. Unfortunately, in literature data on sustained H_2 production by marine microalgae are absent. We also failed to find conditions for H_2 production by sulfur-deprived marine microalgae. We suggested that sea and ocean waters contain an excess of sulfur and this is a reason why microalgae did not produce hydrogen. The aim of this presentation is to develop P-deprived conditions and check these conditions for possible hydrogen production by fresh water and marine microalgae.

Our results show that fresh water and marine microalgae can produce H_2 under P-deprivation and this is the first report about sustained H_2 production by marine microalgae.

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Lecture S3.3

Redox potential of the secondary quinone electron acceptor Q_B in photosystem II as revealed by FTIR spectroelectrochemistry

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Photosystem II (PSII) drives photo-induced electron transfer from the Mn_4CaO_5 cluster to two plastoquinones, the primary quinone electron acceptor Q_A and the secondary quinone Q_B. The driving force of the electron transfer between these quinones is their redox potential (E_m) gap. However, the E_m gap remains unclear, because E_m of Q_B has not yet been directly measured. Meanwhile, $E_m(Q_A^-/Q_A)$ has been extensively investigated and determined to be ca. −100 mV in intact PSII. It was further found that $E_m(Q_A^-/Q_A)$ shifts by about +150 mV by inactivation of the Mn_4CaO_5 cluster [1, 2, 3].

In this work, by applying FTIR spectroelectrochemistry [4], we measured $E_m(Q_B^-/Q_B)$ to reveal the energetics of the electron transfer between the two quinones. $E_m(Q_B^-/Q_B)$ was determined to be +134 mV in intact PSII, which was shifted by −17 mV by Mn depletion. We thus conclude that Mn depletion decreases the E_m gap from ~230 mV to ~70 mV due to the slight negative shift of $E_m(Q_B^-/Q_B)$ in contrast to the large positive $E_m(Q_A^-/Q_A)$ shift. This decrease in the E_m gap promotes the reverse electron transfer from Q_B to Q_A, leading to photoprotection when the Mn_4CaO_5 cluster is inactivated.

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Lecture S3.4

Role of the hydrogen bond network around Y_Z in photosynthetic water oxidation

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The X-ray crystal structure of photosystem II revealed two major proton transfer pathways, i.e., the hydrogen bond network around Cl^- ion and that around the redox-active tyrosine Y_Z. To examine the role of the Y_Z network during water oxidation, we analyzed a site-directed mutant of D1-N298, which interacts with D1-H190 hydrogen-bonded with Y_Z, using the cyanobacterium *Synechocystis* sp. PCC 6803. The D1-N298A mutant grew photoautotrophically with a rate slightly slower than WT. O_2-evolving activity of the D1-N298A mutant was ~15% of WT. Fourier transform infrared (FTIR) analysis showed that the D1-N298A mutation decreased the efficiency of the $S_2 \rightarrow S_3$ transition and virtually blocked the $S_3 \rightarrow S_0$ transition. FTIR spectra of the mutant further showed the structural changes in a hydrogen bond network and in the interactions of a weakly hydrogen-bonded water molecule(s). The kinetics of the proton-coupled electron transfer was examined by time-resolved infrared measurements. Both of the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions were dramatically slowed by the mutation, while electron transfer from the Mn cluster to $Y_Z^\bullet$ during the $S_3 \rightarrow S_0$ transition was not detected. The block of the final O_2-evolving step by the mutation was consistent with the FTIR analysis. These results indicate that the hydrogen bond network around Y_Z functions as a proton transfer pathway during the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions.

Lecture S8.3

Chloroplastic NAD(P)H dehydrogenase complex in C_4 photosynthesis

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Chloroplast NAD(P)H dehydrogenase-like complex (NDH) is an enzyme complex located on the thylakoid membrane. NDH involves in cyclic electron flow around photosysem I (CEF I), which supplies ATP without producing NADPH. However, the phenotype of NDH defective mutants of C_3 plants is so subtle that its physiological contribution to photosynthetic electron transport is still remained controversial. Recent proteomics analyses have revealed that accumulation of NDH increases in C_4 plants. During, evolution, C_4 plants have developed a mechanism to concentrate CO_2 and achieved high photosynthetic efficiency to prevent photorespiration. However, to drive CO_2 concentration pump, C_4 plants need more ATP to compare to C_3 plants. This means that C_4 photosynthesis is "high cost – high return" photosynthesis and it is believed that the function of NDH to supply ATP has become more important during C_4 evolution to meet the high energy requirement of C_4 photosynthesis. To clarify the role of NDH in C_4 photosynthesis, we have generated NDH-suppressed transformants of a C_4 plant: *Flaveria bidentis* (Asteracea) by RNAi technique.

Preliminary results suggest that when NDH-mediated CEF I is impaired, C_4 plants cannot operate efficient photosynthesis under light limited condition because of ATP shortage. C_4 photosynthesis to concentrate CO_2 at the expense of ATP is beneficial under light excess condition. However, once light available for photosynthesis is insufficient, the resulting energy shortage suppresses plant growth. NDH plays a crucial role to cover this disadvantage and adapt to low light in C_4 plants.

Lecture S3.5

Protein Structure and dynamics in photosystem II investigated by neutron scattering

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Light harvesting and excitation energy transfer in photosystem II are relatively well understood at cryogenic temperatures up to ~100K (see e.g. [1] and references therein), while at physiological temperatures protein dynamics and also structure may generally differ from the crystalline or low temperature regime.

In this regard, neutron scattering techniques can be used for investigations of structural and dynamical properties of photosynthetic pigment-protein complexes, because neutron wavelengths fall in the range of interatomic distances and neutron energies are in the order of low-energy dynamical excitations [2].

We have used small-angle neutron scattering (SANS) to study the structure of light-harvesting complex II (LHC II) and photosystem II in buffer solution at room temperature. Neutron spectroscopy was employed to characterize vibrational and conformational dynamics of LHC II [3, 4]. The INS spectrum of LHC II reveals a distinct vibrational band peaking at ~2.5 meV at 80K that shifts towards lower energies if the temperature is increased to 285K. This effect is interpreted in terms of a "softening" of the protein matrix along with the dynamical transition at ~240K. Our findings indicate that SANS and INS are valuable methods to characterize protein structure and dynamics at physiological temperatures.

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Lecture S7.4

Structural and functional parallels between the biological water oxidation site and a synthetic manganese-oxide catalyst

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For the sustainable production of non-fossil fuels, water oxidation is pivotal. Many efforts have been spent in the last years to develop an artificial catalyst that mimics the biological water oxidation catalyst, Mn_4CaO_5 cluster embedded in Photosystem II [1], structurally and functionally [2–5]. An attractive strategy to emulate the natural catalytic system is to combine an electrocatalytically active inorganic material with a photovoltaic semiconductors.

Recently we developed an efficient protocol to electrodeposit a Mn-based oxide catalyst under benign conditions [6]. Here we apply quasi in-situ X-ray absorption spectroscopy combined with electrochemical characterization to study the structure of the catalyst during the catalytic cycle [7]. We demonstrate that the electrochemically-induced Mn oxidation state and structural changes in the catalytically active material closely resemble those occurring in the natural photosynthetic. Based on a comparison to both a similarly deposited but inactive oxide material and the biological catalyst, we identify key structural features that determine the catalytic activity of synthetic manganese oxides.

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Lecture S8.4

Long and short term acclimatization and organization of photosynthetic apparatus under salt grown *Chlamydomonas reinhardtii*

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Salt is a major threatening factor for growth of plants/algae, here, our study was mainly focused on the organization of photosynthetic apparatuses to the long and short term responses of NaCl in *Chlamydomonas reinhardtii*. We have ascertained cell wall as the reason for palmelloid formation in *C. reinhardtii* cells when grown under salt and it was determined using a cell wall mutant CC-503. Increase in Fo and drop in the Fv/Fm ratio, derived from Chlorophyll fluorescence transients reflect the redox state i.e., reduction of PQ pool. For the first time, we are showing the STN7 kinase dependent light harvesting complex (LHC) II phosphorylation in both short and long term treatments with various NaCl concentrations and furthermore, confirmed the STN7 kinase mediated LHCII phosphorylation using *stt7* mutant, which is defective in state transitions. Thus, we report here the short and long term NaCl induced state transitions, similar to the light induced state transitions. Surprisingly, as a part of acclimation responses in the long term treatments, increase in trimeric LHCII complexes and LHCII phosphorylation reflects the necessity of continuous PQ pool reduction. Further, blue native PAGE, protein profile analysis had shown that the increased LHCII trimers along with the damaged photosystem (PS) II reaction centers quench the excess unused energy and protect PSII from salt. Protein profile analyses indicate that PSII core proteins were more prone to damage by salt stress, however, most of the PSI core and LHCI proteins were increased since they may facilitate the protection during salt stress. Additionally, glycine betaine has retarded the salt effect since it has the highest cellular osmoprotective efficiency. Inference drawn from our results is that STN7 plays a key role in implementing the state transitions as short term and photosystem stoichiometry adjustment as long term responses by sensing the redox state of PQ pool.

Lecture S8.5

Why anthocyanic leaves show lower risk of photoinhibition? Let the stomata speak

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Foliar anthocyanins are implicated in photoprotection of the photosynthetic apparatus. However, despite that they absorb part of the photosynthetically active radiation, the exact mechanism of their action remains elusive because the absorption spectrum of the anthocyanic optical filter does not match the action spectrum of photoinhibition. In the current study, anthocyanic leaves of *Berberis thunbergii* and *Ocimum basilicum* showed higher rates of gas exchange compared to their green (acyanic) counterparts and at the same time they showed higher values of effective quantum yield of PSII photochemistry. The occurrence of anthocyanins positively affected gas exchange rates and other photosynthetic parameters, even under drought stress, and this effect was apparently due to the increase of stomatal opening. These results indicate that the supply of CO_2 to the chloroplastic stroma results in higher photosynthetic rates, probably lessening the level of overexcitation of the electron transport chain and therefore the risk of photoinhibition. Our results can be added to similar published results which, however, have never been discussed under this perspective. The mechanism by which the anthocyanic filter regulates stomatal opening is unknown. One hypothesis is that the selective absorption of the green waveband from the anthocyanic molecules results in less extensive reversal of the blue light-induced component of the stomatal opening signal cascade.

Lecture S10.1

Sustainability of photosynthesis research

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The International Meeting "Photosynthesis Research for Sustainability – 2015" has been organised in honour of Dr. George C. Papageorgiou. George is a well known and a greatly respected scientist. What is most probably less known is that he has been also an outstanding, passionate teacher, who has guided his students to attain the essence of knowledge, the deep understanding of the fundamentals. Without considering anything as obvious, he was explaining with patience both theoretical and experimental aspects, working with his students side by side at the bench and with the fluorimeter, teaching them how to write publications, motivating, inspiring and fascinating them, triggering thus the best in them. I had the privilege to experience this at first hand as his post-graduate student in the early 1970s, to appreciate it and to benefit from it, and I am happy for the opportunity to acknowledge it publicly. By honouring George's virtues, I also outline how, in my opinion, Photosynthesis Education should be, ascribing to it a meaning much broader than that of formal lecturing. I refer to the continuous process of dissemination of knowledge that is advanced by research – an educating process in itself – and which, in turn, leads to research progress and further knowledge advancement. On the basis of this strongly dialectical relation between education and research, I arrived at the title of my talk by inverting that of the meeting to "Sustainability of Photosynthesis Research", with arguments that apply to any scientific research. However, this sustainability requires more than a meaningful and inspiring education. I will bring to the discussion other issues that, I am afraid, jeopardise sustainability of research, undermining also the prospects for substantial education. These issues are created by the currently predominant bureaucratic system, which has turned to "measuring" and ranking scientific "production", to suppressing heterodox ideas and their bearers, and to establishing accordingly designed funding policies. Direct consequence is the struggle for impact factors and other "metrics" and for grant hunting – endlessly writing proposals and filling application forms. This system impedes research rather than promotes it, creates a counter-productive antagonism among researchers and labs, and drives the potentially creative researcher away from originality and discovery, and away from the unique satisfaction that these bring.

Lecture S7.5

Nanostructured manganese oxide on silica aerogel toward water oxidation

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Among different compounds [1], Mn oxides are very interesting [2] because they are not only cheap and environmentally friendly but also a Mn oxido cluster is efficiently used by Nature for water oxidation [3]. Nano-sized Mn oxide/silica aerogel is a good catalyst toward water oxidation. The composites were synthesized by a simple, low-cost procedure with different ratio of Silica aerogel and Mn oxide, and characterized by different methods. Then, the water-oxidizing activities of these composites was considered in the presence of cerium(IV) ammonium nitrate and photo-produced $Ru(bpy)_3^{3+}$. The composites with high ratio of Mn oxide to silica aerogel are good Mn-based catalysts with turnover frequencies of ~0.4 (mmol O_2/mol Mn·s). In addition to the water-oxidizing activities of these composites under different conditions, their self-healing reaction in the presence of cerium(IV) ammonium nitrate was also studied.

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Lecture S8.6

Regulation of photosynthesis by cyclic and pseudocyclic electron flow

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In linear electron transport from water to $NADP^+$, protons are translocated across the thylakoid membrane. Resulting ΔpH is utilized in ATP synthesis. In the process, the ratio of proton translocation per electron movement is fixed, which does not satisfy the ATP/NADPH ratio required by the Calvin-Benson cycle. In angiosperms, PSI cyclic electron transport, which generates ΔpH without accumulation of NADPH, is considered to contribute to the adjustment of ATP/NADPH production ratio. It is also essential for the regulation of electron transport via lumenal acidification; introduction of a qE component of NPQ and the down-regulation of the cytochrome *b6f* complex. Flavodiiron proteins (Flv) mediate O_2 reduction directly to water, catalyzing pseudocyclic electron flow in cyanobacteria. Genes encoding Flv are conserved in *Chlamydomonas reinhardtii* and *Physcomitrella patens* but not in angiosperms. We cloned two genes encoding FlvA and FlvB from *Physcomitrella patens* and introduced the genes into the *Arabidopsis* wild type and *pgr5* mutant defective in PSI cyclic electron transport. Flv partly complemented the function of PGR5 in *Arabidopsis*. I will discuss on the physiological difference between cyclic and pseudocyclic electron flow.

Lecture S8.7

Redox state of the plastoquinone pool depends both on wavelength distribution and intensity of incident light

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The plastoquinone (PQ) pool mediates electron transfer from photosystem II (PSII) to photosystem I (PSI), and the redox state of the PQ pool is a key regulator of photoacclimation responses in plants. Our measurements show that the PQ pool functions in the light as a bistable switch that becomes easily fully reduced or fully oxidized. To study the PQ pool, we developed a chlorophyll fluorescence method to find wavelengths favoring PSII or PSI and then used this information to measure the effects of key wavelengths on the redox state of PQ in intact leaves with HPLC. Short illumination with 470, 560 or 660 nm light fully reduced the PQ pool whereas 440, 520, 630 or 690 nm light oxidized it. These wavelengths also respectively triggered phosphorylation and dephosphorylation of light-harvesting complex II, a typical PQ-mediated acclimation response. Different white light sources oxidized or reduced PQ, indicating that the redox state of PQ does not only change in different monochromatic lights, but also reacts to the wavelength distribution of polychromatic incident light. In the dark, the PQ pool appeared to relax to an intermediate state between fully reduced and fully oxidized states, and low intensities of natural sunlight appeared to oxidize the PQ pool when compared to this dark state. Short illumination with high light causes reduction and fluctuating light caused respective fluctuations in the redox state of the PQ pool. We also tested the effect of absorption by chlorophylls by filtering simulated sunlight through a thin leaf of an aquatic plant. This caused oxidation of the PQ pool, indicating that towards the downside of the leaf, the PQ pool tends to remain more oxidized than on the surface. The data show that wavelength-dependent and light-intensity-effects on the redox state of the PQ pool are deeply intermixed.

Lecture S8.8

Modeling the slow SMT phase of chlorophyll *a* fluorescence induction in green alga *Chlamydomonas reinhardtii*

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In higher plants and algae, Chlorophyll (Chl) *a* fluorescence transient has a fast (under a second) increasing OJIP phase, and a slow (few minutes) PS(M)T decreasing phase, where, O is for origin, the minimum fluorescence, J and I for intermediate levels, P for peak, S for a semi-steady state, M for a maximum (which is sometimes missing), and T for the terminal steady state level. We have used a photosynthesis model of Ebenhöh et al. (2014) (Phil Trans R Soc B 369; see http://dx.doi.org/10.1098/rstb.2013.0223) to simulate the slow PS(M)T phase, in order to determine the origin of the S-M rise in *Chlamydomonas* (*C.*) *reinhardtii* cells. Our simulation results show that the maximum M situated around 100 s (as observed, e.g., by Kodru et al. (2015) Photosynth Res 125:219–231) can be reproduced only if the samples are initially in a so-called "state 2" (s2), when the absorption cross section (CS) of Photosystem II (PSII) is significantly lower than that of PSI, and Chl *a* fluorescence is low. In this case, an illumination *in silico* induces a gradual s2 to state 1 (s1) transition (qT_{21}), and a slow S-M fluorescence rise in the simulated Chl *a* fluorescence curve, since the fluorescence yield is known to be higher in s1, when CS of PSII is larger than that of PSI. State transitions are suggested to result from structural modifications in thylakoid membranes induced by changes in the redox state of the plastoquinone pool (see e.g., a review by Papageorgiou and Govindjee (2011) (J Photochem Photobiol B: Biol 104:258–270). Additionally, we will discuss how light intensity and several photosynthetic processes influence the degree of this qT_{21}.

With this presentation, we honor George C. Papageorgiou, an authority on slow Chl *a* fluorescence changes in algae and cyanobacteria.

Lecture S8.9

Development of chimeric sensor to analyze function of histidine kinases in the cyanobacterium *Synechocystis* sp. PCC 6803

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We developed a technique to analyze function of histidine kinases by expression of chimeric proteins consisting of a signal-input domain of the uncharacterized histidine kinase and a kinase-domain of a phosphate-deficient sensor, SphS, from *Synechocystis*. We analyzed function in Hik2 in *Synechocystis* by the method and found that Hik2 responded to the concentration of Cl^-. We also applied this method on the study of ethylene-sensors from a land plant, *Arabidopsis*. *Arabidopsis* has five ethylene sensors, ETR1, ERS1, ETR2, ERS2 and EIN4. We expressed chimeric sensors including signal-input domain from the ethylene-sensors and kinase domain of SphS in *Synechocystis* cells. The chimeric sensors of ETR1, ETR2 and EIN4 were constitutively active and others were constitutively inactive. In order to identify the reasons of differences in the phenotype we constructed domain swapping mutants between the signal input-domain of the active ETR1 and that of the inactive ERS1, because these primary sequences were similar to each other. The results indicated that when linker region between the transmembrane and the GAF domain from ETR1 was coexisted with either these domain from ETR1, the chimeric sensors became active. These results will give us a cue to consider the ways of activation of histidine kinases *in vivo*.

