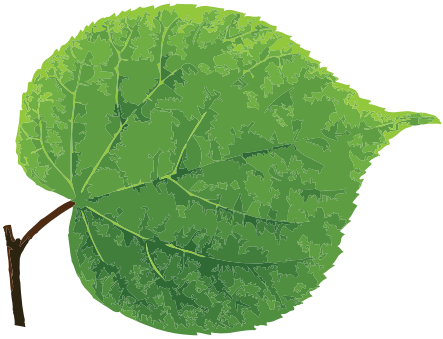

10th International Conference

Photosynthesis and Hydrogen Energy Research for Sustainability

in honor of Kimiyuki Satoh, Tingyun Kuang, Cesare Marchetti, and Anthony Larkum

June 23 – 28, 2019
Saint Petersburg, Russia

ABSTRACTS AND PROGRAMME

Institute of basic biological problems
of the Russian Academy of Sciences
Russian Society for Photobiology
Komarov Botanical Institute of the Russian Academy of Sciences

10th International Conference

“Photosynthesis and Hydrogen Energy Research for Sustainability-2019”

in honor of Kimiyuki Satoh, Tingyun Kuang, Cesare Marchetti, and Anthony Larkum

June 23 – 28, 2019
Saint Petersburg, Russia

Abstracts and Programme

St. Petersburg – 2019

10th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainability-2019" in honor of Kimiyuki Satoh, Tingyun Kuang, Cesare Marchetti, and Anthony Larkum"

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This book contains the abstracts of the lectures and poster presentations at the 10th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability-2019: in honor of Kimiyuki Satoh, Tingyun Kuang, Cesare Marchetti, and Anthony Larkum", held from June 23 to June 28, 2019 at the Palace of Beloselsky-Belozersky in Saint Petersburg. Both the experimental and theoretical aspects of Photosynthesis and Biohydrogen production are covered. Topics range from the primary process of electron transfer and energy bioconversion to the physiology of photosynthesis, as well as the applied aspects of hydrogen production. Special attention is given to discussion of the structural organization of photosynthetic reaction centers, abiotics stress effects on photosynthesis, and mechanisms of hydrogen production. We expect the content of this publication to be of broad interest to all researchers, teachers, and students interested in photosynthesis and/or biohydrogen production.

The 10th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainability-2019" is supported by the Ministry of Education and Science of the Russian Federation, grant No. 075-02-2019-1443.

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Additional information is available on our website: https://icprs.ru

WELCOME!

Dear Colleagues!

You are mostly welcome to 10th International Conference on Photosynthesis and Hydrogen Energy Research for Sustainability-2019.

The Conference will be held from June 23 to June 28, 2019, at the Beloselsky-Belozersly Palace.

The plenary lectures will be given by leading scientists in the fields of photosynthesis and hydrogen energy. Furthermore, we will be honoring four distinguished scientists - Kimiyuki Satoh, Tingyun Kuang, Cesare Marchetti and Anthony Larkum - , who have made pioneering contributions to photosynthesis and hydrogen energy research.

This Conference is a great occasion for discussions of previous, present, and future research on photosynthesis from molecular to global, biohydrogen production, from mechanisms to applied aspects, and to meet researchers of photosynthesis and biohydrogen from around the world. The Meeting provides a forum for students, postdoctoral 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 the Conference range widely. The photosynthetic part includes presentations on primary processes of photosynthesis, structure, function, and biogenesis of photosynthetic machinery, photosystems 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 hydrogen research part includes mostly topics of biohydrogen production, storage, and bioconversion of it into electricity.

The multidisciplinary nature of the conference is obvious from the list of topics. In total, more than 200 participants are expected.

Second half of June is the best time to visit the city of Saint Petersburg and to enjoy its world-famous museums and architecture in the mystic twilight of the White Nights.

We are looking forward to seeing you in Saint Petersburg!