We also utilized the sensing system to regulate autolysis of the cells. When the cells were exposed to phosphate (Pi)-deficiency, half of the cells were lysed in 3 d and the cellular proteins were released in the media. The remaining living cells could resume to proliferate by supply of Pi in the media. This system might develop a novel method to recover the useful compounds produced via photosynthesis and accumulated in the cells.

Lecture S11.1

Localization of auxiliary proteins on photosystem II by surface plasmon resonance spectroscopy and chemical cross-linking in combination with mass-spectrometry

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Photosystem II (PSII), a large multi subunit membrane protein complex localized in the thylakoid membrane of cyanobacteria and chloroplasts, is the only known enzyme that catalyzes the light-driven oxidation of water. In addition to the membrane intrinsic part of PSII, efficient oxygen evolution requires soluble protein subunits at its luminal interface. In contrast to the detailed crystal structure of the active cyanobacterial complex the characterization of intermediate PSII species related to its assembly and repair is hampered by their instability or low abundance. As most structural variations of the corresponding PSII species are based on a different set of protein factors bound to the luminal interface of the complex, we developed a method for interaction analysis between PSII and its soluble interaction partners based on surface plasmon resonance (SPR) spectroscopy and chemical cross-linking in combination with mass-spectrometry (CX-MS). The well-known binding positions of the extrinsic PSII proteins PsbO, PsbV and PsbU were used for validation of this combined approach and the binding positions of several auxiliary proteins (e.g. CyanoP [1], Psb27, Psb32) that are not present in any of the recent PSII crystal structures, were mapped successfully on the luminal PSII surface.

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Lecture S8.10

Physiological significance of photosystem I photoinhibition in wheat leaves

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Photosystem I (PSI) photoinhibition represent mostly irreversible damage with a slow recovery; however, its physiological significance has not been sufficiently characterized. The aim of the study was to assess the effect of PSI photoinhibition on photosynthesis *in vivo*. The inactivation of PSI was done by a series of short light saturation pulses applied by fluorimeter in darkness (every 10 s for 15 minutes), which led to decrease of both PSI (~60%) and PSII (~12%) photochemical activity. No PSI recovery was observed within 2 days, whereas the PSII was fully recovered. Strongly limited PSI electron transport led to an imbalance between PSII and PSI photochemistry, with a high excitation pressure on PSII acceptor side and low oxidation of the PSI donor side. Low and delayed light-induced NPQ and $P700^+$ rise in inactivated samples indicated a decrease in formation of transthylakoid proton gradient (ΔpH), which was confirmed also by analysis of electrochromic bandshift (ECSt) records. In parallel with photochemical parameters, the CO_2 assimilation was also strongly inhibited, more in low light (~70%) than in high light (~45%); the decrease was not caused by stomatal closure. PSI electron transport limited the CO_2 assimilation at low to moderate light intensities, but it seems not to be directly responsible for a low CO_2 assimilation at high light. In this regard, the effects of PSI photoinhibition on the redox signalling in chloroplast and its role in downregulation of Calvin cycle activity are possible.

Lecture S8.11

The signaling state of orange carotenoid protein

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Orange Carotenoid Protein (OCP) is the photoactive protein responsible for high light tolerance in cyanobacteria. We studied the kinetics of the OCP photocycle by monitoring the changes in its absorption spectrum, intrinsic fluorescence and fluorescence of the Nile red dye, bound to OCP. It was demonstrated that all of these three methods provide the same kinetic parameters of the photocycle, namely, the kinetics of OCP relaxation in darkness occurred to be biexponential with the ratio of two components equal to 2:1 independent from temperature. While the changes of the absorption spectrum of OCP characterize the geometry and environment of its chromophore, intrinsic fluorescence of OCP reveals the changes in its tertiary structure, and fluorescence properties of Nile red indicate the exposure of hydrophobic surface areas of OCP to the solvent following the photocycle. From the results of molecular dynamics studies, the presence of two metastable conformations of 3'-hydroxyechinenone was concluded, which is consistent with characteristic changes in the Raman spectra. We assume that rotation of the β-ionylidene ring in the C-domain of OCP could be one of the first conformational rearrangements during photoactivation. The obtained results suggest that the photoactivated form of OCP represents a molten globule-like state, which is characterized by increased mobility of tertiary structure elements and solvent accessibility.

Lecture S8.12

Flavodiiron 2 and 4 proteins mediate an O_2-dependent alternative electron flow in *Synechocystis* sp. PCC 6803 under CO_2-limited conditions

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This study aims to elucidate the molecular mechanism of an O_2-dependent alternative electron flow (AEF) functioning under suppressed (CO_2-limited) photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 (S. 6803) but not in *Synechococcus elongatus* PCC 7942 (S. 7942). Photosynthetic linear electron flow, evaluated as the quantum yield of photosystem II [Y(II)], reaches a maximum shortly after the onset of actinic illumination. Thereafter, Y(II) transiently decreases concomitantly with a decrease in the photosynthetic O_2 evolution rate, and then, in S. 6803, recovers to a rate that is close to the initial maximum. These results show that CO_2 limitation suppresses photosynthesis and induces AEF in S. 6803. The mutants deficient in the genes encoding flavodiiron proteins (FLV) 2 and 4 show no recovery of Y(II) after prolonged illumination. In contrast to S. 6803, S. 7942 has no gene encoding FLV2 and 4 proteins, which may explain why AEF is not induced in S. 7942. As GST-FLV4-fusion protein overexpressed in *Escherichia coli* exhibits NADH-dependent O_2 reduction to H_2O, we suggest that FLV2 and 4 mediate O_2-dependent AEF in S. 6803 when electron acceptors such as CO_2 are not available. On the other hand, in S. 6803, photorespiration appears not to function as an electron sink, different from land plants. We propose the evolutionary process of AEF under CO_2-limited photosynthesis in cyanobacteria, eukaryotic algae, and land plants.

Lecture S11.2

Which technique is better for studying photosynthetic apparatus? Modulated, prompt or delayed chlorophyll fluorescence?

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There are many known techniques to measure chlorophyll *a* fluorescence signals which allow to study the efficiency and structure of photosynthetic apparatus in plants and other photosynthesizing living organisms. For long years, scientists have been able to observe the changes in prompt chlorophyll fluorescence, and delayed chlorophyll fluorescence that can be measured under continuous illumination of photosynthesizing samples, pre-adapted to the dark. However, the introduction of modulated measurements of chlorophyll fluorescence in the PAM system (pulse-amplitude-modulation) revolutionized the means fluorescence can be read. By the application of this tool chlorophyll *a* fluorescence can be measured in the presence of an additional source of actinic light of any spectral composition, e.g. sunlight. During the last 50 years, a two clear "schools" were established and the users were divided into 2 groups, unwittingly. A part of the scientists are a proponents of modulated measurements and the other part advocate prompt or/and delayed fluorescence. However, we believe that, each method brings another type of information about the efficiency and structure of photosynthetic apparatus. They are complementary and they should be applied simultaneously in photosynthesis researches. The pros and cons of above mentioned three types of measurements are also discussed.

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Lecture S3.6

Theoretical study of the EPR signal of the $S_3TyrZ^{\bullet}$ metalloradical intermediate state

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The intermediates of the S oxidation states of the Oxygen Evolving Complex (OEC) of Photosystem II (PSII) contain the free radical $TyrZ^{\bullet}$ magnetically interacting with the Mn-cluster (Mn_4). The EPR signal of the $S_3TyrZ^{\bullet}$ metalloradical intermediate has been recently detected in MeOH-containing PSII preparations [1]. Previous studies of these samples support an S=3 ground state for the S_3 state [2]. On the other hand, the synthesis and the study of the asymmetric [Mn_3CaO_4] cubane core of the OEC suggests that the S=3 ground state of the S_3 state arises from the antiferromagnetic exchange coupling between the S=9/2 of the Mn_3CaO_4 and the S=3/2 of an external Mn(IV) of the OEC [3]. By application of the above assumption, and considering dipolar interaction between $TyrZ^{\bullet}$ and Mn_4, we performed a theoretical study of the EPR spectrum of the $S_3TyrZ^{\bullet}$ metalloradical state. The results of the analysis are consistent with the structural model of the OEC, which has been reported at a resolution of 1.9 Å [4].

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Lecture S4.1

Polyamines in chemiosmosis: a cunning mechanism for the regulation of photosynthetic ATP synthesis during growth and stress

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Polyamines are low molecular weight amines that occur in every living organism. The three main PAs (putrescine, spermidine and spermine) are involved in several important biochemical processes. As rule of thumb, increase of the cellular titer of polyamines in plants is related to cell growth and cell tolerance to abiotic and biotic stress. In the present contribution, we describe recent findings from plant bioenergetics that bring to light a previously unrecognized dynamic behavior of the polyamine pool. Traditionally, polyamines are described by many authors as organic polycations, when in fact they are bases that can be found in a charged or uncharged form. Although uncharged forms represent less than 0.1% of the total pool, we propose that their physiological role could be crucial in chemiosmosis. This process describes the formation of a polyamine gradient across membranes within seconds and is difficult to be tested *in vivo* in plants due to the relatively small molecular weight of polyamines and the speed of the process. We tested the hypothesis that polyamines act as permeable buffers in intact leaves by using recent advances *in vivo* probing. We found that an increase of polyamines increases the electric component ($\Delta\psi$) and decreases the ΔpH component of the proton motive force (*pmf*). These findings reveal an important modulation of the energy production process and photoprotection of the chloroplast by polyamines. We explain in detail the theory behind polyamine pumping and ion trapping in acidic compartments (such as the lumen in chloroplasts) and how this regulatory process could improve either the photochemical efficiency of the photosynthetic apparatus and increase the synthesis of ATP or fine tune antenna regulation and make the plant more tolerant to stress.

POSTERS

Section 1: Primary Processes of Photosynthesis

Poster S1.5

Detection of both monovinyl chlorophyll *b* and divinyl chlorophyll *b* in a picoplankton *Prochlorococcus sp.* NIES-2086

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In 1983, an unfamiliar chlorophyll which had the absorption maxima 436 and 661 nm in diethyl ether was isolated from the fractions with the particles of less-than-one μm in surface waters of the tropical Atlantic Ocean [1, 2], and its absorption spectrum was identical to that of divinyl Chl *a* (DV-Chl *a*). In 1988, a free-living marine prochlorophyte, *Prochlorococcus marinus*, was discovered in seawater samples, and the cells contained DV-Chl *a* characterized by a red-shift of the Soret peak by 8–10 nm compared to normal Chl *a* (MV-Chl *a*) [3]. To date, there is only scarce information on DV-Chls in *Prochlorococcus*, most probably due to the difficulties in (1) mass cultivation of *Prochlorococcus* and (2) the separation of DV-Chls from MV-Chls by almost all available HPLC techniques. In 2012, we have achieved, for the first time, the separation of DV-Chls from MV-Chls on a reversed-phase HPLC column with isocratic mode, and found that *Prochlorococcus* sp. NIES-3376 possesses DV-Chl *a*' and DV-Phe *a* [4, 5], instead of MV-Chl *a*' and MV-Phe *a* [6], as minor key components in the RCs, as well as the major DV-Chls *a* and *b*. In this paper, we performed the precise pigment analysis of *Prochlorococcus* sp. NIES-2086, by means of the combination of reversed-phase and normal-phase HPLC with isocratic eluent mode. We found for the first time that this picoplankton possesses MV-Chl *b* as well as DV-Chl *b*. Note that this picoplankton does not have any MV-type Chls, namely, MV-Chl *a*, *a*' and MV-Phe *a* except MV-Chl *b*.

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Poster S1.6

The LIR1 protein regulates membrane tethering of ferredoxin-$NADP^+$ oxidoreductase (FNR)

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The *LIR1* (*Light Induced Rice 1*) gene encodes a small chloroplast-targeted protein of 13 kDa, which is distributed between the thylakoid membrane, soluble stroma and the inner envelope membrane. The LIR1 protein contains two almost identical repeated motifs of unknown function, both possessing two highly conserved cysteine residues. Co-immunoprecipitation, pull-down assays and yeast two-hybrid analysis show that LIR1 interacts with the two chloroplast ferredoxin-$NADP^+$ oxidoreductase (FNR) isoforms, which function in the last step of photosynthetic linear electron transfer by catalyzing reduction of $NADP^+$ to NADPH. LIR1 and FNR form high molecular weight thylakoid protein complexes together with the Tic62 and TROL proteins, which have previously been shown to anchor FNR to the membrane. The rapid light-triggered degradation of the LIR1 coincides with the release of the FNR from the thylakoid membrane and provides a mechanism to control membrane attachment of FNR. Loss of *LIR1* resulted in a marked decrease in the accumulation of FNR-containing thylakoid protein complexes without concomitant decrease in total FNR content. In rice (*Oryza sativa*), photosynthetic capacity and growth of the *Oslir1* plants were slightly impaired, whereas no such effect was observed in *Arabidopsis* knock-out mutants. The consequences of LIR1 deficiency in different species are discussed.

Poster S1.7

The F_0 level of chlorophyll *a* fluorescence induction: Does it reflect a standard and reproducible physiological state?

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F_0, or level O fluorescence, is defined as the first recorded chlorophyll *a* (Chl *a*) fluorescence signal upon illumination of a dark-adapted photosynthetic specimen. At the same time, F_0 is also the absolutely minimal Chl *a* fluorescence signal a photosynthetic specimen emits. Following F_0, Chl *a* fluorescence traces a characteristic two two-wave time course known as Chl *a* fluorescence induction. As defined above, the magnitude of the F_0 must depend on the technical characteristics of the measuring fluorometer, particularly the excitation rise time, intensity, wavelength, and response time, as well as on the conditions that prevail during the dark adaptation of the photosynthetic specimen. Since, however, F_0 is also the minimal fluorescence signal dark-adapted specimens emit, irrespectively of the fluorometer and of the nature of the photosynthetic specimen, it can be reasoned that it probably reflects a similar quasi-steady physiological state in all cases. This justifies the widespread usage of F_0 as constant yield Chl *a* fluorescence, and the remainder of the fluorescence induction trace as representing the variable yield fluorescence (Fv). In this paper, we question this assumption and we explore the dependence of F_0 on factors that impact on the photosynthetic specimen both during the dark incubation period as well during the light period that intervenes from the onset of excitation to the registration of the F_0 signal. In particular, we examine the effects on F_0 of the oxidation state of the plastoquinone pool during dark adaptation and the effects of singlet oxygen formation during the light preregistration period.

Poster S1.8

Slow phase signal enhancement method using convolution for chlorophyll fluorescence

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A new signal enhancement method using a convolution function is proposed for evaluating chlorophyll fluorescence characteristics. The chlorophyll fluorescence induction curve given after the commencement of light excitation exhibits the photosynthetic status of the plant physiology. However, most reported studies have analyzed the induction curve directly using no signal processing. As described herein, a method is proposed to enhance the slow phase signal of the induction phenomenon using convolution. We use signal enhancement method with convolution operation. The convolution $C(t)$ is defined as

$$C(t)=\int_0^t f'(t-\tau)f(\tau)d\tau,$$

where $f(t)$ stands for the chlorophyll fluorescence induction curves and function $f'(t)$ is the differential of the $f(t)$ with respect to time t. When eq. (1) is calculated and approximated using the Laplace transform method, the convolution $C(t)$ can be expressed simply as

$$C(t)\approx A_1 e^{-\frac{t-L_1}{\tau_1}}+A_2(t-L_2)e^{-\frac{t-L_2}{\tau_2}},$$

where A_1 and A_2 are gains.

When cultivating the petunia in three plant chambers with different light environments, the chlorophyll fluorescence induction curves of petunia were changed. The second term of eq. (2) exhibits larger values for the conditions of red light and red and blue light than in the blue light condition. These tendencies are emphasized using the convolution method, which can emphasize the induction curve of the chlorophyll fluorescence and which can readily determine the induction curve parameters.

Poster S1.9

Carotenoid composition determines the structural and functional properties of the phycobilisomes and photosystems

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In photosynthetic organisms the carotenoid pigments participate in the regulation of membrane dynamics and in the protection against (oxidative) stress; they also have structural role in some protein complexes. Due to their hydrophobic character, carotenoids are preferentially present inside of the protein complexes or in the membrane not bound to any proteins. Although wealth of information is available on the role of carotenoids in thylakoid membrane of plants, less is known about their impact in cyanobacteria. We have studied the specific effects of different carotenoid forms on functional organisation of thylakoid membrane under non-stress conditions, which have been hitherto not completely unfolded in cyanobacteria. We obtained modified carotenoid composition by using Synechocystis sp. PCC6803 mutants impaired at various steps of the carotenoid biosynthesis. In cyanobacteria the photosystem I (PSI) complexes mostly present as trimers, while functional PSII is in dimeric form. Although, it is generally believed that xanthophylls are not part of the photosystems, we found that they stabilise and influence the structure of PSI trimers and PSII dimers. The changes induced by the xanthophyll-deficiency can also influence the PSI functions and result in the decrease of the capability of cells to adapt to modified light conditions. Furthermore, in completely carotenoid-deficient cells the extramembraneous light-harvesting complexes, the phycobilisomes (PBSs) are also distorted. This alteration is surprising because carotenoids have never been found as part of the PBSs. In this mutant the number of phycocyanin rods connected to the PBSs core is strongly reduced with an increased amount of unattached phycocyanin units. We show that β-carotene is essential for the assembly of PBSs complex by assuring the required level of rod linker proteins. Our results obtained from carotenoid-deficient mutant also cover a new "linker" function of β-carotene in PSI complex. One might speculate that xanthophylls and carotenes are essential ingredients of the assembly and maintenance of the machinery of the photosynthetic complexes in the cyanobacterial cells.

Section 2: Structure, Function and Biogenesis of the Photosynthetic Apparatus

Poster S2.6

Narrow-band red and blue light affect chloroplast ATP-synthase structure and function in barley seedlings

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In our previous works we have shown that photophosphorylation activity is one of the photosynthetic parameters that are most strongly affected by light spectrum. One of the reasons for this may be differences in ATP-synthase structure and function. We studied the effects of narrow-band red and blue light on ATP-synthase structure and function: photophosphorylation activity in isolated chloroplasts, Ca^{2+}-ATPase activity of isolated CF_1 complexes and their subunit composition, expression of chloroplast genes encoding ATP-synthase subunits (mRNA level). We used 9-day-old barley (*Hordeum vulgare* L.) seedlings gown with 70 μmol PAR/(m^2 s) and LED light sources with narrow-band light of different spectrum: red (emission maximum 660 nm) and blue (450 nm). Plants grown with white fluorescent lamps were used as a control. Cyclic photophosphorylation activity in plants grown with red light was nearly two-fold lower, and in plants grown with blue light – 1.6-fold higher than in control plants. The same pattern, although less pronounced, was observed in Ca^{2+}-ATPase activity of isolated CF_1 complexes: in plants grown with red light it was 27% lower, and in plants grown with blue light – 34% higher than in control plants. CF_1 denaturing electrophoresis (10% SDS-PAGE) showed two distinct protein bands in the region of the nuclear-encoded γ-subunit, which showed different density in plants grown with light of different spectrum. This may indicate a structural heterogeneity of CF_1 complexes. No difference was observed in mRNA levels of plastid-encoded ATP-synthase subunits. Our data suggest that red and blue narrow-band light may regulate ATP-synthase structure in barley at posttranscriptional and posttranslational level. This might explain the observed changes in photosynthetic phosphorylation in plants grown with red or blue narrow-band light.