On behalf of the organizing committees,
Suleyman I. Allakhverdiev
Anatoly Tsygankov
Maria Borisova-Mubarakshina
Olga Voitsekhovskaja

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Graffiti L Hostel
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+7 800 700-38-60,
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Old City (Blue Sky) Hotel
146, Nevsky pr.
+7 (812) 600-79-01
+7 (921) 864-64-41

Arka Hotel
17, Bolshaya Konyshennaya str.
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- From 300 rub

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- From 900 rub

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- From 900 rub

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- From 500 rub

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- From 1000 rub

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- From 1000 rub

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- 59, Liteyniy pr.
- From 1000 rub

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- From 1000 rub

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- 8 minutes away
- 51, Nevsky pr.
- From 700 rub

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Burger King
- 10 minutes away
- 18, Nevsky pr.
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Jager Resto-Bar
- 10 minutes away
- 84-86, Nevsky pr.
- From 700 rub

Tokyo-City
- 11 minutes away
- 90-92, Nevsky pr.
- From 600 rub

Manneken Pis
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Diner
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- 5 minutes away
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- From 3000 rub

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- From 700 rub

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- 7 minutes away
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- From 1000 rub

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- 7 minutes away
- 28, Karavannaya str.
- From 500 rub

Пельмения (Pelmenia)
- 6 minutes away
- 25, Fontanka emb.
- From 500 rub

Теремок (Teremok)
- 7 minutes away
- 60, Nevsky pr.
- From 350 rub

Villa Aston
- 10 minutes away
- 5, Vladimirsky pr.
- From 700 rub

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SCHEDULE: PHOTOSYNTHESIS RESEARCH FOR SUSTAINABILITY-2019

22 JUNE, 2019

19:00 – 21:00 REGISTRATION.
HOTEL "AGNI" (90-92, NEVSKY PR.)

23 JUNE, 2019

14:00 – 18:00 REGISTRATION.
BELOSELSKY-BELOZERSKY PALACE (41, NEVSKY PR.)

15:00 – 15:50 OPENING CEREMONY (MIRROR HALL)

15:50 – 16:00 **Jian-Ren Shen** Introducing Kimiyuki Satoh

16:00 – 16:30 **Kimiyuki Satoh** Sixty years since the decease of Robert Emerson: History of uncovering the chemical entities laying the basis for his great findings

16:30 – 17:10 **Guangye Han** Photosynthesis Research in Tingyun Kuang's Laboratory

17:10 – 17:50 **Cesare Marchetti** Self sinking capsules, a final solution to radioactive disposal

17:50 – 18:00 **Julian Eaton-Rye** Introducing Anthony Larkum

18:00 – 18:30 **Anthony Larkum** Stromatolites old and new

18:30 – 18:45 Meeting of participants in front of the Beloselsky-Belozersky palace to go to the "get together evening" in Komarov Botanical Institute of the Russian Academy of Sciences. The buses will be provided.

19:30 – 22:00 GET TOGETHER EVENING AND EXCURSIONS TO THE GREENHOUSES OF THE BOTANICAL GARDEN (TROPICAL PATHWAY AND VICTORIA HOUSE)
2, PROFESSORA POPOVA STR.

24 JUNE, 2019

09:10 – 18:00 Registration
Beloselsky-Belozersky Palace (41, Nevsky pr.)

Plenary lectures (Mirror Hall)

09:10 – 09:50 **Jian-Ren Shen** Structural biological studies on photosynthetic systems

09:50 – 10:30 **Mahdi Najafpour** Najafpour-Feizi reaction under water-oxidation conditions

10:30 – 10:45 coffee break

10:45 – 11:25 **Tatsuya Tomo** Analysis of energy transfer pathway in cyanobacterium during the process of the chlorophyll *f* accumulation

Section 1.1 (Mirror Hall)

11:30 – 11:55 **Chikahiro Miyake** P700 oxidation suppresses the production of reactive oxygen species (ROS) in photosystem I and induces ferredoxin-independent cyclic electron flow within PS I

11:55 – 12:20 **Alexey Semenov** Electron transfer in Photosystem I upon limited protein conformational mobility

12:20 – 12:45 **Kentaro Ifuku** Thylakoid lumenal proteins supporting the assembly and function of photosynthetic supercomplexes

Sections 2.1 – 2.9 (Oak Hall)

11:30 – 11:55 **Dhruv Shah** Future prospect and challenges in production of hydrogen

11:55 – 12:20 **Naman Tejaswi** Prospects and challenges of mainstreaming hydrogen-fueled automobiles

12:20 – 12:45 **Bolatkhan Zayadan** Perspectives of cyanobacterial strains for biodisiel and biohydrogen production

12:45 – 14:00 Time for lunch

Plenary lectures (Mirror Hall)

14:00 – 14:40 **Andrey Rubin** Biophysics of the primary processes of photosynthesis

14:40 – 15:20 **Sumanta Kumar Padhi** Electro-catalytic activity for H_2 evolution by a polypyridyl copper complex

15:20 – 15:35 coffee break

Talks of young speakers (Mirror Hall)

15:35 – 15:50 **Zahra Abdi** Water oxidation by Vitamin B12: Questions and challenges

15:50 – 16:05 **Daisuke Takagi** Phosphorus toxicity decreases both electron sink activity and anti-oxidative activity in rice leaves

16:05 – 16:20 **Eugene Maksimov** Temperature sensor derived from the photoactive Orange Carotenoid Protein

16:20 – 16:35 **Valerya Dmitrieva** Photosynthesis controls plasmodesmata permeability in *Arabidopsis thaliana*

16:35 – 16:50 **Sasan Aliniaeifard** γ-aminobutyric acid confers cadmium tolerance in maize plant by concerted regulation of polyamines metabolism and antioxidant defense system

16:50 – 17:05 **Jack Forsman** Hydrophobic interactions between the D1 and PsbT subunits of Photosystem II stabilize the iron-quinone acceptor complex

17:05 – 17:20 **Kseniya Nikerova** The increase in the activity of AOS enzymes is an indicator of abnormal growth of woody plants, which differ in the heartwood/sapwood ratio

Sections 2.1 – 2.9 (Oak Hall)

15:35 – 16:00 **Gadi Schuster** Harnessing photosynthesis to produce electricity and hydrogen using spinach thylakoids and live cyanobacteria

16:00 – 16:25 **Taras Antal** Photosynthetic hydrogen production as acclimation mechanism in green algae

16:25 – 16:50 **Szilvia Z. Toth** Keeping the Calvin-Benson cycle inactive is the key to sustained and photoautotrophic H_2 production in green algae

16:50 – 17:15 **Sergey Kosourov** Understanding the mechanism of H_2 photoproduction in the pulse-illuminated green alga, *Chlamydomonas reinhardtii*

17:15 – 17:40 **Fang Huang** Potential for sustained H_2 photoproduction in the *Chlamydomonas* mutant hpm91

17:20 – 19:00 Poster viewing

25 JUNE, 2019

09:10 – 18:00 Registration
Beloselsky-Belozersky Palace (41, Nevsky pr.)

Plenary lectures (Mirror Hall)

09:10 – 09:50 **William Cramer** Structure-function of the cytochrome b6f complex: (i) Lipid-protein interactions; (ii) Structure-based control of the electron transport rate; (iii) Arabidopsis

09:50 – 10:30 **Matthias Rögner** Remodeling of photosynthetic electron transport in *Synechocystis* sp. PCC 6803 for future hydrogen production from water

10:30 – 10:45 coffee break

Sections 1.2 and 1.3 (Mirror Hall)

10:45 – 11:10 **Jörg Pieper** Vibrational dynamics and excitation energy transfer in LHC II

11:10 – 11:35 **Seiji Akimoto** Modification of light-harvesting and energy-transfer processes in diatoms under different light conditions

11:35 – 12:00 **Marina Kozuleva** The role of oxygen in evolution of photosystem I

12:00 – 12:25 **Lyudmila Vasilieva** Features of bacteriochlorophylls axial ligation in the photosynthetic reaction center of purple bacteria

Section 1.9 (Oak Hall)

10:45 – 11:10 **Natalia Belyaeva** Evaluation of the thylakoid membrane processes in *Synechocystis* sp. PCC 6803 by modeling fluorescence induction on the time scale from microseconds to several minutes

11:10 – 11:35 **Olga Voitsekhovkaja** The good and the bad of lacking chlorophyll *b*: photosynthesis and growth in barley *chlorina f2*^{3613} mutant

11:35 – 12:00 **Vasily Ptushenko** Apparent lack of stomatal growth irradiance response in four *Tradescantia* species

12:00 – 12:25 **Vladimir Chikov** The communication of chloroplasts with the stomatal leaf apparatus in the regulation of photosynthesis

12:25 – 14:00 Time for lunch

Plenary lectures (Mirror Hall)

14:00 – 14:40 **Julian Eaton-Rye** Mutation of D1 and D2 residues associated with bicarbonate and bound waters on the acceptor side of PS II impair the quinone-Fe-acceptor complex

14:40 – 15:20 **Marc Nowaczyk** Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer

15:20 – 16:00 **Nathan Nelson** Photosynthetic reaction centers – Robustness with increased complexity

16:00 – 16:15 coffee break

Sections 1.4 – 1.6 (Mirror Hall)