Poster S2.7

The soluble carbonic anhydrase in thylakoids of higher plants

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Carbonic anhydrases (CA) are zinc metalloenzymes catalyzing the reversible interconversion of CO_2 and HCO_3^-. They widely distributed in all living organisms, from prokaryotes to humans, due to the participation of all components of the catalyzed reaction in many metabolic processes in cell. Today, CAs are classified into six independent families – α, β, γ, δ, ε and ζ. However, there is no significant sequence homology between families and they appear to be examples of convergent evolution of catalytic function. The genome of higher plant *Arabidopsis thaliana* possesses 19 genes encoding CAs, but evidences to explain physiological role of at least one of them are not enough, and the locations of the most of these enzymes are still unknown.

The presence of soluble carbonic anhydrase in the thylakoid lumen (the inner space of thylakoids) was firstly shown in pea thylakoids [1]. We identified this protein as CA of β-family. In further studies we used mutant of arabidopsis with knocked out gene of β-CA1 for eliminating any contaminations with this enzyme during isolation of thylakoids. The absence of other stromal proteins was confirmed by Western blot analysis with antibodies against the large subunit of Ribulose-1,5-bisphosphate carboxylase/oxygenase, the most abundant stromal enzyme. Using thylakoids purificated from any extrathylakoid proteins, we have shown that thylakoid lumen of arabidopsis also contained protein with CA activity. Triton X-100 had no effect on activity of this lumenal CA from both pea and arabidopsis thylakoids and that implied this CA to be a soluble protein. The specific carbonic anhydrase inhibitor, ethoxyzolamide, had the same effect on both preparations of lumen and stroma, where the last one was enriched with β-CA1. These data is one more evidence that CA of lumen belongs to β-family. Native electrophoresis of a preparation obtained using affinity chromatography of thylakoids from arabidopsis revealed one protein band of 130 kDa with CA activity.

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Poster S2.8

Heterologous expression of genes for thermophilic phycocyanin in the mesophilic cyanobacteria *Synechococcus elongates* PCC 7942

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Phycocyanin (PC) is a component of the photosynthetic antenna complex, i.e. phycobilisome, attached on the photosystem (PS) II or PSI, in cyanobacteria. The PC includes phycocyanobilin as a chromophore and exhibits blue color. Although PC extracted from mesophilic cyanobacteria is used as a unique natural food coloring agent, the PC loses the blue color easily due to the thermal denaturation. If we would obtain enough amounts of thermostable PC, the utilities of the natural blue in the industry will be expanded. In this work, we isolated thermophilic cyanobacteria from a hot spring in Japan. We extracted PCs and semi-purified by DEAE chromatography. In order to investigate thermostability of the PCs, the semi-purified PCs were incubated at 60°C and then determined loss of absorption of 615 nm of light as an indication of the blue color. The PCs from the thermophilic cyanobacteria did not lose the absorption after incubation at 60°C for 90 min, but PCs isolated from the mesophilic cyanobacteria did. These results suggested that the thermophilic cyanobacteria have thermostable PCs. We prepared the genomic DNAs from them, and determined the draft genome sequences using next-generation sequencing (GS 454 FLX+). Then, we found that the genome contains the single *cpc* operon. We are attempting to express the thermostable PC in the mesophilic cyanobacterium *Synechococcus elongatus* PCC 7942. In this work, we will analyze the photosynthetic activities in the each type of genetically modified *Synechococcus* strain, which expresses the thermostable PC or which expresses both the mesophilic and the thermostable PC. At this moment, we attempt to analyze phycobilisome extracted from the wild-type and mutants of *Synechococcus* by Native-PAGE. This is the first trial to produce thermostable PC in the mesophilic cyanobacteria and may also provide a nice platform to study energy transfer between photosystems and antenna including components from heterologous origin.

Poster S2.9

Interactions of photosynthetic core complexes with light harvesting antenna proteins in centric diatom *Cyclotella meneghiniana*

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Diatoms are most common types of phytoplankton, the starting point of food chain in the oceans. Their photosynthetic apparatus is different from that of green plants and algae. Diatom fucoxanthin-chlorophyll proteins (FCP) are homologic to light harvesting complexes of higher plants but the nature of FCP interactions with photosystems is still unclear. In this work we studied the isolated photosynthetic complexes from centric diatom *Cyclotella meneghiniana* by biochemical and electron microscopy methods, to reveal association of FCP light harvesting systems to both photosystems.

Poster S2.10

The cyanobacterial PsbP orthologue assists the assembly of photosystem II

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The cyanobacterial photosystem II complex (PSII) is assembled by consecutive association of distinct sub-complexes (modules), each consisting of one of the main chlorophyll-binding proteins (D1, D2, CP43, and CP47), small PSII subunits, pigments, and auxiliary protein factors.

We have recently shown that the purified PSII reaction centre assembly sub-complex (RCII) of *Synechocystis* 6803, composed of the D1 and D2 modules but lacking both the CP47 and CP43, contains CyanoP (Sll1418). This protein is an ancestral orthologue of eukaryotic PsbP, one of the three lumenal subunits stabilising the oxygen-evolving cluster of PSII in green algae and plants. However, CyanoP is not present in the crystallographic models of the cyanobacterial PSII, and its location and role are apparently different from those of PsbP. Moreover, it is modified by lipidation, a feature absent in its eukaryotic relatives.

The association of CyanoP with the reaction centre has been confirmed by the purification of the RCII using a FLAG-tagged CyanoP expressed in wild-type strain. The accumulation of newly synthesized unassembled D1 protein observed in *Synechocystis* strains lacking CyanoP and the co-purification of CyanoP-FLAG with D2 and PsbE in the strain lacking D1 indicate the involvement of CyanoP in early steps of RCII assembly. Nevertheless, a small amount of PSII core complexes has been also co-purified with tagged CyanoP implying that the protein may accompany PSII on most of its assembly pathway.

Pull-down preparations of FLAG-tagged CyanoP from strains lacking either D1 or D2 contained also other lumenal PSII-interacting lipoproteins, Psb27, CyanoQ, and Ycf48. This finding raises an idea of a specifically located lipoprotein network assisting the newly synthesized chlorophyll-proteins to take the optimal position and conformation for their fast and correct assembly.

Poster S2.11

Post-translational modifications of ferredoxin-NADP$^+$ oxidoreductase in *Arabidopsis* chloroplast

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Chloroplast metabolism and photosynthetic machinery need to respond to sudden changes in environment. Rapid changes in protein function can be achieved through post-translational modifications (PTMs), which are known to affect the activity, interactions and localization of proteins. Recent studies have evidenced that, besides protein phosphorylation, many other types of modifications, including acetylation, methylation and glycosylation, have a crucial role in the regulation of chloroplast proteins. In *Arabidopsis thaliana*, two nuclear genes (*At5g66190* and *At1g20020*) encode two distinct chloroplast targeted isoforms of ferredoxin-NADP$^+$ oxidoreductase (FNR): AtFNR1 and AtFNR2. FNR is a key enzyme linking the light reactions of photosynthesis to stromal metabolism by transferring electrons from two ferredoxin molecules to NADP$^+$. We have investigated the regulation of AtFNR isoforms by studying PTMs, since both isoforms exist as two distinct forms with different isoelectric points (pIs) after 2D-PAGE. Our results show that both AtFNR1 and AtFNR2 isoform contain alternative N-termini, which are partially N$^\alpha$-acetylated causing the change in pI. Moreover, light appears to change the ratio of N$^\alpha$-acetylated and non-acetylated forms. A conserved lysine residue near the active site of AtFNR was found to be acetylated in both isoforms, which may affect enzyme activity. Both AtFNR isoforms contain potential phosphorylation and glycosylation sites. However, no evidence for *in vivo* phosphorylation or glycosylation was gained.

Poster S2.12

Application of the TwinStrep-tag/Streptactin system for the analysis of Photosystem II assembly intermediates from *T. elongatus*

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Photosystem II (PSII), nature's unique water-splitting catalyst, is composed of 20 protein subunits and various cofactors like metal ions, lipids, carotenoids and chlorophyll molecules. In order to yield a fully functional PSII complex the assembly and repair of PSII occurs in a step-wise manner orchestrated by numerous transiently binding protein factors. These protein factors are, however, not resolved in the detailed crystal structure of the active cyanobacterial complex [Umena, Y. et al., 2011, Nature, 473:55–60] and the characterization of intermediate PSII species is hampered by their instability and low abundance. Although, a number of auxiliary PSII factors have been identified in the past, the precise function for many of them is still elusive. One way to elucidate the role of such transiently binding PSII factors is to enrich PSII complexes using a tagged-version of the PSII factor. The conventionally used histidine-tag is thereby of limited use due to its susceptibility for unspecific binding. This is specifically true for the purification of low abundant assembly and repair intermediates. We were looking for an alternative way to purify PSII from the thermophilic cyanobacterium *Thermosynechococcus elongatus* that offers higher specificity than the histidine-tag.

Here we present the first purification of intact PSII complexes using a TwinStrep-Tag – Streptactin System. The PSII complexes obtained from a "CP43-TwinStrep-Tag" mutant are characterized by very high purity thus reducing samples complexity in their downstream analyses. This allows for future characterization of PSII intermediates under normal growth and nutrient deficient conditions.

Poster S2.13

Architecture of light harvesting apparatus of eustigmatophyte algae

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Light harvesting apparatus of oleaginous *Nannochloropsis oceanica* (Eustigmatophyta, Stramenopila) was analyzed biochemically and specific light harvesting protein (Lhc) functions were determined. The phylogenetic relationships of these Lhcs were also described. *N. oceanica* utilizes Lhcs of multiple classes: Lhcr-type proteins (related to red algae LHCI), Lhcv (VCP) proteins (violaxanthin-containing Lhcs related to Lhcf/FCP proteins of diatoms), Lhcx proteins (related to Lhcx/LhcSR of diatoms and green algae) and Lhc proteins related to Red-CLH of *Chromera velia*. Alltogether, 17 Lhc-type proteins of the 21 known from genomic data were found in the proteomic analyses. Three proteins were found closely associated with Photosystem I – two conserved Lhcr-type protein and an ELIP-type protein (early light inducible protein). The ELIP-type protein is a member of a novel Lhc protein clade specific for Stramenopile, Haptophyte and Red algae. Three Lhc proteins, of the Red-CLH lineage, were found associated with Photosystem II, whether they work as analogs of monomeric Lhc proteins of land plants remains to be seen. The free antenna fraction is formed by trimers of a mixture of Lhcs of varied origins (Lhcv, Lhcr, Lhcx and relatives of Red-CLH). Despite possessing several proteins of the Red-CLH-type Lhc clade, *N. oceanica* is not capable of chromatic adaptation. On the other hand it seems likely that *C. velia*, a relative of apicomplexan parasites, has some Lhcs of eustigmatophycean origin. A naming scheme of *Nannochloropsis* Lhcs is proposed to facilitate further work.

Poster S2.14

The antarctic psychrophile, *Chlamydomonas* sp. UWO241, preferentially phosphorylates a PSI-cytochrome b_6/f supercomplex

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Chlamydomonas sp. UWO 241 is a psychrophilic green alga isolated from Antarctica. A unique characteristic of this algal strain is its inability to undergo state transitions coupled with the absence of LHCII phosphorylation. We show that UWO 241 preferentially phosphorylates specific polypeptides associated with a ~1000 kDa pigment-protein supercomplex that contains components of both photosystem I and the cytochrome b_6/f complex. Nano-LC-ESI-MS/MSwas used to identify 3 major phosphorylated proteins associated with this PSI-Cyt b_6/f supercomplex which includes two 17 kDa PsbP-like proteins, and a 70 kDa ATP-dependent zinc metalloprotease FtsH. The PsbP-like protein sequence exhibited 70.6% similarity to the authentic PsbP protein associated with the oxygen evolving complex of photosystem II in *Chlamydomonas reinhardtii*. Tyrosine-146 was identified as a unique phosphorylation site on the UWO 241 PsbP-like polypeptide. Assessment of PSI cyclic electron transport by *in vivo* P700 photo-oxidation and the dark relaxation kinetics of $P700^+$ indicated that UWO 241 exhibited PSI cyclic electron transport rates that were 3 times faster and more sensitive to antimycin A than the mesophile control, *Chlamydomonas raudensis* SAG 49.72. The stability of the PSI-Cyt b_6/f supercomplex was dependent upon the phosphorylation status of the PsbP-like protein and the zinc metalloprotease FtsH as well as the presence of high salt. We suggest that adaptation of *Chlamydomonas* sp. UWO 241 to its unique low temperature and high salt environment favours phosphorylation of a PSI-Cyt b_6/f supercomplex to regulate PSI cyclic electron transport rather than regulation of state transitions through the phosphorylation of LHCII proteins.

Poster S2.15

Specific Lhcb4 and Lhcb5 phosphorylation sites are absent in the psychrophilic state transition variant, *Chlamydomonas* sp. UWO241

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Chlamydomonas sp. UWO241 is a unique, psychrophilic green alga, isolated from Lake Bonney, Antarctica. UWO241 represents the first known natural variant which is deficient in state transitions. Furthermore, in contrast to all other photosynthetic organisms, phosphorylation of light harvesting complex II proteins associated with state transitions, has never been detected in UWO241 using immunoblot analysis with antibodies specific for phospho-threonine. Despite these negative results, radioactive ^{33}P labelling revealed that phosphorylation of LHCII does indeed occur in UWO241. Compared to *Chlamydomonas reinhardtii*, the model green alga, phosphorylation of the major LHCII (Lhcbm1-10) protein bands was observed at lower levels in the psychrophile. Futhermore, UWO241 exhibited minimal phosphorylation of minor LHCII (Lhcb4 and Lhcb5) protein bands. It has been proposed that Lhcb4 and Lhcb5 play an important role as linker proteins during state transitions, as these minor LHCII proteins are located at the interface between the PSII core and major LHCII trimers. To examine the phosphorylation sites present in monomeric LHCII proteins of UWO241, Lhcb4 and Lhcb5 genes were cloned from a cDNA library. UWO241 Lhcb4 and Lhcb5 shared 66.3% and 72.6% amino acid identity with *C. reinhardtii*, respectively. However, examination of the Lhcb4 amino acid N-terminal domains revealed that only one of five phosphorylated threonine residues (Thr18) is present in UWO241, compared to *C. reinhardtii*, and the only phosphorylated threonine (Thr10) residue of Lhcb5 is replaced with a serine in UWO241. We suggest that these altered phosphorylation sites contribute to significantly reduced levels of phosphorylation of minor LHCII proteins in UWO241, resulting in the failure to transition to State II and an overall state transition deficiency in the Antarctic psychrophile, *Chlamydomonas* sp. UWO241.

Poster S2.16

In-vitro enzymatic assay for 13^2-demethoxycarbonylation in chlorosomal bacteriochlorophyll biosynthesis

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Some groups of photosynthetic bacteria, green sulfur bacteria, filamentous anoxygenic phototrophs, and acidobacteria, have large and efficient light harvesting antenna complexes, called chlorosomes. A large number of bacteriochlorophyll (BChl) *c*, *d*, or *e* molecules are found in the chlorosome, and self-aggregate without any assistance of proteins to form well-ordered supramolecules. The J-type self-aggregates are tightly constructed by the hydrogen bond between the $C3^1$-hydroxy and $C13^1$-keto groups and the coordination between the $C3^1$-hydroxy and central magnesium. Except for these chlorosomal pigments, all the other chlorophyllous pigments have a methoxycarbonyl group at the $C13^2$ position for specific interaction with some proteins. In contrast, the $C13^2$-demethoxycarbonylation is important for the self-aggregation of the chlorosomal BChls, because the presence of such a sterically bulky substituent would suppress the formation of their protein-free chlorosomal self-aggregates [1]. In the biosynthetic pathways of the chlorosomal BChls, an enzyme encoded by *bciC* gene, was proposed to catalyze the removal of the $C13^2$-methoxycarbonyl group from chlorophyllide *a* molecule [2]. The details of the reaction mechanism is still not clear. Here we perform the in-vitro assay to elucidate demethoxycarbonylation and substrate specificity of the BciC enzyme using various chlorophyll derivatives [3]. We will discuss the role of the BciC in the biosynthetic pathway.

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Poster S2.17

Truncated chlorophyll *b*-less antenna of *chlorina* f2 3613 barley mutant can provide light-tolerance and wild type level productivity

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Barley mutant *chlorina* f2 3613 lacking functional chlorophyllide-*a*-oxygenase (Mueller et al., 2012) is characterized by truncated chlorophyll *b*-less antenna. Its productivity can be enhanced considerably by a special cultivation practice (Tyutereva, Voitsekhovskaja, 2011). As a result, *chlorina* f2 3613 develops high tolerance to light and drought stress, and a seed yield comparable to that of the wild type. The light-sensitive phenotype of *chlorina* f2 3613 exhibits compromised stomata control and a very low level of photoprotection reflected by high singlet oxygen production, high levels of grana degradation and low NPQ. Contrarily, the light-tolerant phenotype shows the restoration of stomata control and the build-up of efficient photoprotection as judged by wild type levels of singlet oxygen production, restoration of grana, and development of zeaxanthin-independent NPQ. While the light-sensitive phenotype shows delayed flowering and early senescence, the transitions between the ontogenetic stages were partially restored in the light-tolerant phenotype. Transcript patterns were compared in both phenotypes vs. the wild type. Minor antenna proteins accumulated in the light-tolerant *chlorina* f2 3613 phenotype as compared to the light-sensitive phenotype. We speculate that this partially restored the arrangement of PSII supercomplexes in the grana thylakoids, improving linear electron transport and repair of PSII, and consequently photosynthesis and productivity. Moreover, as the minor antenna proteins play a role in ABA signaling (Xu et al., 2012), this might be responsible also for the increased drought stress tolerance of the light-tolerant phenotype of *chlorina* f2 3613.

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Section 3: Photosystem II and Water Oxidation Mechanism

Poster S3.7

Trapping $Tyr_Z^{\bullet}$ during $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions of the water oxidizing complex of Photosystem II

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Low-temperature EPR spectroscopy has been employed in the last decade to study transient intermediates between S states, identified as oxidized Tyr_Z magnetically interacting with the Mn_4CaO_5 cluster. At the lower S states, S_0 and S_1, Tyr_Z can be oxidized even at liquid helium temperatures. At S_2, due to a positive charge accumulated near the Mn_4CaO_5, Tyr_Z can be oxidized only at temperatures >77K (Ioannidis et al. 2006, Biochemistry 45, 6252–6259), at which proton rearrangements around Mn_4CaO_5 are possible. In the above cases, oxidized Tyr_Z decays at approximately 10 minutes by recombination with Q_A^-.

This study extends to higher temperatures, where S-transitions are feasible, and specifically near the half-inhibition temperatures of the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions (Styring & Rutherford 1988, BBA 933, 378–387). With a flash at ~230K and rapid freezing to cryogenic temperatures, characteristic signals attributed to $S_2Tyr_Z^{\bullet}$ and $S_3Tyr_Z^{\bullet}$ were trapped in samples poised in the S_2 and S_3 respectively. $S_2Tyr_Z^{\bullet}$ is a critical intermediate in which structural changes occur in the cluster, possibly involving binding of a water molecule. $S_3Tyr_Z^{\bullet}$ is the final step before O_2 formation and thus, the one that could give the most information about it, in future. These signals show a notable stability – even at 77K for days – which will be useful in related studies by advanced spectroscopic techniques.

Poster S3.8

PsbO protein isoforms in angiosperms: parallel subfunctionalisation revealed by phylogenetic analysis and mapping of sequence variability onto protein structure

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PsbO, the manganese-stabilising protein, is an indispensable extrinsic subunit of Photosystem II. It plays a crucial role in the stabilisation of the water-splitting Mn_4CaO_5 cluster and is also involved in the D1 protein turnover. Our analysis of psbO sequences from a wide range of plant species showed that many angiosperm species express two psbO isoforms. Phylogenetic analysis of these pairs of paralogs revealed that psbO duplication occurred many times independently, frequently in ancestors of modern angiosperm families. In spite of this, the level of isoform divergence is similar in different species. Moreover, mapping of the differences on the protein tertiary structure showed that the isoforms in individual species differ from each other on similar positions, mostly on the luminally exposed end of the β-barrel structure. This suggests that similar subfunctionalisation of PsbO isoforms occurred parallelly in various lineages. Location of the differences on PsbO structure showed potential interaction surfaces and indicated various selection pressures in PsbO evolution. We speculate that the presence of two PsbO isoforms helps the plants to finely adjust the photosynthetic apparatus in response to variable conditions. This could be mediated by diverse GTPase activity of PsbO isoforms, since the isoform differences predominate near the predicted GTP-binding site.

Poster S3.9

Temperature dependence of photoinhibition

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Photoinhibition, light induced decay in the activity of photosystem II (PSII), occurs under all light intensities, and the synthesis of a new D1 protein is needed for recovery. We measured the temperature dependence of photoinhibition from isolated pumpkin thylakoid membranes in visible (blue, red and white) and in ultraviolet light (at 254, 312 and 360 nm). In all the wavelengths, the rate constant of photoinhibition showed similar, slight increase with temperature, except that red light (>650 nm) showed somewhat stronger temperature dependence. Similar temperature dependence of photoinhibition was measured also from intact cells of *Synechocystis* sp. PCC 6803, indicating that the observed results are not a property of isolated systems. The temperature dependence of the redox state of Q_A did not explain the results. Also, the temperature dependence of singlet oxygen production by the thylakoid membranes was measured, through the reaction with singlet oxygen and histidine, and the temperature dependence resembled that of photoinhibition.

It has been suggested that visible-light-induced photoinhibition depends on singlet oxygen produced by the charge recombination reactions between the reduced electron acceptors of PSII and the S-states of the oxygen evolving complex. However, the observed temperature dependence of photoinhibition was much weaker than the temperature dependence of the $S_2Q_A^-$ recombination. This indicates that these thermally activated recombination reactions cannot have very significant role in photoinhibition, or in singlet oxygen production. The results support our suggestion that light absorption, both in ultraviolet and visible range, by the manganese ions of the oxygen evolving complex inhibits electron donation to P_{680}^+ and the inhibited reaction centers spread the damage to nearby still functioning centers by producing singlet oxygen via charge recombination reactions.

To test the effect of photoinhibition and singlet oxygen on the cold-acclimation process, four differently cold-tolerant *Arabidopsis thaliana* accessions were cold-treated and photoinhibition was measured. One peculiar thing about photoinhibition in cold (at 0–10°C) is that when measured with F_v/F_m, photoinhibition appear to proceed faster than when measured with the oxygen evolving activity of PSII. This result may suggest that chilling induces quenching of fluorescence on top of photoinhibition.

Poster S3.10

FTIR evidence for proton release into the bulk upon photooxidation of tyrosine D in photosystem II

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Photosystem II (PSII) has two symmetrically located redox-active tyrosine residues, D1-Tyr161 (Y_Z) and D2-Tyr160 (Y_D). Y_Z mediates electron transfer from the water oxidizing center to P680, whereas Y_D functions as a peripheral electron donor to P680 due to its slower redox reaction. Our recent FTIR study strongly suggested that the proton from oxidized Y_Z is trapped by the neighboring D1-His190. However, the fate of the proton from Y_D after its oxidation remains to be clarified. To understand the mechanism of the functional difference between Y_Z and Y_D, in this study, we examined whether the proton from Y_D is released out of the protein or not using Fourier transform infrared (FTIR) spectroscopy.

The proton detection method previously used for water oxidation in PSII [Suzuki et al. (2009) J. Am. Chem. Soc. 131, 7849–7857] was applied to Y_D. In this method, protons released from PSII into the bulk were trapped by high-concentration MES buffer and its protonation reaction was monitored by FTIR difference spectroscopy. It was shown that 0.84 ± 0.10 protons are released into the bulk by oxidation of Y_D for one PSII center. This result indicates that the proton from Y_D is not transferred to the neighboring D2-His198 but is released out of the protein. This proton release is attributed to the H-bonded structure of the Y_D-His couple determined by D2-Arg294 as an H-bond donor to the His Nπ, in contrast to the corresponding residue, D1-Asn298, near Y_Z functioning as an H-bond acceptor of D1-His190. It is thus concluded that the significantly slow redox reaction of Y_D in comparison with Y_Z is caused by the long distance proton transfer to the bulk, which is determined by the H-bond partner of the coupled His.

Poster S3.11

High-field EPR characterization of the redox centers of photosystem II

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High-field (W-band) electron paramagnetic resonance (EPR) is a versatile technique for the study of transition metal cofactors. It provides information on the geometric and electronic structure of the metal complex itself, its interaction with its substrate(s) and solvent environment, and the chemistry that the complex promotes [1, 2]. This can be demonstrated in nature's water splitting catalyst, a pentaoxygen tetramaganese calcium (Mn_4O_5Ca) cofactor, found in the unique pigment–protein complex Photosystem II (PSII) [1, 3]. Recently we have exploited high field EPR to obtain structural information on the last metastable intermediate of the reaction cycle (S3) [4]. Our results indicate that this "activated" intermediate structure represents an all octahedral Mn(IV) complex, similar to the structure observed by X-ray crystallography [3], but requires the coordination of an additional water molecule. We have now extended this study to examine the same state in samples where the Ca^{2+} ion is replaced with Sr^{2+}, providing new information on the role of the redox inactive metal in biological water splitting catalysis. Similarly, we have been able to characterize the acceptor side of PSII, specifically a non-heme iron (Fe^{2+}) species, located between the electron acceptors Q_A and Q_B. Our EPR study is one of a handful of examples of an isolated biological Fe^{2+} center.

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Poster S3.12

Tracking structural, energetic and kinetic properties of cyanobacterial Photosystem II variants

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The primary photochemistry of light-driven charge separation and water oxidation in all oxygenic phototrophs is catalyzed by photosystem II (PSII). In PSII, dioxygen is formed from water in a four-stepped process (S-state cycle), which is driven by four quanta of light and catalyzed by the Mn_4CaO_5 cluster. The photochemical events that precede water oxidation take place in a heterodimer of D1 (PsbA) and D2 (PsbD) polypeptides and a heterodimer of CP43 (PsbC) and CP47 (PsbB) polypeptides. The D1 protein provides most of the ligands to Mn_4CaO_5 cluster. The genomes of cyanobacteria contain multiple genes, especially for the D1 protein. From all so far annotated cyanobacterial genomes, 5 extremely divergent PsbA forms can be classified. A substitution by different D1 variants affects growth, viability and photosynthetic capacity *in vivo* and changes structure, kinetics and energetics *in vitro*. Towards a better understanding of both the role of divergent D1 proteins and the mechanism of photosynthetic water oxidation in natural PSII variants, especially of O-O bond formation, knockout mutants have been generated. The photosynthetic properties of the mutants and of the extracted PSII thylakoid complexes have been characterised and compared from a thermophilic and a mesophilic species, namely *Thermosynechococcus elongatus* BP-1 and *Synechococcus* sp. PCC 7002.

Section 5: Photosystem I and Bacterial Photosynthesis

Poster S5.8

A comparative study of *Rhodobacter sphaeroides* mutants without peripheral light harvesting antenna

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Purple non-sulfur bacteria (PNSB) are able to grow in a variety of conditions and dispose a wide range of organic substrates. Under anaerobic light conditions at the lack of nitrogen, these microorganisms can produce hydrogen at rates close to the practical significance using organic acids from wastewater. There are some restrictions that effectively prevent using the biohydrogen industrially so far, specifically insufficient volumetric rate of hydrogen production. To increase the rate of hydrogen production by PNSB two approaches are possible: increasing the rate of hydrogen production by one cell and increasing the number of cells per volume unit. However, increasing the amount of photosynthetic cells per unit of volume at higher cell concentrations than 2–3 g/L leads to self-shading effect and switch from the nitrogen limitation to the limitation by light, which reduces the rate of hydrogen evolution. Using the mutant strains with reduced amount of pigment can increase the biomass in photobioreactor (PBR) without self-shading effect.

The aim of this study was to compare the influence of light intensity on the growth and hydrogen production of *Rba. sphaeroides* parental strain and their mutant lacking peripheral antenna.

Obtained results showed that the growth rates of turbidostat cultures of parental strain and the mutant increased with the increase of light intensity. The saturating light intensity depended on the organic substrate and was the lowest (13 W m^{-2}) with the mixture of lactate and acetate for both parental and mutant strain. The highest saturating light intensity (40 W m^{-2}) was observed for turbidostat cultures growing with succinate for both strains. The following correlation for both strains was found: higher growth rate organic substrate supported – higher saturating light was observed.

The parental strain and mutants were able to produce hydrogen under light anaerobic conditions in the light. The saturating light intensity for hydrogen production by parental strain with lactate and acetate was 10 W m^{-2}. This corresponded to the saturating light intensity for the growth. Surprisingly, mutant did not reached saturating light intensity for hydrogen production even at 2000 W m^{-2}. Parental strain and the mutant have other peculiarities that are discussed in the presentation.

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Poster S5.9

Ferredoxin-binding modulates the redox reaction rates between $NADP^+/H$ and ferredoxin-$NAD(P)^+$ reductase from the green sulfur bacterium *Chlorobaculum tepidum*

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Ferredoxin-$NAD(P)^+$ oxidoreductase (FNR, [EC 1.18.1.2], [EC 1.18.1.3]) is a flavoprotein catalyzing the reversible redox reaction between $NAD(P)^+/H$ and a soluble small iron-sulfur protein ferredoxin (Fd). FNR from the green sulfur bacterium *Chlorobaculum tepidum* (*Ct*FNR) conserves its protein topology with bacterial NADPH-thioredoxin reductases. Its homologues have been found in many heterotrophic bacteria including low GC Gram-positive bacterium *Bacillus subtilis* (*Bs*FNR). In our previous stopped-flow work, *Bs*FNR promotes NADPH oxidation in the reaction with $NADP^+/H$, although the redox midpoint potential of B. subtilis Fd is rather lower (−385 mV). In this work, we examined the reaction of *Ct*FNR with $NADP^+/H$ by a stopped-flow spectrophotometry.

The mixing $CtFNR_{ox}$ with NADPH resulted in a rapid increase of the absorption of CT band. Its intensity reached maximum within the dead time of the instrument and did not change significantly further. The absorption change of the flavin band I involved two phases. The apparent rate of the rapid phase was independent from NADPH concentration, while that of the slower phase decreased with increasing NADPH concentration. When $CtFNR_{red}$ was mixed with $NADP^+$, no CT absorption band appeared in the course of the reaction. The absorption of the flavin band I increased slowly with an apparent rate of ~4 s^{-1}. The equilibrium between *Ct*FNR and $NADP^+/H$ indicated that the redox potential of *Ct*FNR was more negative than that of $NADP^+/H$. Interestingly, an addition of *Ct*Fd drastically increased the rate of $NADP^+$ reduction by $CtFNR_{red}$ (>500 s^{-1}).

Poster S5.10

Analysis of energy transfer system in chlorophyll *f* containing cyanobacterium

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Chlorophylls (Chls) play important roles in light harvesting, energy transfer, and electron transfer during photosynthesis. Chl *f* containing cyanobacterium, which name was *Halomicronema hongdechloris*, was found in living Stromatolites by Chen and co-workers. The absorption maximum of Chl *f* in organic solvents occurs at a wavelength approximately 40 nm longer than that of Chl *a*. This Chl is most red-shifted Chl in oxygenic photosynthesis, so far. The structure of Chl *f* was determined to be [2-formyl]-Chl *a* using mass spectroscopy and NMR analysis. The Chl content of *H. hongdechloris* varied under different light conditions. When under far-red light (>700 nm), the Chl *f* content increased to ca. 10% of total Chl. When under white fluorescence light, the Chl *f* content decreased negligibly. The photochemical and photophysical functions of Chl *f* are not known in photosystem I complexes. Therefore, we isolated photosystem I complexes from cells grown under far-red light or white light, and measured steady and time-resolved fluorescence spectrum. We observed a plural energy transfer pathways from Chl *a* to Chl *f*. We will discuss the characterizations of photosystem I complexes isolated from chlorophyll *f* containing cyanobacterium.

Section 7: Artificial and Applied aspects of Photosynthesis

Poster S7.6

Heterologous production of monoterpene hydrocarbons in cyanobacteria (*Synechocystis*)

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Heterologous production of the monoterpene β-phellandrene ($C_{10}H_{16}$), a chemical with commercial value and a potential biogasoline-type fuel, was achieved in *Synechocystis* upon expression of a codon-optimized β-phellandrene synthase (PHLS) gene from *Lavandula angustifolia* (lavender). This work offers proof of concept that cyanobacteria can be exploited as photosynthesis-to-fuel platforms for heterologous generation of terpene hydrocarbons from the primary products of photosynthesis. β-Phellandrene is particularly attractive for photosynthetic production, as compared to longer chain hydrocarbons, because it spontaneously diffuses out of the cells and further escapes from the extracellular aqueous phase and accumulates as a hydrophobic floater molecule at the surface of the culture, where it can be easily siphoned-off and collected. This property facilitates product segregation and harvesting, a parameter that impacts the economics of a microbial production system, while alleviating product inhibitory or toxic effects on cellular metabolism. However, a problem in high-yield of β-phellandrene production is the slow K_{cat} (low V_{max}) of terpene synthases, which limits rates and yield of monoterpene synthesis. Overcoming this bottleneck in heterologous terpene production requires high levels of enzyme concentration to enable improved rates and yield of product formation. This approach was employed by nature with the Rubisco, where high levels of enzyme concentration compensate for the slow K_{cat} (low V_{max}) of the Rubisco carboxylation reaction. Thus, our work focused on heterologous terpene synthase over-expression in cyanobacteria. While bacterial proteins have been heterologously over-expressed in cyanobacteria, heterologous expression of proteins from higher plants, e.g. terpene synthases, has not achieved high levels of recombinant protein in cyanobacteria so far. This limitation in the expression of plant genes in cyanobacteria negatively impacts product yield, thus undermining commercial exploitation of these photosynthetic microorganisms in the generation of plant-based products. In the present work we addressed this problem and, for the first time, we show heterologous expression of a terpene synthase up to 20% of total protein in *Synechocystis*. High levels of β-phellandrene synthase enhanced rates and yields of β-phellandrene accumulation, resulting in an improved carbon partitioning ratio of 1% β-PHL:Bms (w:w). Moreover, we show that integration and expression of the PHLS via homologous recombination and deletion of the cpc operon, encoding for the peripheral rods of the phycobilisome light-harvesting antenna, can be a strategy to simultaneously improve sunlight-utilization and β-phellandrene production in *Synechocystis.*

Poster S7.7

The study of genetic diversity and determination of the heritability in promising lines of bread wheat in the Moghan – Iran

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Genetic diversity is the basis of plant breeding. Evaluation of genetic diversity in the crops is critical for breeding programs and protecting the successive reserves. This study was conducted in order to evaluate the genetic diversity and determine the heritability of 12 genotypes and promising lines of bread wheat as randomized complete block design with three replications in the Agriculture and Natural Resources Research Centre of Ardabil Province at pars Abad city in 2012–2013. Analysis of variance showed that there was significant difference between genotypes in terms of all traits except peduncle and spike length. It was also showed comparing the average data that, genotype 11 was able to produce the highest yield, while the lowest yield was related to the genotypes 4 and 5. According to the cluster analysis using Ward's method, 12 genotypes were evaluated in the three groups. The results showed the second group included high yielding genotypes. In the contrast, the first group genotypes produced fewer products. Degree of general heritability for all the studied traits was between 14.08 and 74.55 percent, so that, the general heritability for grain yield was 28.25 percent.

Poster S7.8

Probing the photosynthetic efficiency of green microalgae used for bioremediation and valorization of anaerobic digestion effluents

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Anaerobic Digestion (AD) is an established waste treatment technology, the final products of which are biogas, a renewable energy source mainly consisting of methane and carbon dioxide and digestate, a nitrogen-rich residual mixture, which can be used as fertilizer. However, excessive disposal of AD Effluent (ADE) can adversely affect natural ecosystems. The aim of this work is to investigate the effectiveness of cultivating green microalgae in ADE and evaluate their nutrient removal capacity as well as biomass and lipid production, under different dilution ratios of this waste stream. In the framework of this study, *Parachlorella kessleri* and *Acutodesmus obliquus* were batch-cultured in water diluted digestate, derived from the co-digestion of end-of-life dairy products with a given mixture of agro-industrial wastes, as well as in BG-11 (synthetic medium) which was used as control. Prior to use, ADE was centrifuged at 4500 rpm and filtered through 0.7 μm pore size in order to remove large particles. Sterilization was also applied so as to eliminate the presence of existing microorganisms. ADE was fully characterized both prior and after sterilization. For each strain, cultures of a total volume of 300 mL were inoculated at 10% v/v and incubated at 25±2°C, using a filter-sterilized air flow rate of 0.5–1.0 L min^{-1} and continuous illumination of about 200 μmol m^{-2} s^{-1}, for 12 days. Throughout each experiment, the concentrations of ammonium nitrogen, dissolved phosphorus and COD were monitored; biomass production was evaluated as g DW L^{-1}, while microalgal lipid content was determined at the end of each experiment, at a dry weight (DW) basis. Concerning photosynthetic efficiency, several bio-physical parameters derived from the JIP-test were used to assess the stepwise energy flow through PSII and the vitality of the photosynthetic apparatus of these microalgae grown on ADE.