16:15 – 16:40 **Yuki Kato** FTIR study on the water oxidation reaction in photosystem II microcrystals

16:40 – 17:05 **Franz-Josef Schmitt** How different wavelengths of light change energy transfer and trapping in far red light adapted cells of the Chl *f* containing cyanaobacterium *Halomicronema hongdechloris*

17:05 – 17:30 **Kevin J. Sheridan** Characterisation of a phylogenetically distinct group of D1 proteins from cyanobacteria

17:30 – 17:55 **Roman Pishchalnikov** Relationship between pigment-protein structure and dynamics of the energy transfer and trapping in cyanobacterial PS I

Section 1.9 (Oak Hall)

16:15 – 16:40 **Rajagopal Subramanyam** Expression of LHCSR3 induces change in supercomplexes of thylakoid membranes under PEG stress from *Chlamydomonas reinhardtii*

16:40 – 17:05 **Natalia Rudenko** Carbonic anhydrases in chloroplasts of C3 higher plants

17:05 – 17:30 **Galina Smolikova** Embryonic photochemical activity is crucial for the seed maturation

17:30 – 17:55 **Liudmila Kabashnikova** Effect of β-1,3-glucane on the structure and function of photosynthetic apparatus and oxidative status of tomato leaves under fusarium wilt

17:55 – 19:00 Poster viewing

26 JUNE, 2019

09:10 – 17:00 Registration
Beloselsky-Belozersky Palace (41, Nevsky pr.)

Plenary lectures (Mirror Hall)

09:10 – 09:50 **Govindjee** On finding ways to make plants, especially rice, more efficient: A perspective

09:50 – 10:20 **Anatoly Tsygankov** Hydrogen electrode with HydSL hydrogenase from Thiocapsa roseopersicina in fuel cell

10:20 – 10:35 coffee break

Section 1.8 (Mirror Hall)

10:35 – 11:00 **Imre Vass** Photosynthetic electron transport *in silico*

11:00 – 11:25 **Kostas Stamatakis** *Synechococcus* sp. PCC7942: A cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions

11:25 – 11:50 **Rachel Nechushtai** Artificial photosynthesis with electron acceptor/photosensitizer-aptamer conjugates

11:50 – 12:40 **Agrisera Workshop** "Tips and tricks of antibody production and western blot. How to obtain good results." Dr. Joanna Porankiewicz Asplund, Agrisera AB

Section 1.9 (Oak Hall)

10:35 – 11:00 **Tatyana Savchenko** Comparative analysis of corticular photosynthesis in grapevine varieties contrasting in freeze tolerance

11:00 – 11:25 **Boris Ivanov** Production of hydrogen peroxide within thylakoid membrane with involvement of the plastoquinone pool, and the role of this production for signalling

11:25 – 11:50 **Gregory Pozhvanov** Microgravity modelling by 3D-clinorotation affects actin cytoskeleton, ROS production, photosynthesis and metabolome of *Arabidopsis* plants

11:50 – 12:15 **Hitoshi Nakamoto** Novel molecular chaperones in cyanobacteria: groEL and clpB paralogs

12:40 – 13:50 Time for lunch

Plenary lectures (Mirror Hall)

13:50 – 14:30 **Giovanni Venturoli** Room temperature immobilization of photosynthetic reaction centers in amorphous matrices: testing the role of protein dynamics in electron transfer

14:30 – 15:10 **Dmitry Dunikov** Hydrogen energy technologies: resent developments and prospects in Russia

15:10 – 15:50 **Mats Hansson** Employing barley mutants to dissect a chlorophyll biosynthetic enzyme

15:50 – 16:10 coffee break and free time

The meeting of the committee on the topic of selection of the best oral and poster presentations of young scientists. (Oak Hall)

Committee members: Marian Brestic, Marc Nowaczyk, Seiji Akimoto, Anatoly Tsygankov, Rajagopal Subramanyam, Suleyman Allakhverdiev, Tatsuya Tomo, Julian Eaton-Rye, Mahdi Najafpour

16:10 – 16:50 **Giuseppe Spazzafumo** Comparison of different system layouts to generate a substitute of natural gas from biomass and electrolytic hydrogen

16:50 – 17:10 **Talk of the gold sponsor of the conference Sergey Antsypovich LabInstruments Company (Mirror Hall)**

Section 1.11 (Mirror Hall)

17:15 – 17:40 **Natalya Kaznina** Photosynthetic activity of barley plants under zinc deficient and excess stress conditions