Poster S7.9

Evaluation of antibacterial activity of a novel anionic hyperbranched dendritic polymer and its effect on photosynthesis

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In the last decades, the research on antibacterial polymers and mainly on functionalized dendritic polymers has gained significant interest. In this study, an amine-rich polymer, hyperbranched polyethylenimine (PEI) having molecular weight 25 KDa, was functionalized by a simple sulfopropylation reaction, affording an *N*-sulfopropylated PEI derivative (PEI-SO_3). This anionic hyperbranched derivative was spectroscopically characterized by FTIR, ^{1}H NMR and ^{13}C NMR in order to determine the degree of substitution of primary and secondary amines of the parent PEI. It was found that the average number of attached sulfopropyl groups per polymer was 80. Subsequently, the antibacterial activity of PEI-SO_3 was investigated against two types of bacteria, an autotrophic (cyanobacterium *Synechococcus* sp. PCC 7942) and an heterotrophic (*Escherichia coli*). Various concentrations of PEI-SO_3 were employed in order to determine the EC_{50} values, which were found to be 14.6 μg/mL and 40.0 μg/mL for cyanobacterium *Synechococcus* sp. PCC 7942 and *Escherichia coli*, respectively. The difference in the EC_{50} values can be attributed to the effect of PEI-SO_3 on photosynthesis. It was found that the activity of photosystem II was inhibited by ~64% at 200 μg/mL of PEI-SO_3, while the activity of photosystem I was unaffected. In conclusion, PEI-SO_3 is a very promising candidate to be used as antibacterial agent as well as potential inhibitor of the photosystem II.

Poster S7.10

Potassium deficiency, a "smart" cellular switch for sustained high yield photosynthetic hydrogen production by green algae

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Hydrogen is considered to be the future optimal energy carrier, and is expected to contribute to the growth of the world's economy by facilitating a stable supply of energy. The ability of green algae to produce hydrogen was discovered 75 years ago. Since then, several attempts were made, to increase hydrogen production yields, sulfur starvation being the best known. The main concern during these attempts was that the achievable increase in yield was not sustainable. In this contribution, potassium deficiency is presented as a biochemical/bioenergetic switch for a sustained high yield of hydrogen production via the photosynthetic apparatus. Potassium can partially be replaced by sodium in the majority of biochemical processes and as a result the system remains functional. However, sodium cannot replace potassium in the conversion procedure of glucose to starch. This fact significantly increased the yield of hydrogen production through the Photosystem II independent pathway, since electrons originating from the metabolism of glucose used for continuously donating electrons to the plastoquinone-pool of the photosynthetic electron chain. Additionally, PSII inactivation (and therefore the inhibition of O_2-production), the further synthesis and over activation of Photosystem I and plastidic hydrogenase, generated a sustained increase in hydrogen production, mainly through the PSII-independent pathway. The self regulation of these multistage processes in hermitically closed static systems of *Scenedesmus obliquus* cultivation, permitted the establishment of anoxic conditions and the continuous electron supply to highly activated hydrogenase, resulting in the sustained high yield hydrogen production and paving the way for future usage in an industrial scale application.

Poster S7.11

Sucrose production: *Synechococcus* sp. PCC 7942, an ideal candidate

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Advancements in application of the rich knowledge on the mechanisms of photosynthesis can be used to tackle a serious problem, which is the energy sources limitation. Photosynthetic cyanobacteria (oxygenic photoautotrophic prokaryotes) have unique advantages that make them excellent candidates for producing, under specific conditions, high energy chemicals. They accumulate compatible organic osmolytes, such as sucrose, which is a high energy compound (unique feature of cyanobacteria), as a defensive response. Sucrose enables them to survive and proliferate under salinity stress conditions. The objective of this study is to examine possible ways of increasing the sucrose accumulation in the cyanobacterium *Synechococcus* sp. PCC 7942 in extreme environments (abiotic stress). In the present study we investigated: sucrose accumulation and photosynthetic activity of the cyanobacterium (wild type PAM) and its genetic transformant strain (PAMCOD) under salinity stress conditions (up to 0.4 M NaCl) as well as in alkaline environments (pH 7.5 and 8) at ambient carbon dioxide condition.

Section 8: Regulation of Photosynthesis and Environmental Stress

Poster S8.13

Spectrally resolved fluorescent signatures of light treated *Synechocystis* PCC 6803

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Cyanobacteria (but also plants) have developed photoprotective responses to maintain the balance between energy absorbed and energy used so that accumulation of reactive oxygen species is avoided [1]. When exposed to high light, fluorescence quenching at the level of the phycobilisome (PBS) antenna has been observed: excitation energy gets dissipated before reaching the reaction centers. When exposed to low intensities of light that modify the redox state of the PQ-pool, state transitions redistribute energy between photosystem (PS) I and II. Methods to investigate the underlying mechanisms of photoprotection such as pulse-amplitude modulated (PAM) fluorometry are based on spectrally integrated signals. To preserve the spectral information R. Kaňa, et al. [2] introduced a spectrally resolved fluorometry method with a time resolution of 90 ms. We collect this valuable spectral information and introduce a method to interpret it in terms of principal components related to pure states such as open/closed reaction centers, un/quenched PBS or state 1 vs. state 2. Thus, spectral differences in the time-dependent fluorescent signature of photosynthetic organisms under varying light-conditions can be traced. We have adapted a multiple LED set-up [3] for measurements in solutions so that full fluorescent spectra of whole cells of model organisms were acquired and fluorescence intensity maps as a function of wavelength and time were obtained. We developed a method to resolve from the data matrix the spectra and time-dependent concentration profiles of state 1 and state 2 in *Synechocystis* PCC 6803.

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Poster S8.14

The effect of ionising radiation on pigment production, photochemical efficiency, ptotein level and generation of reactive oxygen species in plants

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Exposure all living organisms to ionizing radiation causes damage to living tissue and can result in mutation and radiation sickness. This aim of this work is the study content of photosynthetic and non-photosynthetic pigments, photochemical activity of chloroplasts, thylakoid membrane proteins, the level of generation of hydrogen peroxide and superoxide radicals in plants exposed to the chronic ionizing radiation. Syrian bean-caper (*Zygophyllum fabago* L.), reed (*Phragmites australis*) (Cav.), siberian sea rosemary (*Argusia sibirica* (L.) Dandy), oleaster (*Eleagnus caspica*) (Sosn.) Grossh. plants, spread on an area polluted with oil, located in Ramani village (near Baku), were used in the present study as investigation objects. Plants grown under 250 μR/h background level radiation and natural (4–8 μR/h) conditions were used. Analysis revealed that besides *Eleagnus caspica*, total chlorophyll content (Chl_{a+b}) increased under the influence of ionizing radiation as compared to non-irradiated plants. The amount of carotenoids and non-photosyntetic pigment anthocyan sharply increased in *Phragmites australis* under the effect of ionizing radiation; it was 0.045 mg/g in the control plants and 0.07 mg/g (fresh weight) in the stressed plants of *Phragmites australis*. The content of anthocyan was 2.9 and 4.4 μM/g, respectively. The photochemical activity of PS II significantly increased in *Phragmites australis*, *Zygophyllum fabago* and *Elaeagnus caspica* plants under the high dose of ionizing radiation compared to control plants. It was determined that in reed photochemical activity of PS II increased approximately 181% as compared to the control (with the control sample at 26 μmol O_2 mg^{-1} Chl h^{-1} and stressed sample at 47 μmol O_2 mg^{-1} Chl h^{-1}), and photochemical activity of PS I increased 128% (control: 132 μmol O_2 mg^{-1} Chl h^{-1}, stress: 169 μmol O_2 mg^{-1} Chl h^{-1}). However, photochemical activity of PS II and PS I in chloroplasts of *Argusia sibirica* decreased. Under the background radiation, some changes were detected in the protein level of thylakoid membranes of studied plants. Histochemical analysis detected increase in generation of hydrogen peroxide in irradiated leaves of syrian bean-caper, siberian sea rosemary and oleaster, as well as a high level of superoxide radicals in irradiated leaves of *Zygophyllum fabago* and *Argusia sibirica* as compared to healthy leaves.

Poster S8.15

Drought-induced changes in photosynthetic apparatus and antioxidant components of wheat (*Triticum durum* Desf.) varieties

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Water deficit is a key factor influencing the yield and quality of crops. In the present study the photosynthetic responses of wheat (*Triticum durum* Desf.) plants differing in drought sensitivity were analyzed by means of thylakoid membrane proteins, chlorophyll a fluorescence, photochemical activity of chloroplasts and as well as antioxidant components. Two durum winter wheat varieties, Barakatli-95 (drought tolerant) and Garagylchyg-2 (drought sensitive) were grown under field well-watered and drought conditions. It was found out that contents of the PS I core (CPI) with *Mr* of 123 kD and apoprotein P700 with *Mr* of 63 kD were relatively higher in Barakatli-95 variety under drought stress compared with the control plants. Synthesis of α- and β-subunits of CF_1 ATP synthase complex with *Mr* of 55 and 53.5 kD also increased significantly in drought tolerant Barakatli-95. Contents of these polypeptides decreased markedly in the drought sensitive variety Garagylchyg-2. A sharp decrease in the intensity of 30 kD band and increase in the intensity of 29.5 kD band were observed in this variety. A new 27 kD band was detected for tolerant Barakatli-95. The band intensity decreased sharply in the region of ~25 kD for Barakatli-95 contrary to Garagylchyg-2, in which significant increases in the intensities of 25–16 kD bands and also contents of low-molecular bands occurred. The intensity of peaks at 687, 695 and 740 nm increases in the fluorescence spectra (77K) of the chloroplasts isolated from the sensitive genotype Garagylchyg-2 and there is a shift from 742 to 740 nm under soil drought. However, there were no marked changes in the drought tolerant variety Barakatli-95. Drought caused also a significant decrease in the photochemical activity of PS II in the drought-sensitive variety Garagylchyg-2. Higher concentrations of ascorbate and glycine betaine were found in the drought tolerant variety throughout the different periods of growth in comparison with those in the control plants.

Poster S8.16

Impact of soil water deficit on morphophysiological parameters of durum (*Triticum durum* Desf.) and bread (*Triticum aestivum* L.) wheat genotypes

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Drought is a serious problem for wheat production around the world, including in Azerbaijan. We studied the effect of drought stress on leaf gas exchange parameters-photosynthesis rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E), leaf assimilating area (LA), leaf area index (LAI) and dry mass, chlorophyll a, b (Chl a,b), carotenoids (Car (x+c)) content, relative water content (RWC), proline content and canopy temperature of field grown 8 durum and 14 bread wheat genotypes. Water deficiency resulted in a decrease of P_n, g_s, E and an increase of C_i. The P_n was regulated mainly through mesophyll conductance (g_m). Drought stress led to a decrease of Chl a, b and Car (x+c) content, Chl (a+b)/Car (x+c) ratio. RWC decreased under the influence of drought stress. Water stress more affected on LA than leaf dry mass of wheat genotypes. LAI decreased significantly under water deficiency. Proline content was increased under drought stress. There were genotypic differences in response to drought stress. Physiological traits may be reliable for selection of drought tolerant wheat genotypes.

Poster S8.17

Photochemistry of Photosystem II: Is it a reliable tool for selecting cotton (*Gossypium hirsutum* L.) with improved flooding tolerance?

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Cotton plant requires well drained soils. Cotton producing areas are vulnerable to flooding and caused heavy economic losses. Flooding tolerant plants have greater ability to cope with oxygen deficiency due to metabolic modifications. It is hypothesised that growth and photosynthesis of cotton cultivars decreased due to short term flooding stress but tolerant species may recovered faster and have greater cotton yields. The present study was focused on assessing genotypic differences for structural and operational status of PSII under soil flooding stress in selected cotton germplasm using fast chlorophyll *a* induction kinetic analysis, chlorophyll fluorescence quenching analysis and NPQ induction and relaxation analysis. Identification of chlorophyll fluorescence based selection criteria for flooding tolerance in cotton may contribute substantially to improve cotton productivity under flooding stress. Growth inhibition in selected eight cotton cultivars under short-term flooding was mainly due to depletion of oxygen. Flooding reduced the photosynthetic capacity of cotton plants by reducing photosynthetic pigments. However, all flooding tolerant cotton cultivars do not have greater chlorophyll *a* and *b* than in flooding sensitive cotton cultivars. Fast chlorophyll *a* fluorescence analysis suggested that flooding stress reduced the quantum efficiency of the primary photochemistry, reaction centre density and quantum efficiency of end electron acceptor of PSI. Comparative semi-quantitative and JIP-test analysis of flooding tolerant and sensitive cotton genotypes suggested that flooding stress caused more drastic effect on PSII grouping/connectivity (appearance of L-band at 150 us), donor end of PSII (oxygen evolving complex, appearance of K-band at 300 us) and acceptor end of PSII due to inhibition electron transport from Q_A to Q_B (increase in fluorescence level at O-J phase). Flooding stress also caused a significant increase in fluorescence level at I step in all cotton cultivars which suggested that maximum inhibitory effect of flooding stress on electron transport in intersystem and PSI. The quantum yield of reduction of PSI end electron transport was markedly reduced by flooding. In terms of the parameters representing the energy utilization efficiency or probability that photon captured by the PSII drive the electron transport chain from reduced PQ to PSI final electron acceptor decreased due to flooding stress in all cotton cultivars. Minimum biochemical transformation capacity was found in NIAB-KRISHMA under flooding stress conditions. Moreover, OJIP analysis can be used to select elite genotypes with improved flooding tolerance.

Poster S8.18

Adjustment of photosynthetic electron transport in wheat under drought stress

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Adjustmentrs of photosynthetic electron transport in wheat (*Triticum aestivum* L.) leaves to different levels of drought stress were analyzed in potted plants in growth chamber under moderate light. Low to medium drought stress was induced by limiting irrigation, maintaining 20% of substrate water content for 14 days followed by 3 days without water supply to induce severe stress. Measurements of gas exchange and chlorophyll fluorescence were followed by simultaneous records of P700 and chlorophyll fluorescence. Measurements of electrochromic bandshift at 520 nm were performed on part of samples. Leaf water status was determined. Drought stress gradually decreased PSII electron transport, but the capacity for non-photochemical quenching only increased in severely stressed plants (where the photosynthetic rate had decreased by half or more). In addition to the major effects of stomatal closure, non-stomatic effects related to low leaf water potential were also identified. Cyclic electron flow was induced in all samples, but mainly when there was an excess of light; however, in severely stressed plants enhancement of cyclic flow even occurred below ambient light intensity. The rate of cyclic flow increased in parallel with an increase of non-photochemical quenching and accumulation of $P700^+$ and a decrease in reduction level of PSI acceptors. We observed also the major effects of drought on light energy distribution between photosystems, which can play a role in acclimation processes; moreover, it may cause errors due to overestimation of PSII electron transport.

Poster S8.19

Early response of photosynthetic apparatus efficiency to nitrogen deficiency in radish plants

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The aim of this research was to study the changes of efficiency and structure of photosynthetic apparatus in radish plants grown under nitrogen deficiency before the appearance of visible symptoms on plants. *Raphanus sativus* var. sativus plants were grown in hydroponics system with modified Hoagland solution under a set of controlled conditions (Phytotron). The followings were measured: (i) prompt fluorescence using a HandyPEA (Hansatech Instruments Ltd., UK), (ii) net photosynthetic rate (P_N) using an IRGA (LI-6400, LI-COR Inc., USA); (iii) chlorophyll and flavonoid content using a Dualex Scientific+™ Polyphenol & Chlorophyll-Meter (Force-A, France), (iv) chloroplast distribution and structure, using an electron microscope Morgagni 268D (FEI Ltd., USA) and confocal microscope Leica TCS SP5 II (Leica Microsystems GmbH, Germany). Acquired data were statistically analysed and a significant divergence (α=0.05) was observed for nitrogen deficient plants as compared to control plants for OJIP test parameters, P_N, chlorophyll content, as well as flavonoid concentration values. Fast kinetics induction curve analysis revealed a reduction in connectivity of photosystem II light harvesting complexes, exhibited as an L-band in the initial phase of induction curve 0.02–0.3 ms. Whereas, K-band appearance at 0.02–2 ms indicated the inhibition of oxygen-evolving complex. Fluorescence rise at the J-step (F_J=2ms) and I-step (F_I=30ms) indicated an accumulation of reduced primary quinone Q_A^- and plastoquinones PQ. Severe disorders in chloroplasts structure were confirmed by confocal and electron microscopy images. Statistically significant changes of some OJIP test parameters, e.g. a reduction of maximum quantum yield of primary photochemistry (φ_{Po}) as well as chlorophyll and flavonoid content were observed before decrease in P_N and the appearance of visible symptoms.

Poster S8.20

Photosynthesis in juveniles and adults of three mediterranean species with different life forms during leaf developmental period

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Juveniles (prereproductive plants) and adults (fully reproductive plants) of three woody species growing under mediterranean climatic conditions were used for field measurements in order to investigate their photosynthetic performance during leaf developmental period. Individuals of *Nerium oleander* L., an evergreen sclerophyllous shrub, *Phlomis fruticosa* L., a semi-deciduous dimorphic shrub and *Cercis siliquastrum* L., a deciduous tree with pre-leafing flowering, were selected and leaves marked after their emergence. Field measurements were conducted at distinct developmental stages during maturation of the marked leaves. Leaf dimensions and surface, specific leaf mass (*SLM*) and pigments concentration were estimated in complement with photosynthetic characteristics. For each developmental stage, PSII performance was examined by chlorophyll fluorescence measurements (modulated fluorescence and OJIP transients). Photosynthetic rates and efficiencies were approached with gas exchange measurements (CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance to water vapor (g_s) and water use efficiency (*WUE*). More specific parameters like quantum yield, carboxylation efficiency, relative stomatal limitation and fractions of electron transport rates diverted into carboxylation or photorespiration were calculated after the construction of light curves and *A*:c_i response curves. Any differences in most of the photosynthetic parameters between adults and juveniles of *P. fruticosa* and *N. oleander* during leaf maturation were finally eliminated between fully developed leaves. However, *WUE* was higher and transpiration lower in matured leaves of adults. Adults of *N. oleander* showed higher carboxylation efficiency and lower stomatal limitation, while ETR and stomatal limitation were higher in adults of *P. fruticosa*. On the other hand, most of the photosynthetic parameters in *C. siliquastrum* exhibited higher values in adults compared to juveniles during leaf maturation as well as in fully developed leaves. It seems that in the perennial and the semi-deciduous species, any advantage of the adults' photosynthetic machinery, possibly relating with more efficient exploitation of the resources, is counteracted by the lower demands for resources of the juveniles, resulting in similar photosynthetic characteristics of their fully developed leaves. However, it is interesting, that new leaves of adult *C. siliquastrum*, possess a more efficient photosynthetic machinery compared to that of juveniles, despite that the adults use significant amounts of their reserves for early flowering.