17:40 – 18:05 **Elena Tyutereva** Potassium and glucose as putative signals regulating processes in shoots and roots

Section 1.9 (Oak Hall)

17:15 – 17:40 **Eugene Lysenko** Specificity of Cd, Cu, and Fe action on cation contents in chloroplasts and activities of PS II and PS I

17:40 – 18:05 **Tatiana Pluysnina** Model of microalgae *Chlamidomonas reinhardtii* adaptation to sulfur starvation

18:05 – 18:45 Selected talks for best posters of young scientists and summing up the competition for best oral and posters presentations of young scientists. Awarding best talk/best poster prizes for early career researchers by the committee and by the AGRISERA company (Mirror Hall)

18:45 – 19:00 Closing Ceremony (Mirror Hall) and Conference Photograph on the Stairs of the Beloselsky-Belozersky palace.

Banquet
Jazz restaurant White Night Music Joint.
40, Fontanka emb.

27 JUNE, 2019

Excursion day

28 JUNE, 2019

Departure

Part 1.
Photosynthesis Research for Sustainability

Section 1.1: Primary Processes of Photosynthesis

Lecture

Sixty years since the decease of Robert Emerson: History of uncovering the chemical entities laying the basis for his great findings

Kimiyuki Satoh

Okayama University, Japan

Lecture

Analysis of energy transfer pathway in cyanobacterium during the process of the chlorophyll *f* accumulation

Toshiyuki Shonoda[1], Seiji Akimoto[2], Min Chen[3], Suleyman I. Allakhverdiev[4], Tatsuya Tomo[1*]

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2 – Graduate School of Science, Kobe University, Kobe, Japan
2 –School of Life and Environmental Sciences, University of Sydney, Sydney, Australia
4 – K.A. Timiryazev Institute of Plant Physiology, RAS, Moscow; Institute of Basic Biological Problems, RAS, Pushchino, Russia
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Chlorophylls (Chls) are photosynthetic pigments functioning in light-harvesting, energy-transfer, and electron transport. Oxygenic photosynthetic organism uses Chl *a*, *b*, and *c* as antenna pigments. Chl *a* also acts as an electron transfer component. In 2010, Chl *f* containing cyanobacterium was found in the living stromatolite in western Australia [1]. The Chl content of this cyanobacterium depends on the cultivation light. Chl *f* is synthesized only in cells grown under far-red (FR) light (>700 nm). The mechanism of energy transfer in Chl *f* has recently been reported [2–5]. However, establishment of energy transfer pathway associated with Chl *f* is not known in photosystems in their accumulation process. Therefore, we cultured this cyanobacterium under white to FR light transition. We analyzed steady and time-resolved fluorescence spectra for investigations of cells in the accumulation process of Chl *f*. We will discuss characteristics of energy transfer within Chl *f* containing cyanobacterium.

The reported study was funded by JSPS and RFBR according to the research project № 19-54-50002.

References:
1. Chen et al. (2010) *Science*, 329, pp.1318–1319
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Lecture

P700 oxidation suppresses the production of reactive oxygen species (ROS) in photosystem I and induces ferredoxin-independent cyclic electron flow within PS I

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O_2-evolving photosynthetic organisms oxidize reaction center chlorophyll, P700, in photosystem (PS) I of thylakoid membranes to suppress ROS production [1]. The oxidation of P700 is a robust strategy for them to survive under natural environments. P700 carries electrons from plastocyanin to ferredoxin (Fd) in the photo-oxidation reduction cycle of P700 in PS I. Accumulation of excited P700, P700*, increases chances to electron donation to O_2 and energy transfer to O_2 producing superoxide radical in Mehler reaction and singlet O_2, which oxidatively inactivate PS I to inhibit photosynthesis. Photosynthetic organisms have diverse mechanisms to oxidize P700: to stimulate oxidation of P700*, flavodiiron proteins and photorespiration can be an effective electron sink; to suppress reduction of oxidized P700, $P700^+$, proton conductance, gH^+, can regulate the acidification of the lumen of thylakoids to control the activity of Cyt b_6/f-complex. These factors cooperate to suppress ROS production in PS I as "P700 oxidation system" [2, 3].