Poster S8.21

Chlorophyll catabolism under light deficiency in *Datura suaveolens*

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Datura suaveolens, Solanaceae, plants were placed in permanent darkness. The leaves were left to fall like yellow ones. Yellow leaves extract was subjected to spectroscopic analysis. Chlorophyll catabolites determined allowed the chlorophyll catabolic pathway identification in *Datura suaveolens.*

Poster S8.22

Effect of silver nanoparticules on *Arthrospira platensis*

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Silver nanoparticles (AgNPs) are widely used in industry therefore we can meet with them in many consumer products including paints, laundry additives, textiles, cosmetics, food storage containers, food supplements and medical tools such as wound dressings and catheters. AgNPs are added to these products in order to prevent their contamination with bacteria and fungi. Antibacterial and antifungal properties of AgNPs are stronger than the effects of silver ions. However, in consequence of increasing applications, it is a real danger that AgNPs dissolve in aquatic environment where could be harmful both for natural ecosystems and agriculture. While there are detailed investigations on toxicity of AgNPs in bacteria, the mechanism of toxicity in photosynthetic cyanobacteria is hardly studied. *Arthrospira platensis* is a worldwide known cyanobacterium that is cultured in large scale and utilized in cosmetics, drug development and primarily as food supplement. Usually, *Arthrospira* is cultured in huge open ponds with high risk of contamination from air pollution. In this work the effect of 2 nm size AgNPs on *Arthrospira platensis* cultures was studied. The inhibitory effect of AgNPs on photosynthetic electron transport chain was investigated by quenching analysis of chlorophyll fluorescence using Imaging-PAM system. Flash-induced chlorophyll fluorescence changes and OJIP transients were measured by a fluorimeter. The morphological consequences of AgNP toxicity was observed by light and electron microscopes. The amount of incorporated silver was detected by ICP-MS elemental analysis. Differences were found in the characteristics of toxicity of AgNPs and silver ions both in photosynthetic parameters and morphological alterations.

Poster S8.23

Diurnal changes in photosynthetic enzyme activities and their regulation in some C4 species of Chenopodiaceae family

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Dynamics of the diurnal changes in photosynthetic enzyme activities and their regulation in some C4 species of Chenopodiaceae family grown under natural conditions have been studied during the active vegetation period of ontogenesis. Activities of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) appeared to be relatively stable in Salsola dendroid and Suaeda altissima species belonging to NAD-ME subtype of C4 photosynthesis. However, increases in ALAT and mitochondrial ASAT activities and a decrease in the cytosolic isoform activity of ASAT were observed at evening time in another NAD-ME type plant *Amaranthus cruentus*. The diurnal changes in activities of these enzymes correlated in the studied plants. But NADP-malate dehydrogenase activity in a C4 plant *Kochia scoparia* belonging to NADP-ME subtype was higher in the daytime.

It was established that activities of fructose 1,6-bisphosphatase and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase were higher in morning and evening hours than during daylight hours.

The study of the inhibitory effect of aspartate on PEPC activity during the day showed that the enzyme activity under the influence of the inhibitor decreased basically in accordance with its normal activity. Similar results were obtained for the activation effect of glucose-6-phosphate on PEPC. However, in *Salsola dendroid* and *Suaeda altissima* the inhibitory effect of malate on PEPC was observed to increase mostly during evening hours, suggesting a decrease in the phosphorylation degree during this time.

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Poster S8.24

Photosynthetic activity in degradation of xenobiotics, a possible connection with organochlorine formation?

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The influence of light on the biodegradation of trichloroacetic acid (TCA) was investigated using radiotracer method with sterile plant seedlings. TCA is metabolized in plants to carbon dioxide, in case of radioactively labelled TCA (1,2-^{14}C[TCA]) it is mineralized into $^{14}CO_2$, and its emission is then determined in a flow-through trap by liquid scintillation. Sterile Norway spruce (*Picea abies* L.) seedlings showed uptake of TCA, and emission of $^{14}CO_2$. Degradation showed a distinct pattern connected with the light, showing photosynthetic activity involved in the process. The emission of $^{14}CO_2$ was slightly elevated by application of a wide spectrum antibiotics mix applied into the medium. Antibiotics application confirmed that no microorganisms are involved in the degradation process, either in the medium or endophytic organisms living on the surface of the plants. The photosynthetic activity is generally connected only with dechlorination activity in lower plants and microorganisms. Chlorinated compounds often act as inhibitors, TCA similarly therefore it was used as an herbicide. Transformation of chlorinated compounds by photosynthetic processes can be in connection with formation of novel compounds.

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Poster S8.25

In vivo phenotyping of photosynthetic light reactions in leaves of two ecotypes of *Platanus orientalis* L. during water stress and after re-watering

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Platanus orientalis is almost extinct in the natural ecosystems of Western Europe and, because of its hydrophilic habitat, may also be strongly affected by increasing water limitations in Eastern Europe. The effects of drought stress were studied in two ecotypes of *P. orientalis*. Plane seedlings were grown from seeds originated from areas with different environmental conditions in Bulgaria and Italy. Experiments were performed with 4-month-old seedlings. The dynamics of drying and followed re-watering was monitored by changes in photosynthetic activity *in vivo*. Signals of prompt and delayed chlorophyll fluorescence as well as modulated signal of 820 nm light reflection by leaf samples were simultaneously measured. A number of specific experimental protocols were designed to evaluate reactions in light stage of photosynthesis, as activities of linear electron transport in PS I, PS II and between photosystems, cyclic electron transport in PS I, photochemical and non-photochemical quenching of chlorophyll excited states in PS II.

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Poster S8.26

An alternative approximation of the ABS/CS parameter when the JIP-test is applied in the field

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The quantitative analysis of the fast fluorescence rise (JIP-test) is a rapid, non-destructive technique which has been used for studying the effects of many abiotic and biotic factors on the physiological state of photosynthetic samples with different complexity like thylakoids, chloroplasts, or leaves. An advantage of the JIP-test is the ease of use in the field and, for this reason it has become a very popular technique in ecophysiological or agricultural studies, in complement to traditional photosynthetic measurements. The mathematical quantification of the fluorescesne transient, describes, the stepwise flow of energy through photosystems and can be made on a reaction center (RC) basis (specific fluxes) or on an excited cross section (CS) basis (phenomenologigal fluxes). The key parameter ABS/CS which is used for the estimation of all the per CS parameters, describes the number of photons absorbed by the antenna molecules of both active and inactive RCs of the excised cross-section and is related to the chlorophyll concentration of the tested sample. ABS/CS value can be approximated by reflection or transmission measurements using the appropriate instruments. However, in field studies, when screening of large populations is required, measurements of that kind are often inconvenient and the approximation of ABS/CS parameter is based on F_O (ABS/CS_O) or F_M (ABS/CS_M) basis. During the last decade, our laboratory has conducted thousands of field measurements during different projects and with various plant species. Our results showed an occasional correlation of F_O and/or F_M values with chlorophyll concentration. However, after thorough meta-analysis, we found out a satisfactory correlation between chlorophyll concentration and the S_m parameter of the JIP-test. This correlation could be ascribed to a relatively stable stoichiometry between chlorophyll molecules and PQ pool within a given species. In the present work, we present results in favor of this correlation under different environmental conditions and developmental stage of the leaf and we discuss the potential use of S_m for ABS/CS approximation as an alternative of F_O and F_M in ecophysiological studies.

Poster S8.27

Effect of terminal drought stress on yield, yield components and morphological traits of bread wheat in Moghan

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Drought, the most important factor limiting crop production worldwide has been successfully studied. This factor is created as result of a combination of physical and different environmental factors that cause stress in the plants and thus reduce production. The agronomic performance of seven varieties and promising lines of bread wheat 2013–2014 on two separate tests in normal and stress conditions based on randomized complete block design with three replications were conducted in plain MOGHAN Pars Abad city. In this study nine traits including yield and component yield and indices MP, GMP, SSI, STI and TOL were evaluated. The results showed that in normal circumstances, between the lines and wheat cultivars for most traits except tiller number, straw weight was significant. In drought stress conditions between the lines and cultivars tested except number of tillers, straw weight and harvest index were significantly lower. The grain yield in normal conditions, line No. 5 with 7.41 tonnes/ha compared to other genotypes 6 and 5.82 tonnes/ha highest and the lowest yield was produced. Under drought stress, lanes 7 to 5.28 tonnes per hectare, the yield was superior lines. The minimum requirements for the Line 6 to 37.4 tonnes/ha. TOL based on lines 6 and 7 and the homeland, with the 1.46, 1.85 and 1.78 in terms of drought tolerant lines after pollination. The SSI index of lines 6 and 7, respectively, with values equal to the homeland 0.81, 0.84 and 0.83 most tolerant lines in wheat under drought stress after an thesis were in the study.

Poster S8.28

Alternating functions of terminal oxidases under light and dark conditions in *Synechocystis* sp. PCC 6803

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The respiratory terminal oxidases (RTOs), cytochrome c oxidase (Cox) and quinol oxidase (Cyd), are directly involved in the electron flow occurring in the thylakoid membranes of cyanobacteria. Traditionally RTOs have been thought to function mainly in darkness in respiration and their activity has been considered to become inhibited under a transition to dim light (Kok 1949). However, it was recently shown that in *Synechocystis* sp. PCC 6803 the presence of at least one of the thylakoid-based RTOs is essential to survive under the growth regime of 12h dark/12h high light square cycles (Lea-Smith et al. 2013).

To investigate the role of RTOs under the light, MIMS gas-exchange analysis using the $^{18}O_2$ isotope was applied to cyanobacterial cells. This enabled a differentiation of O_2 uptake occurring during illumination from photosynthetic O_2 evolution. We demonstrate that Cyd is competing for electrons with Cyt *b6f* and is able to redirect excess electrons from the PQ pool to O_2 thus contributing to redox poising of PQ pool in the presence of DBMIB. Significant contribution of Cyd to the light-induced O_2 uptake was also observed in the Δ*flv1/3* mutant under fluctuating light, where the linear electron transport is known to be inhibited due to the malfunction of Photosystem I and down-regulation of CO_2 fixation (Allahverdiyeva et al. 2013). Moreover our results indicate that Cox can accept electrons in darkness from the PQ pool, bypassing the Cyt *b6f* complex. In addition, the Q_A re-oxidation and P700 oxido-reduction kinetics, and the Chl *a* fluorescence induction analyses strongly suggest a highly reduced PQ pool in darkness in the Δ*cox*/*cyd* double mutant.

Our data suggests that Cyd represents the “slow” sink for Photosystem II-driven electrons at the PQ pool level whilst flavodiiron proteins are able to operate in a much faster time scale under the light. Cox is mostly active in dark respiration but can also act in regulation of electron transport between Cyt *b6f* and Photosystem I under light.

Poster S8.29

Photosystem I rather than photosystem II is selectively inhibited under short term salinity stress in two halophyte species *Sulla carnosa* and *Atriplex halimus*

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The present study compares the capacity of two halophytes *Sulla carnosa* Desf. and *Atriplex halimus* L. to maintain the structural and functional integrity of the photosynthetic apparatus under moderate (150 mM KCl) and severe (300 mM KCl) salinity stresses. Exposure of *A. halimus* and *S. carnosa* plants to moderate KCl stress for 7 days caused only minor changes in the content and composition of the photosynthetic pigments (Chl a, Chl b, carotenoids), while at 300 mM KCl both *S. carnosa* and *A. halimus* exhibited significant decrease of photosynthetic pigments of (10–15%) and (23–25%), respectively. Low temperature (77K) chlorophyll fluorescence measurements of isolated thylakoid membranes from both species showed a substantial linear decrease (24%) of F735/F685 ratio with increasing the severity of KCl stress in *S. carnosa*, while much smaller decrease (14%) of the F735/F685 ratio was registered only at high (300 mM KCl) salinity in *A. halimus* plants. These results clearly indicate that although salinity stress induces noticeable changes in the excitation energy distribution and/or stoicheometry between PSII and PSI chlorophyll-protein complexes in both species, *A. halimus* plants seem to be less susceptible to short (7 days) and long (24 days) term salinity stress. The increased salinity stress was also associated with a steady, although small decline (15%) of the maximal photochemical efficiency of PSII measured *in vivo* as Fv/Fm in *S. carnosa* plants at 300 mM KCl. Under the same conditions *A. halimus* did not exhibit measurable changes of Fv/Fm values. The higher susceptibility of PSII to salinity stress in *S. carnosa* was also confirmed by *in vitro* measurements of PSII activity (O_2 evolution) in isolated thylakoid membranes. Interestingly, the relatively moderate effects of KCl stress on PSII primary photochemistry (Fv/Fm) in *S. carnosa* plants was accompanied by much stronger inhibition of photochemical quenching qP (45%) and the yield of PSII-driven electron transport (73%). This implies that PSII could not be the major target of salinity stress in these plants. Indeed, in contrast to PSII, photochemical activity of PSI measured as photoxidation of P700 ($P700^+$) in *S. carnosa* was strongly affected and was decreased by 61 and 81% at moderate and high salinity levels, respectively. Moreover, the steady state levels of P700 photooxidation were also reduced although to a lower extend (43 and 55%) in *A. halimus* under the same conditions. Taken together, our results clearly demonstrate that PSI, rather than PSII is preferentially targeted and inhibited under short term salinity stress conditions and that *A. halimus* plants are less susceptible to salinity stress by developing enhanced capacity for light use efficiency and more effective photoprotective mechanisms.

Poster S8.30

Hydrogen peroxide signaling – implications for photosynthetic cold stress tolerance in *Zea mays* L.

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The role of specific mechanisms of cold stress tolerance and the rate limiting steps in photosynthetic performance at low temperatures were investigated by comparing the effects of cold stress on a cold sensitive maize line (Null line) and an oxalate oxidase (OxO) over-expressing transgenic maize line (OxO-OE line) over-producing H_2O_2. OxO transgenic lines exhibited several unique phenotypic features, such as faster field emergence, improved seedling vigour, earlier maturity and increased growth rates under non-saturating light conditions. In addition, control OxO overexpressing plants exhibited increased abundance of the PSII reaction center protein D1, decreased level of phosphorylation of D1 and LHCII polypeptides, increased abundance of chloroplastic NAD(P)H dehydrogenase complex, increased stromal and intersystem electron pool sizes, slower Q_A to Q_B electron transfer and possible alterations in PQ binding to the Q_B-site, compared to Null plants. In response to cold stress the CO_2 assimilation and linear electron transport (ETR) rates decreased similarly in Null and OxO plants. However, under the stress conditions OxO plants managed to maintain higher photochemical efficiency of PSII than Null plants under the same cold stress conditions. Moreover, OxO-OE plants exhibited improved growth during cold stress and recovery and increased tolerance to low temperature induced photoinhibition of both PSII and PSI. These features of cold-stressed OxO-OE plants were associated with higher cold-induced expression of chloroplast targeted *FtsH*, larger stromal and intersystem electron pool sizes, higher capacity for PSI-dependent cyclic electron transport, increased stability of D1 protein and alterations in light energy management by the reaction center and light harvesting antennae, when compared to the cold-stressed Null plants. The enhanced tolerance to photoinhibition and D1 stability was confirmed in OxO transgenic lines representing different transformation events and parental backgrounds. Since hydrogen peroxide has been long implicated in cell signalling, signal transduction and plant photosynthetic adaptation we suggest that an increased level of endogenous H_2O_2 might stimulate the native maize defence system responsible for cold stress response and acclimation. Indeed, the constitutive over-expression of OxO in transgenic corn plants was accompanied by induction of gene expression of chloroplast targeted proteins in particular genes involved in jasmonic acid signalling pathway and glutathione biosynthesis.

Poster S8.31

Strategies of *Chlamydomonas reinhardtii* to cope with fluctuating light

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We have investigated the role of flavodiiron proteins (FDP), the proton-gradient-regulation 5 (PGR5) and the PGR5-like protein 1 (PGRL1) as electron valves in photosynthetic light reactions under fluctuating light condition, which is the natural environment for aquatic organisms.

FDPs are present in Archea and Bacteria, being involved in detoxification of NO and/or O_2. The cyanobacterium *Synechocystis* PCC 6803 possesses 4 genes encoding FDPs (Flv1–Flv4). The Flv2/Flv4 heterodimer is involved in the photoprotection of PSII in cells acclimated to air level CO_2, however exact electron acceptor and donors are not know yet (Zhang et al. 2009, Zhang et al. 2012). Whereas, Flv1 and Flv3 proteins function in a Mehler-like reaction, donating electrons from downstream of PSI directly to molecular oxygen and thus playing an important role as a strong sink of excess electrons (Helman et al. 2003, Allahverdiyeva et al. 2011). Besides, Flv1 and Flv3 enable the growth of cyanobacteria under fluctuating light conditions by protecting PSI against photodamage (Allahverdiyeva et al. 2013).

Phylogenetic analysis revealed two genes (*flvA* and *flvB*) in the genome of *C. reinhardtii* with high homology to *Synechocystis flv1* and *flv3* genes. We observed an upregulation of *flv* transcripts and high FDP accumulation during the shift to high light, from high to air-level CO_2 and during S-deprivation (Jokel et al. 2015). These results strongly suggest a possible function of *C. reinhardtii* FDPs as an electron sink, similarly to that in *Synechocystis*. However, different from FDP mutants in *Synechocystis*, we did not observe a strong growth phenotype from FLVA and FLVB knock-down mutants of *C. reinhardtii* under fluctuating light intensities. In contrast, the PGR5 knock-out mutant of *C. reinhardtii* displayed an impaired growth phenotype under fluctuating light, suggesting an important role for this protein in regulation of electron flow during fast changes in light intensities. Interestingly, the PGRL1 knock-out mutant of *C. reinhardtii* shows impaired growth only under very harsh fluctuating light conditions. Furthermore, it needs to be mentioned that the PGRL1 knock-out mutant exhibits elevated levels of FDPs, demonstrating a possible cross-talk between these proteins (Dang et al. 2014).

We discuss the coordinated function of FDPs, PGR5 and PGRL1 proteins in *C. reinhardtii* in the regulation of the photosynthetic electron flow under fluctuating light.

Poster S8.32

Enhanced thermal and light stability of the thylakoid membranes from spruce

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Responses to photosynthetic reactions to the temperature changes are important for higher plant, particularly if we consider the global warming and the increasing frequency of extreme temperature fluctuations. High temperature stress decreases photosynthetic assimilation through the inactivation of the most heat sensitive photosystem II (PSII). Recently, we have found higher thermostability of PSII photochemistry in the spruce needles compared to control plants such as *Arabidopsis*. In this contribution we have focused our attention on the impact of elevated temperature on the pigment-protein complexes (PPCs) organization in the spruce thylakoid membranes by circular dichroism (CD) spectroscopy and native electrophoresis.