We furthermore researched the regulation of P700 oxidation by ferredoxin (Fd) in intact leaves of higher plants using DUAL/KLAS-NIR (WALZ, Germany), which Ulrich Schreiber and Christof Klughammer developed [4, 5]. The electron flux in PS I, evaluated as redox reaction of P700, did not show any linear relationship with that in PS II, evaluated as quantum yield of PS II [Y(II)] by Chl fluorescence analysis. The electron flux in PS I was always larger than that in PS II, which was induced by P700 oxidation. On the other hand, Y(II) clearly showed the positive linear relationship with turnover rate of Fd. These data showed that P700 oxidation induced the extra electron flux in PS I, not coupled with the redox reaction of Fd, which was driven by photosynthesis and photorespiration. We proposed charge recombination could drive Fd-independent cyclic electron flow within PS I, and will discuss its physiological functions in alleviating oxidative damages in PS I by ROS.

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Lecture

Electron transfer in Photosystem I upon limited protein conformational mobility

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The rate of electron transfer (ET) in membrane proteins depends on the distance between redox-cofactors, free energy difference and activation energy of ET reactions. All these factors may be affected by conformational mobility of the protein. Photosystem I (PS I) is an excellent model for the study of the effect of conformational mobility on the ET reactions for several reasons: 1) the availability of 3D-structure at 2.5 Å resolution, 2) the variety of site-directed mutants, 3) the possibility to monitor the single flash-induced kinetics of ET reactions in the wide time range. The effects of conformational mobility on the ET in PS I were studied mostly at cryogenic temperatures in water-glycerol mixtures [1, 2]. An alternative approach is the alteration of protein functioning in dry trehalose glassy matrices at room temperature [3, 4]. A number of similarities are apparent in the charge recombination kinetics of PS I immobilized in the frozen glycerol at 170 K and in the trehalose glass at 298 K. At high temperature/humidity, recombination occurs predominantly from the terminal iron-sulfur clusters F_A/F_B, but on transition to the glassy state, its contribution decreases at the expense of an increase in the recombination from both cluster F_X^- and phylloquinone A_1^-. The backward ET from the F_X in both cases is significantly heterogeneous, covering the wide time range from 0.5 ms to 30 ms. The distinctions between two glass states include different amplitudes of recombination from F_X^- and A_1^- and different kinetics of recombination from A_1^-. We propose that desiccation of trehalose matrix at room temperature and freezing of the water-glycerol solution below its glass transition point cause similar changes in the protein, altering apparent activation energy. In case of trehalose matrix, these changes are more pronounced in peripheral regions of PS I, while at low temperature this effect is evenly spread through the whole protein.

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Lecture

Thylakoid lumenal proteins supporting the assembly and function of photosynthetic supercomplexes

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The thylakoid lumen of chloroplasts provides the environment for oxygen evolution, electron transfer, and photoprotection. There are more than eighty thylakoid lumenal proteins that have been revealed to play roles in numerous processes, most often linked with regulating the biogenesis, activity, and turnover of photosynthetic protein complexes. The oxygen-evolving complex (OEC) proteins – membrane-extrinsic subunits of photosystem II (PS II) – are major lumenal proteins optimizing the water-oxidizing reaction. It is known that the composition of the OEC proteins are largely differed among photosynthetic organisms. Recent structures from X-ray and cryo-electron microscopy studies allow us to describe the structural basis of the binding and function of the OEC proteins in eukaryotic PS II. In addition, multiple isoforms and homologs for the OEC proteins have been found in the chloroplast thylakoid lumen and recent studies have revealed their various roles in photosynthetic electron transfer. We propose that functional diversification of the OEC family proteins during evolution would be important to regulate efficient photosynthesis under changing environments.

Lecture

Biophysics of the primary processes of photosynthesis

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The modern ideas about the mechanisms of energy transformation during electron transfer through the electron transport chain are considered. These mechanisms are based on electron-conformational interactions, the nature of which depends on the physiological and biochemical factors and changes under stress. Fluorescence induction curves provide important information about the nature of these processes, including the effectiveness of photosynthesis under conditions of excessive illumination and the lack of mineral nutrition elements in microalgae cells that are subject to oxidative stress. Analysis of the fluorescence induction curves provides information on the individual stages of the primary processes of photosynthesis. The report discusses the use of these approaches in environmental monitoring and photobioreactors using the appropriate instrumentation base.