We have found that higher thermal stability of PSII photochemistry (F_V/F_M) of spruce needles is related to the maintenance of PSII macro-organisation under temperature stress as revealed from the temperature dependence of so-called Y-type CD signals (transient temperatures: spruce ~54°C; Arabidopsis ~48°C). Clear-native electrophoresis has demonstrated that the decrease of Y-type CD amplitudes induced by heat stress correlate with reduction of PSII-LHCII supercomplexes content. On the other hand, the thermal disruption of individual PPCs observed on electrophoreograms and drop of excitonic CD amplitudes were observed roughly at the same temperature for both plant species (~60°C). Preliminary results indicated that the light stability of the PPCs organization in spruce thylakoid membranes is also higher than in *Arabidopsis* ones.

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Poster S8.33

Function of prenyllipids during photo-oxidative stress in plants

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Plants under natural environments have to cope with conditions that are far from optimal for their growth and are called stress conditions. The most frequent abiotic stresses are caused by excess light, temperature (low and high) and drought. All these unfavorable conditions result in enhanced production of reactive oxygen species (ROS) that may cause deleterious effects. To prevent this, plants produce enhanced levels of a variety of antioxidant molecules under stress conditions. Among antioxidant prenyllipids, tocopherols were most early recognized. However, other compounds of similar function were recently identified, such as plastoquinol, plastochromanol or its hydroxyl derivative, especially under high light stress. Plastochromanol, synthesized by tocopherol cyclase, was shown in many *in vitro* experiments as very versatile antioxidant. Its oxidation product during singlet oxygen action, hydroxyl-plastochromanol was identified in *Arabidopsis* whose level increases during high-light stress and aging. Therefore, it can be regarded as a marker of singlet oxygen stress in plants. Recently, we have identified in *Arabidopsis* also other oxidation products of prenyllipids, such as plastoquinol-C and plastoquinone-B. The oxidation products of prenyllipids, such as hydroxyl-plastochromanol and plastoquinol-C could still fulfill antioxidant action in chloroplasts.

Poster S8.34

Photosynthetic characteristics of contrasting european beech (*Fagus sylvatica* L.) Provenances

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The study presents the result of variability of chlorophyll *a* fluorescence parameters, and leaf stomatal characteristics of European beech (*Fagus sylvatica* L.) provenances in the Slovak trial area at the locality Tále (800 m a.s.l.). We selected five provenances located along an altitudinal gradient: Farchaus 72 A (Germany; 55 m a.s.l.), Belzig (Germany; 140 m a.s.l.), Jaworze 178 F (Poland; 450 m a.s.l.), Postojna Javor (Slovenia; 1040 m a.s.l), and Eisenerz (Austria; 1100 m a.s.l.). The measurements of chlorophyll *a* fluorescence and stomatal imprints were taken in August 2012. The stomatal characteristics, specifically stomatal density (SD), stomatal pore surface (SPS) and stomatal resistance (R_S), and characteristics of primary photosynthesis response such as RC-absorbance ratio (RC/ABS), performance index (PI), Area, and reaction time for F_M (Tfm), were measured. Results indicate that performance index showed a significant correlation with the altitude of origin ($r=0.8954$; $p=0.04$). For the other parameters, the altitudinal trend was not confirmed, and also there was found no effect of amount of precipitations and average temperature during the vegetation season of the place of origin on the evaluated parameters. On the other hand, analysis of variance confirmed significant differences in stomatal pore surface and all determined parameters of chlorophyll *a* fluorescence among provenances. Moreover, the Slovenian and Austrian proveniences, coming from the highest altitudes, showed the best vitality, as they exhibited significantly higher values of performance index, number of active RC, Area, and also reaction time for F_M.

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Poster S8.35

Screening 16 plant species by *in vivo* reflectance spectroscopy indicate a common photosynthetic pigment profile for green fruits, as a response to their distinct internal microenvironment

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Green fruits' chloroplasts function in a particular microenvironment, completely different from that of leaves, characterized by hypoxia and extremely high internal CO_2 concentrations. These conditions, which are shaped by fruit metabolic and anatomical features, may affect the pigment profile and the processing of the absorbed light energy. Previous investigations of our laboratory have shown that, compared to leaves, fruits display lower total chlorophyll and carotenoid levels, but higher Chl b/a and Car/Chl ratios, combined with enhanced xanthophyll cycle pools and functionality.

In the present study we asked whether the above photosynthetic traits constitute a generalized fruits' pattern, responding to the peculiarities of their aerial internal. To this aim, 16 different plant species were selected, in order to assess the photosynthetic pigment profiles of their fully exposed green fruits, with corresponding leaves serving as controls. Since conventional spectrophotometric and HPLC analyses of crude extracts could not easily be applied in large sample sizes, we examined whether appropriate spectral reflectance indices already used for the rapid and non-destructive pigment estimation in leaves and twigs, could also be utilized accurately in fruits. Regression lines of the above indices vs actual pigment levels were used to assess their reliability.

Our results indicate that the higher pigment ratios and enhanced VAZ cycle activity represent a common photosynthetic pattern for green fruits, which may serve the enhanced thermal dissipation needs imposed by the gas exchange restrictions of their internal microenvironment.

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Poster S8.36

Foliar photosynthesis under non-perpendicular illumination: the contribution of leaf optical properties

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Leaf anatomy, in particular the monocot vs. dicot leaf structure and the homobaric vs. heterobaric character (i.e. the occurrence of perpendicular bundle sheath extensions that separate the mesophyll into areoles), was examined in relation to light propagation inside the mesophyll and the photosynthetic rate under non-perpendicular illumination (NPI). According to our results, reflection of NPI was minimized when light rays were parallel to the leaf axis and was maximized when light rays were perpendicular to the leaf axis. This result was evident only in heterobaric monocot leaves. Leaf transmittance was also minimized when light rays were parallel to the leaf axis but this was evident also in homobaric monocot leaves. Thus, the above favourable arrangement between light rays and the leaf axis resulted in higher leaf absorptance compared to non-favourable arrangements. Also, photosynthetic rate (A_n) under NPI was higher than expected (according to the PFD calculated from the Lambert's cosine law) in monocot leaves regardless of the homo/heterobaric character. This surplus of A_n was maximized again when light rays were parallel to the leaf axis. Our results show that leaf anatomy significantly determines photosynthetic performance under NPI. This is important since NPI is the light regime under which leaves photosynthesize during most of the day under natural conditions.

Poster S8.37

Physiological traits and local adaptive potential of beech populations in the Central Europe

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Climate changes in progress are leading to declining of local beech populations fully adapted to current climate conditions. In this context arise a need to replace these populations for those that have a greater tolerance to new ecological conditions. For the stability of future forests is particularly important choosing the right, most adaptable but also genetically variable provenances. In the aspect of character and intensity of climate change is helpful to replace these populations in the new, coming from areas that have a similar climatic character. In our study we focused on assessing climate adaptation of selected beech populations based on an integrated physiological response within the provenance trial. Field experiment was conducted on provenance plots Tále during summer 2013. We used 15 years old saplings originated from 5 contrasting regions of Central Europe, differing mainly in terms of altitude and annual rainfall, signed as 26D (55 a s. l. Denmark), 30DE (140 a s. l., Denmark), 36AT (1100 a s. l., Austra), 36PL (450 a s. l. Poland) and 55SI (1040, Slovenia). With the purpose to monitoring of physiological adaptation were evaluated parameters of photosynthesis and fluorescence. Provenances originated from higher altitudes responded better to our environment. Provenance 36PL was characterized by the best ability to adapt to our environmental conditions. This provenance reached the highest values of net photosynthesis (P_N) and stomatal conductance (g_s). We monitored the worst adaptation by provenance 30DE originated from dry climate of Denmark. The same trend was recorded in chlorophyll fluorescence parameters (RLCs of Φ_{PSII}, NPQ).

Knowledge of physiological responses of beech to environmental conditions in direct response to its geographical origin, will help to evaluate mitigation options ongoing climate change on populations of forest tree species.

Poster S8.38

Photosynthetic activity of *Larix* trees grown on permafrost soils

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Larix is a dominant tree species occupies vast areas of Eurasia: ca. 3.5 million km^2 (Shvidenko and Nilsson 1994). Due to the large geographic extent, *Larix* has the high potential to sequester significant amounts of atmospheric CO_2. Moreover, its habitat almost completely coincides with the zone of continuous and interrupted distribution of permafrost (Abaimov 2010). This study estimates seasonal dynamics of CO_2 gas exchange and gross primary production (GPP) in mature 105 year-old productive larch (*Larix gmelinii* Rupr. Rupr.) stand located within the continuous permafrost area (Krasnoyarsk region, Russia, 64°17'13" N, 100°11'55" E, 148 m a.s.l.) during growing period from June to August 2013. Soil active layer thickness (maximum thawing depth of soil reaching in September) was about 20–60 cm. Trees selected for experiments represented mean tree of the stand. Studies of seasonal photosynthetic activity with simultaneous measurements of PAR, foliar biomass, needle morphology and pigment content (chlorophyll and carotenoids) were arranged from early growing season (June 8, 2013) until senescence of needles in September 17, 2013. Variation of larch photosynthetic activity values (0.05–3.6 μmol CO_2 m^{-2} s^{-1}) occurred during growing season was due to different PAR, temperature and precipitation. Obtained PAR-relationships of photosynthesis were saturated at PAR=500 μmol m^{-2} s^{-1} in the same manner through entire growing period. GPP value was estimated using seasonal light (PAR)-photosynthesis curves and one-hour resolution PAR data for entire growing period. Monthly cumulated stand photoassimilation (GPP) with LAI 2.81 m^2 m^{-2} was assessed as 41.5, 112.3 and 84.8 g C m^{-2} month^{-1} for June, July and August, respectively. Data obtained allow to characterize productivity of larch stand growing on permafrost soils as rather high (GPP 238 g C m^{-2} 92 day^{-1}) and comparable with more productive permafrost larch stands in Yakutia.

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Poster S8.39

Photosynthesis and transpiration changes after wounding and perception of herbivore elicitors in *Nicotiana attenuata*: role of stomata regulators abscisic acid, OPDA and cytokinins

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Plants attacked by herbivores respond not only at the site of attack but also in systemic, unattacked parts. They respond to damage in the context of their available resources and their need to optimize these resources for defense, growth and development. Understanding photosynthesis and resource allocation in response to herbivory has therefore been used as a mean to understand the cost of defense.

However, our research showed that not only is carbon assimilation altered in *Nicotiana attenuata* in response to herbivory, but transpiration is as well. Wounding and perception of specific cues of *Manduca sexta*, a natural herbivore of *N. attenuata*, decreased transpiration in local and systemic parts of the plant. We further addressed the nature of this response by using different transgenic lines impaired in the perception and signaling of the hormones, absiscic acid, OPDA and cytokinins; all known to regulate transpiration and water use efficiency in response to herbivory. We found that changes in transpiration after herbivory are not necessarily coupled to carbon assimilation, as often assumed. This was observed to be regulated in different ways by the studied hormones, highlighting the complexity and plasticity of the system. Whereas photosynthetic rates were not affected in systemic tissues, water use efficiency was, which may account for growth and resource tradeoffs known to occur after herbivory.

Poster S8.40

Cell and chloroplast size related to long-term acclimation of barley to combined drought and low nitrogen

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Under multiple abiotic stressors, plant acclimation to stress interaction leads to a unique combination of characteristics that do not represent the sum of responses to each individual stress factor. In this study, two genotypes of barley, 'Demetra' and 'Niki', were cultivated in a greenhouse in water (two levels) and nitrogen (three levels) availability gradients. These two parameters represent the most important soil resources of plant growth. According to the results, drought resulted in a significant reduction of leaf water potential while low nitrogen, when combined with water shortage, caused a reduction of leaf nitrogen levels. Drought stress induced a significant reduction in the values of all gas exchange parameters which, however, in the 'Niki' genotype was dependent on the level of soil nitrogen supply. Both stressors caused a reduction of leaf surface area while drought, in particular, reduced the diameter of vascular bundles and xylem vessels. The combination of the two stressors resulted in a very distinctive response of the histological parameters of the leaves. While drought stress alone induced a reduction in cell and chloroplast size similarly in both genotypes, when drought stress was combined with low levels of soil nitrogen, cell dimensions were restored to levels comparable to those of plants grown under sufficient water and nitrogen supply. Cell and chloroplast size restoration was particularly prominent in the 'Demetra' genotype indicating that the genetic background of the phenomenon is very specific.

Poster S8.41

Effect of progressive drought on mesophyll conductance to CO_2 flow in photosynthetizing leaves of wheat at different ploidy levels

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Contribution of mesophyll versus stomatal factors to main limits of leaf photosynthesis and their causality in different wheat species under drought in reproductive growth stage has not been sufficiently studied yet. Also a shift of mesophyll conductance to CO_2 diffusion within a dehydrated photosynthetizing leaf to higher values in modern hexaploid comparing to di- and tetraploid wheat species allowed to improve this characteristics during the evolution of wheat ploidy and enhance wheat photosynthesis and productivity.

In pot vegetation experiment with different wheat genotypes and species of various ploidy level plants were exposed to a progressive dehydration at the beginning of ear formation. During graduating water stress basic photosynthetic characteristics of flag leaves related to the leaf water status were derived from gas exchange measurements and the intrinsic water use efficiency was calculated on a leaf base. Quantification of non-stomatal limits to the leaf photosynthesis were based on A/c_i curve measurements and analyses according to Sharkey et al. (2007). Proportion of mesophyll (Lm) and stomatal (Ls) limitations to the total A_{CO2} limitation was evaluated in relation to the genotype- and species-based variability.

The results show that in modern wheat genotypes the mesophyll (g_m) rather than stomatal (g_s) conductance has an effect on A_{CO2} variation under drought and is determined mostly by biochemical (Vc_{max}) and anatomical (SLA) limits. However, their contribution in *T. monococcum* and *T. dicoccum* was equal. Genotype Biscay reached the highest g_m allowing the highest CO_2 assimilation during whole period of dehydration comparing to the lowest g_m and A_{CO2} in *T. monococcum*.

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Poster S8.42

Effect of nitrate stress on photosynthetic electron transport in *Chlorella saccharophila* grown under high light

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Nitrate assimilation and its dynamics, important in the process of metabolism and photosynthesis, was studied through chlorophyll fluorescence analysis for its effect on the photosynthetic performance of *Chlorella saccharophila* growing under natural irradiance reaching up to 1500 μE. The experimental conditions were designed to ensure limiting nutrient conditions of <100 ppm N, since N adequacy was seen to be achieved in 250 ppm N as observed through its effect on the chlorophyll biosynthesis. Even with limiting nitrate concentrations, the culture exhibited rapid nitrate uptake and increased chlorophyll pigment during the initial growth period. Significant reduction in chlorophyll fluorescence was however observed immediately after N depletion reflecting an altered photosynthetic activity in the growing culture. Effective quantum yield Y (II) decreased by 45% in cultures provided with a low N with increase in non-photochemical quenching (NPQ). Slow kinetics analysis revealed the possible onset of an altered photosynthetic electron transport in cells that were provided with low nitrogen which indicated a diversion of linear electron transport to alternate electron sinks. Comparatively lower levels of alternate electron flow in high N cultures indicated a minimum requirement of alternate electron path for energy generation. The study clearly demonstrated that chlorophyll fluorescence kinetics of *C. saccharophila* differed when supplemented with different N concentrations. This study can be useful in determining the minimum concentration of N required for optimal photosynthetic activity of algal species while attempting to create stress conditions.

Poster S8.43

Foliar anthocyanin accumulation leads to adjustments in photosystem and chlorophyll ratios, compatible to the shade acclimation syndrome

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In some plants leaves appear red due to the accumulation of anthocyanins at concentrations that mask the green chlorophyll color. Foliar anthocyanins impose on the underlying chloroplasts a particular shade, since they absorb strongly in the green and less in the yellow/blue part of the spectrum.

In the present study, we argued that the selective attenuation of green/yellow light by anthocyanins represents a potential loss for PS II, which preferentially absorbs this spectral band. Accordingly, the uneven excitation of the two photosystems within an anthocyanic leaf could be balanced by a re-adjustment of their stoichiometry. To test this hypothesis, 77K fluorescence emission spectra of thylakoid samples obtained from green and red leaves of different plant species were compared. The calculated F_{686}/F_{735} ratio from each leaf type was used as a relative indication of the PSII/PSI functional analogy. In order to avoid possible inter-specific differences in the measured parameters, we have used seven plant species displaying intra-individual, intra-species or intra-leaf variation in the expression of the anthocyanic trait. The selected species, grown under apparently similar field conditions, accumulate anthocyanins transiently or permanently in their young or mature leaves, either in the epidermis or in the mesophyll.

Our results have shown that, regardless of the species tested and the anthocyanin accumulation pattern, red leaves display higher relative PSII/PSI ratio, compared to their green counterparts. In five of the examined species, red leaves displayed also a lower Chl *a*/*b* ratio.

We conclude that anthocyanic leaves develop adaptive adjustments in pigment and photosystem ratios, which are compatible to a shade acclimation syndrome.

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Poster S8.44

New insights into short-term light acclimation in plants – the role of high molecular mass protein complexes

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In higher plants the thylakoid membrane is heterogenic in structure and the photosynthetic protein complexes are unevenly distributed on the membrane. Several thylakoid membrane protein megacomplexes from *Arabidopsis thaliana* has been characterized using a large pore BN PAGE (lpBN-PAGE) optimized for separation of high molecular mass protein complexes from the thylakoid membrane. Redox regulated thylakoid protein phosphorylation and concomitant rearrangements of the pigment-protein complexes represent an important light acclimation mechanism in plants. We addressed the dynamics of the thylakoid pigment-protein complexes by exposing wild type *Arabidopsis* together with the *stn7* and *tap38/pph1* mutants to varying light intensities. We show that the protein megacomplex composition in the non-appressed thylakoid membrane is dependent on the reversible phosphorylation of the light harvesting complex (LHC) II. The dephosphorylation of LCHII resulted in an increase of the amount of photosystem I rich complexes while the phosphorylation of LHCII resulted in an increase of the amount of the largest protein megacomplex that consist both photosystems. The co-migration of photosystem I and photosystem II in this complex indicates that the two photosystems are interacting in the margins of the grana stacks and that the interaction, most importantly, is dependent on LHCII phosphorylation. We conclude that the composition of pigment-protein megacomplexes in the non-appressed thylakoid membranes undergoes redox-dependent changes to maintain the excitation balance between PSII and PSI upon chances in light intensity.

Poster S8.45

Electron flow from PSII to PSI under high light is regulated by PGR5 but not by PsbS

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In plant chloroplast, the absence of protein PROTON GRADIENT REGULATION 5 (PGR5) prevents the formation of trans-thylakoid proton gradient (ΔpH) and consequently also the thermal dissipation of excess excitation energy (NPQ). Likewise, *npq4* mutant lacking PsbS protein, a central component of NPQ, is incapable of inducing the ΔpH-dependent NPQ. The *pgr5* mutant is not only deficient in induction of sufficient NPQ but also unable to oxidize P700 in high light. This results from an uncontrolled electron flow towards PSI, which has been proposed to be caused by a poor control of electron flow via the Cytochrome *b6f* complex and by the lack of PSII down-regulation by NPQ. To clarify, whether NPQ really is a component of such regulation of electron flow from PSII to PSI under high light, the two NPQ mutants, *pgr5* and *npq4*, were characterized. It is shown that the *npq4* mutant, despite its highly reduced PQ pool, does not inhibit but rather enhances the oxidation of P700 in high light as compared to wild type. This clearly demonstrates that the control of electron flow from PSII to PSI cannot even partially be assigned to the down-regulation of PSII by NPQ, but instead, the control appears to take place solely at the state of Cytochrome *b6f* complex. Moreover, it is shown that the *pgr5* mutant can induce NPQ in very high light, while still remaining deficient in P700 oxidation. These results challenge the view that NPQ, induced by PGR5-dependent cyclic electron transfer, would have a key role in regulating the electron transfer from PSII to PSI. Instead, the results are in line with the recent suggestion that both PSII and PSI function under the same light harvesting machinery regulated by ΔpH and the PsbS protein (Tikkanen & Aro 2014, Grieco et al. 2015).

Poster S8.46

Initial disorder in structure and functions of Photosystem II in radish plants under magnesium deficiency

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Mineral deficiencies in plants are frequently diagnosed based on visible symptoms or by using invasive methods such as chemical analysis. It is difficult to draw inference by the visible symptoms, and invasive techniques are time consuming and relatively costly. Magnesium plays molecular and physiological roles in plants, as it is a component of the chlorophyll molecule, a structural stabilizer for various nucleotides and a cofactor for many processes associated with phosphorylation and dephosphorylation. Radish plants were grown in hydroponic system with Hoagland solution in two sets of conditions (in magnesium deficient solution and nutrient enriched solution in a Phytotron). The photosynthetic apparatus efficiency, functioning and structure was assessed by the measurements of chlorophyll *a* fluorescence measurements, chlorophyll and flavonoid content. Changes in photosynthetic structure were also observed by electron and confocal microscopy. Net photosynthetic rate *in vivo* was measured basing on atmospheric CO_2 assimilation, using an infrared gas analyzer (IRGA). Some growth parameters also were assessed. Obtained data suggested that, there was no significant deviation (α=0.05) in chlorophyll and flavonoid content between magnesium deficient and control plants. In plants growing under magnesium deficiency dry weight of leaves increased as compared to control samples leaves. Magnesium deficiency symptoms were expressed as accumulation of starch in the leaves, which could be connected with early reductions in plant growth and decrease of carbohydrates. Plant gas exchange in control samples and magnesium deficiency samples showed varied results. We suggest some parameters related photosynthetic apparatus efficiency to for early detection of magnesium in radish plant.

Poster S8.47

Elevated temperatures facilitate rapid light-dependent accumulation of zeaxanthin in *Picea abies* needles but not in *Arabidopsis thaliana* leaves

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The dynamics and maximal degree of violaxanthin (V) conversion to zeaxanthin (Z) via anteraxanthin (A) differ not only among species, but also in one plant species grown under the different light conditions. Especially, in Norway spruce acclimated to high-light a rapid accumulation of Z together with induction of maximal nonradiative dissipation of excess excitation energy within PS II (NRD) contribute to efficient PS II photoprotection. In order to elucidate presumed interspecific variability of Z-dependent photoprotection at elevated temperatures, the dynamics of violaxanthin (V) de-epoxidation under different illumination regimes was studied together with chlorophyll *a* fluorescence transients (monitoring particularly the induction of NRD) in *Picea abies* seedlings and *Arabidopsis thaliana* leaves pre-acclimated to temperatures ranging from 20 to 40°C. Whereas in spruce seedlings the rapid phase of V deepoxidation (induced by either 10 s illumination or 10 light-pulses of 1 s duration at 1 min interval, 10x1 s) was gradually stimulated upon increasing temperatures, in *A. thaliana* leaves considerable acceleration of V deepoxidation occurred just at 40°C. Moreover, only in spruce seedlings Z was accumulated during 10x1 s illumination at 32°C and higher temperature. In agreement with these results, elevated temperatures stimulated rapid formation of Z-dependent NRD induced by 1 s light pulses only in spruce seedlings. Analysis of initial phase of V deepoxidation during illumination shorter than 10 s indicated a saturation of VDE capacity in spruce needles at elevated temperatures. These new findings about extremely rapid V deepoxidation and NRD induction observed in spruce seedlings at elevated temperatures are discussed with respect to specific fatty acid composition of spruce thylakoid membrane lipids.

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Poster S8.48

Non-photochemical fluorecence quenching in the pigment apparatus of cyanobacteria

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Using the known 3D structure of interacting proteins and computational modeling, we arrived at a rational spatial model of the orange carotenoid protein (OCP) and phycobilisome (PBS) interaction in the non-photochemical fluorescence quenching (NPQ). The site of interaction is formed by the central cavity between the N- and C-protein domains of the OCP monomer and the characteristic external tip of the phycobilin-containing domain (PB) and its folded loop of the core-membrane linker L_{CM}–polypeptide within the PBS core. The same central protein cavity was shown to be also the site of interaction of the OCP and fluorescence recovery protein (FRP) that participates in the reverse reaction interaction. The revealed geometry of the OCP to the PBL_{CM} attachment is believed to be the most advantageous one as the L_{CM}, being the major terminal PBS-fluorescence emitter, gathers, before quenching by OCP, the energy from most other phycobilin chromophores of the PBS. The distance between centers of mass of the OCP carotenoid 3'-hydroxyechinenone (hECN) and the adjacent phycobilin chromophore of the PBL_{CM} was determined to be 24.7 Å. Under the dipole-dipole approximation, from the point of view of the determined mutual orientation and the values of the transition dipole moments and spectral characteristics of interaction of neighboring carotenoid and phycobilin chromophores, the time of the direct energy transfer from the phycobilin of PBL_{CM} to the S_1 excited state of hedroxyechinenone-carotenoid of the OCP was semiempirically calculated to be 36 ps, which corresponds to the known experimental data and implies the OCP is a very efficient energy quencher. The complete scheme of OCP and PBS interaction that includes the mechanism of direct light-dependent reaction of quenching and the reverse light-independent process of the PBS fluorescence recovery is proposed.

Poster S8.49

Electrical signals as potential mechanism of photosynthesis regulation in higher plants

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Regulation of photosynthetic processes under different environmental conditions is the widely known phenomenon. In particular, changes in photosynthesis are observed in intact part of plants after local action of stressors. Electrical signals (ESs) are potential mechanism of connection between stimulated and intact zones after local stressors action. In higher plants main ESs are variation potential (VP) and action potential (AP), induced by damaging and non-damaging stimuli, respectively. It has been shown that ESs induce short-term photosynthetic inactivation (<15 min) which depends on amplitude and propagation parameters of electrical signals. Possibly, ESs participate in development of long-term photosynthetic inactivation (tens of minutes – several hours), but hormone mechanism of the signal transduction is considered, too. Influence of VP on photosynthesis is connected with plasmolemma H^+-ATPase inactivation which induces intracellular acidification and extracellular alkalization. AP influence on photosynthesis is connected with Ca^{2+} influx in Chara algae but mechanism of the response in higher plants requires further investigation. Both AP and VP inactivate photosynthesis dark stage that can be connected with decrease of CO_2 flow into cell. Changes in Calvin cycle enzymes activity, CO_2:HCO_3^- ratio and aquaporins conductivity are possible mechanisms of the dark stage inactivation. Also, ESs directly influence photosynthesis light stage that can be connected with increase of fluorescence non-photochemical quenching and/or changes in ferredoxin-$NADP^+$ reductase localization. ESs-induced photosynthetic responses influence physiological state of higher plants. In particular, ESs increase ATP content in plants that is mainly connected with photosynthetic responses. It is probable that final role of ESs-induced photosynthetic responses is change in photosynthetic machinery resistance to environmental factors and plant adaptation to stressors.

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Poster S8.50

Natural variation in tocochromanols content in *Arabidopsis thaliana* accessions – the effect of temperature and light intensity

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Arabidopsis thaliana accessions originating from variety of climate conditions were grown under the controlled circumstances of different light intensity and temperature. The accessions were analysed for the prenyllipids content and composition, as well as the expression of the genes involved in the tocochromanol biosynthesis (*vte1-5*). It was found that the applied conditions did not strongly affect total tocochromanols content and there was no apparent correlation of the tocochromanol content with the origin of the accessions. However, the presented results indicate that the temperature, more than the light intensity, affects the expression of the *vte1-5* genes and the content of some prenyllipids. An interesting observation was that under the low temperature of growth, the PC-OH to PC ratio was considerably increased regardless of the light intensity in most of the accessions. PC-OH is known to be formed as a result of singlet oxygen stress, therefore this observation indicates that the singlet oxygen production is enhanced under the low temperature. Unexpectedly, the highest increase in the PC-OH/PC ratio was found for accessions originating from the cold climate (Shigu, Krazo-1, Lov-5), even though such plants could be expected to be more resistant to low temperature stress.

Poster S8.51

Role of phosphatidylglycerol in cyanobacterial cells

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Cyanobacteria, the ancestor of higher plant plastids, are good model organisms for investigation of photosynthetic processes and chloroplast development. Nowadays cyanobacteria are in the focus of intensive research because of their potential application in biotechnology. For most biotechnological applications production of high cyanobacterial biomass is needed. Cyanobacterial reproduction depends on cell division processes and, as being photosynthetic bacteria, on the photosynthetic activity. The phosphatidylglycerol (PG) is the only phospholipid component of the cyanobacterial cells. We have demonstrated by the characterization of PG-deficient cyanobacterial mutant strains that this phospholipid has important roles in the structure and function of photosynthetic complexes. Furthermore, our proteomic investigation of PG-supplemented and PG-depleted cells revealed 80 PG-regulated proteins involved in various cellular processes including photosynthesis, respiration, metabolism, transport, transcription and translation, indicating the importance of this lipid species in various cyanobacterial cellular processes. Depletion of PG also resulted in morphological changes and arrested the cell division, suggesting its further role in cyanobacterial cell division. We followed the division ring formation in PG deficient cyanobacterial cells during PG depletion to reveal the role of PG in the cell division.

Poster S8.52

The effect of lanthanides on photosynthesis and cell proliferation

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Lanthanides are biologically non-essential elements with wide applications in technology and industry. Although non-essential, lanthanides are used to produce beneficial effects in plants.

We studied the effect of lanthanides on the growth, chlorophyll content and photosynthetic rate of the green alga *Desmodesmus quadricauda* at different light intensities. The localization of lanthanides was performed by fluorescence microscopy after staining with Fluo-4 dye. Extracts from lanthanides treated algae were tested for inhibiting effect on keratinocytes and melanoma cell lines.

Both lanthanum and neodymium enhanced the growth of *Desmodesmus quadricauda* at all light intensities except the lowest one. They enhanced the photosynthetic rate only at the lowest light intensity. Lanthanum and neodymium treated cells had higher chlorophyll content. While La and Gd were found in the cytoplasm, Nd and Ce were localized in the chloroplast. Extracts from lanthanides treated algae inhibited the growth of keratinocytes and melanoma cells and induced them to apoptosis.

The work was supported by the National Programme of Sustainability I, ID: LO1416.

Section 9: Systems Biology of Photosynthesis:
Integration of Genomic, Proteomic, Metabolomic and Bioinformatic Studies

Poster S9.1

Proteome analysis of enriched heterocysts from three hydrogenase mutants from *Anabena* sp. PCC 7120

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Cyanobacteria are oxygenic photosynthetic prokaryotes and play a crucial role in the Earth's carbon and nitrogen cycles. The photoautotrophic cyanobacterium *Anabaena* sp. PCC 7120 has the ability to fix atmospheric nitrogen in the heterocysts and produce hydrogen as byproduct through the enzyme nitrogenase. In order to improve the hydrogen production, mutants from *Anabaena* sp. PCC 7120 were constructed by inactivation of the uptake hydrogenase (*ΔhupL*), the bidirectional hydrogenase (*ΔhoxH*), and both genes (*ΔhupL*/*ΔhoxH*).

In this study we have investigated the sarcosine-soluble fraction (SSF) and the sarcosine–insoluble fraction (SIF) of heterocysts proteome from *Anabena* PCC 7120, by 2D gel electrophoresis and mass spectrometry. We have identified 110 proteins from both fractions and 24 of them were common. The SSF was found to contain the Photosystem I, Photosystem II, phycobilisomes and ATP-synthase subunits. These proteins are involved in energy metabolism. Further we identified proteins which participate in the carbonhydrate metabolism. The SIF is enriched in outer membrane proteins significant amount of proteins contribute to the process of photosynthesis and unique proteins which expressed in stress conditions, such as Alr2887 protein. As a whole, we have identified proteins which contribute to the differentiation of vegetative cells to heterocysts. Among mutants, we noticed the expression of proteins, resulting that each strain of the bacterium has developed different mechanisms to adapt to the nitrogen deprivation. Basic proteins of metabolic pathways for the survival of heterocyst were expressed to a larger extent for the strain which is not genetically modified, while for the other mutants we could observed the expression of unique proteins.

Poster S9.2

In silico modelling of photosynthetic electron transport

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We have developed a computer model of the complex network of electron transport components in the thylakoid membrane of photosynthetic organisms. The model provides an excellent tool to simulate electron transport processes in a wide range of conditions, and can be used to perform *in silico* experiments, whose predictions can be verified by measuring the kinetics of various electron transport components. The model was used to study the interpretation of transition probabilities in the water oxidizing complex. Our results show that the well-known period-four oscillation of oxygen evolution, as well as of the individual S-states can be described by the equilibrium of forward and backward electron transport reactions without the need for specific "miss" and "double-hit" events. We have also used our *in silico* photosynthesis model to interpret the recently described wave phenomenon in the relaxation of flash-induced Chl fluorescence in cyanobacteria and other microalgae. Our results show that the fluorescence wave reflects changes in the redox level of the PQ pool, which are caused by the imbalance of PSII and PSI electron transport and the feedback of electrons from stromal components to the PQ pool via the NDH-1 complex [1]. This phenomenon provides an excellent tool to study the interaction of photosynthetic and metabolic electron transport and helps to identify the electron transport components, which are involved NDH-1 mediated cyclic electron flow. The model represents a systems biology approach to electron transport studies and has a large potential for optimizing electron transport pathways for biotech purposes.

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Section 10: Photosynthesis Education

Poster S10.2

Is chlorophyll *e* 15[1]-OH-lactone chlorophyll *a* or chlorophyllide *a*?

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Chlorophyll (Chl) *d* is a major pigment in *Acaryochloris marina*, but the biosynthetic pathway of Chl *d* has not yet been clarified [1, 2]. We serendipitously came across the formation of Chl *d* from Chl *a* with a proteolytic and thiol protease, papain (EC 3.4.22.2) in several aqueous organic solvents at room temperature in the dark [3–5]. Bromelain (EC 3.4.22.4) is also a proteolytic and thiol protease present in pineapple. In this paper, in order to clarify the conversion mechanism *in vitro* and the origin of Chl *a* → *d* conversion in nature, we incubated Chl *a* with grated pineapple in acetone/H_2O (10/1, v/v) at 303K in the dark. The expected conversion of Chl *a* → *d* was not observed, but a new peak was detected, and the pigment was found to be 15[1]-OH-lactone Chl *a* [6] Interestingly, absorption spectrum of 15[1]-OH-lactone Chl *a* is very similar to that of Chl *e* found in *Tribonema bombycinum* [7, 8] and *Vaucheria hamata* [9]. Chlorophyll *e* was also reported to resemble Chl *c* with respect to its adsorbality in columns of powdered sugar [8]. If so, Chl *e* might be 15[1]-OH-lactone Chlide *a*.

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Section 11: Emerging Techniques for Studying Photosynthesis

Poster S11.3

Compartment Markers for Plant Science

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Chloroplasts are ideal hosts for transgenic expression. Introduced sequences undergo homologous recombination to integrate into plastid genomes and are thereafter maternally inherited, minimizing pleiotropic effects and containment risks. Production of heterologously expressed proteins within chloroplasts also limits contamination of the cytosol and cytotoxicity toward plant cells and tissues.

Confirmation of chloroplast targeting and assessment of expression levels can be achieved by tracking introduced proteins and measuring their abundance relative to marker proteins. Fractionation of cellular compartments such as cytosol, mitochondria and chloroplasts, and further sub-fractionation into thylakoids, stroma and lumen can be followed by quantitative detection of both introduced and compartment marker proteins.

Agrisera and Environmental Proteomics have created a comprehensive set of antibodies for detection and tracking of protein compartment markers in plants and algae. Several of these are fully quantitative, allowing normalization of expression levels of heterologous proteins. We demonstrate the fractionation of chloroplasts from cytosol and other organelles with antibodies toward RbcL (chloroplast stroma), PsbA or PsbD (chloroplast thylakoid), SPS (cytosol), PEPC (cytosol, mesophyll enriched in C4 plants), AOX (mitochondrial), and VDAC (vacuole). We also show the enrichment of Rubisco and PEP carboxylase using quantitative RbcL and and PEPC markers following fractionation of mesophyll and bundle-sheath cells from maize.

Poster S11.4

Critical assessment of protein cross-linking – a modified model of the interaction between photosystem II and Psb27

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Photosystem II (PSII) is a large multi subunit protein complex that catalyzes the light-driven oxidation of water. The detailed crystal structure of active cyanobacterial PSII [Umena, Y. et al., 2011, Nature 473:55–60] resembles the mature complex, but the PSII lifecycle is characterized by the presence of intermediate complexes that are related to its assembly and repair. Different protein factors bind to the luminal or cytoplasmic surface of PSII and cause structural variations of the PSII species. However, these intermediate PSII complexes are difficult to analyze due to their inherent instability or low abundance. In order to determine the binding positions of auxiliary proteins on the luminal surface of PSII, we developed a method based on surface plasmon resonance (SPR) spectroscopy and chemical cross-linking in combination with mass-spectrometry (CX-MS).

The PSII crystal structure of the mature complex was used for validation of the CX-MS analysis pipeline, as shown previously for the SPR based approach [Cormann et al. 2014, Front Plant Sci 5:595]. The CX-MS results clearly show that reliable structural constrains are only obtained if the assignment of cross-linked peptides is further confirmed by use of isotopically coded cross-linkers in order to avoid false positive results. Interestingly, the majority of identified PSII cross-links involved at least one protein N-terminus. These are the most flexible parts in the complex and therefore they are often not resolved even in the high resolution PSII crystal structure. In contrast, we never observed cross-links in protein-protein interfaces that are hidden in the complex.

The combined approach of SPR spectroscopy based mapping and CX-MS was used to re-evaluate the binding position of the auxiliary PSII factor Psb27. The results support binding of Psb27 close to CP43 and the D1 C-terminus at a position that is occupied by PsbV in the mature complex rather than binding to D2/D1 a-loop region at the opposite side of CP43.

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