Lecture

Water oxidation by Vitamin B_{12}: Questions and challenges

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The water-oxidizing catalysts are necessary for water-splitting systems to store renewable energies [1]. First-row transition metal compounds such as Mn, Fe, Co, Ni, and Cu have been reported as the efficient catalysts for water-oxidation reaction under the different conditions [2]. In 2008, Nocera's group reported a cobalt-based water oxidizing catalyst with remarkable activity and low overpotential under the neutral condition (pH=7) [3].

Herein, the catalytic activity of vitamin B_{12} towards evolution of oxygen was studied. The experiments showed that at pH 11, the compound is active for water oxidation with k_{cat}=930 s^{-1}. A question in this regard is that what is the true catalyst for water oxidation under these conditions. In the presence of heterogeneous catalysts, the Randles-Sevcik equation results in wrong number of the turnover frequency. Thus, such nanosized cobalt oxides/phosphates could be proposed as the true catalysts for water oxidation, and careful analyses are necessary to detect such nanoparticles and the true catalysts for water oxidation. All these experiments showed that to reveal a molecular mechanism for water oxidation by metal complexes, careful analyses are necessary to examine the role of nanoparticles or other heterogeneous catalysts generated during the reactions. Assuming a homogeneous catalyst at pH 11, the experiments showed that the compound has a high turnover frequency (11 s^{-1}) for water oxidation. However, in the presence of a heterogeneous catalyst and assuming that is not a homogeneous catalyst, a turnover frequency significantly lower than 11 s^{-1} was obtained (10^{-3}–10^{-2} s^{-1}).

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Poster

Characterization of a new carotenoid in a kind of land plants

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Carotenoids play important roles in light harvesting, energy transfer, and quenching of excess energy in photosynthesis. More than 750 structurally defined carotenoids are reported in various organisms; land plants, algae, bacteria, fungi, and animals. In land plants, major carotenoids are β-carotene, violaxanthin, neoxanthin, and lutein. These carotenoids belong to the tetraterpenenoid (C40) group, which can be further categorized into two classes, xanthophylls and carotenes. Due to symmetry reasons (C_{2h}), the transition to the lowest excited state (S_1) of carotenoids is forbidden. Therefore, the absorption spectrum of carotenoids originate from S_0 to S_2 state transition. As a result, carotenoids absorb wavelengths ranging from 450–550 nanometers.

In this study, we found a new carotenoid from a kind of plant. This carotenoid absorbs shorter wavelength than general carotenoids. The peak maximum was located at 408 nm. This means that this carotenoid has smaller π-conjugated system than general carotenoids. This property is a first report in land plants. We isolated this carotenoid, and performed spectroscopic analyses. The fluorescence maximum of this carotenoid observed at 614 nm. Fluorescence properties are closely related to the molecular structure of carotenoids.

We will discuss the structure and function of this carotenoid.

Poster

Interaction of the purified photosystem I complexes with artificial redox mediators under continuous illumination

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During the past decade the electron transfer processes in photosystem I (PS I) from *Synechocystis* sp. PCC 6803 were mainly investigated under single-flash excitation. The big progress has been achieved in this field, and several kinetic models, describing these reactions were suggested [1, 2]. However the mechanisms of the electron transfer in PS I under continuous illumination are still unknown. Only few models were presented to date [3].

Here we present the results of the investigation of the dependence of the balance of the P700 oxidation and reduction reactions under steady-state conditions. The P700 redox-state changes were observed under continuous white-light illumination using X-band transient EPR. The F_A/F_B-depleted (F_X-core) complexes of PS I were used in order to decrease the rate of the $P700^+$ oxidation. 2,6-dichlorophenolindophenol (DCPIP) at different concentrations in the presence of the excess of sodium ascorbate was used as the mediator of cyclic electron transfer [4].

In the presence of the low concentration of DCPIP the P700 oxidation is already very fast, it takes several seconds at the lowest DCPIP concentration, while the dark $P700^+$ reduction initially occur in the minute time range. Upon the increase in the mediator concentration the rate of the $P700^+$ reduction accelerates much faster, than the rate of the P700 oxidation. This balance of the oxidation and reduction of P700 could be estimated as the amplitude of the steady-state $P700^+$ signal, which significantly drops upon the increase of the DCPIP concentration. The data clearly shows the difference in the mechanisms of the electron transfer in PS I complexes under single-flash excitation and under continuous illumination.

The study was supported by the Russian Science Foundation (project No. 17-14-01323).

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Poster

Changes of conformational and electronic state of quinones during electron transfer in reaction center of *Rba. Sphaeroides*

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Electron transfer (ET) in the photosynthetic reaction centers (RC) is one of the key steps of energy conversion during photosynthesis. An important question for the modeling of ET is to determine which intermediate states participate in this process.

One of the steps in the whole ET process in RC of *Rba. Sphaeroides* is the transfer between quinone molecules. These molecules are separated by a large distance. Thus, a question if it is a direct transfer arises. Alternatively Fe ion, which is located between these quinones, can participate in this ET step.

The scope of the current research was to determine the prerequisites of the electron transfer between these molecules and to get the energy of the electronic states participating in the process and couplings between them.

The molecular mechanics force fields which fit the quantum chemistry calculations for ubiquinone and its anion in vacuum were obtained.

The molecular dynamical simulations were performed for RC enclosed in DMPC lipid bilayer. The dynamics was carried out for the three states of the system:

1) With both neutral quinones.
2) With neutral quinone QB and anion QA^-.
3) With anion QB^- and neutral QA.

Two stable conformations were detected for the last case. The structures of the quinones and Fe ion with its ligands were picked out from this conformations for the further calculations. The CI method, which is applicable in the case of the non-orthogonal localized orbitals (LOCI) was developed previously by our lab team [1]. This method in particular gives the opportunity to calculate small parts of the large system by standard techniques of well known QC packages with subsequent reunion and CI calculation.

The CASSCF calculations of separated parts (each quinone and Fe ion with its protein environment) were performed for two structures mentioned above. On the next step the LOCI calculations were performed. The couplings and energy levels of the full system was obtained. The results of CASSCF computation of the full system were used for the comparative analysis.

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Poster

Femtosecond dynamics of the primary processes of energy conversion and electron transfer in the photosystem I reaction centers from different types of cyanobacteria

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The spectral dynamics of photosystem I (PS I) complexes from cyanobacteria *Synechocystis* sp. PCC 6803, *Arthrospira platensis* and *Thermosynechococcus. elongatus* were measured by the pump-probe femtosecond spectroscopy. The complexes from *A. platensis* and *T. elongatus* contain long-wave forms of chlorophyll (LWC), in contrast to *S.* sp. PCC 6803. PS I was excited by the 25 fs laser pulses either into the maximum of the chlorophyll absorption band Qy at 670–680 nm or into the far red region at 720–740 nm. Optical dynamics was measured at 400–760 nm at time delays 0.05–500 ps.

In all three samples, an energy migration within the light-harvesting antenna and electron transfer reactions in the reaction center (RC) were observed. In the PS I from *T. elongatus* and *A. platensis* when excited at 730–740 nm, the broad bleaching of LWC with the maximum at 710 and 720 nm, respectively, was observed at the initial delay of 50 fs. The excitation migration to the RC and formation of the secondary ion-radical pair state $P700^+A_1^-$ proceeded in these complexes at 40 ps and 65 ps, respectively (the formation of the primary ion-radical state $P700^+A_0^-$ was not resolved in these complexes). When excited at 670 nm, the energy transfer from the bulk chlorophyll to LWC occurred at 2–3 ps, after which the excitation relocated from LWC to RC with the same kinetics as described above.

In the PS I from *S.* sp. PCC 6803, when excited at 680 nm, the excitation migration from antenna to RC and the formation of the primary ion-radical pair $P700^+A_0^-$ occurred at 3–10 ps. After that, the transfer of an electron to the secondary acceptor A_1 occurred at 30 ps. When excited at 720 nm, a double-well bleaching at 690 nm and 704 nm was observed at shortest 50 fs delay; it was assigned to the direct excitation of the RC, were several chlorophyll molecules form an exciplex with significant mixing between exciton and charge transfer (CT) states.

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Poster

ESEEM study of electron transfer in photosystem I complexes embedded into dry trehalose matrix at different temperatures

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The study of the stabilizing and cryoprotective effects of trehalose at different degrees of humidity on photo-induced charge separation in photosynthetic pigment-protein complexes of photosystems (PS) I and II is important for creating drought-resistant varieties of agricultural crops and for long-term preservation of proteins at room temperature without losing their activities, and can be widely used in biotechnology. Therefore, the study of molecular dynamics and mechanisms of the stabilizing and cryoprotective effects of trehalose on photo-induced charge separation in photosynthetic reaction centers remains an actual problem.

In the present work, we used the ESEEM (electron spin echo envelope modulation) signal of the charge-separated cofactor spins, to determine the distance between the spins of P_{700}^+ (chlorophyll dimer) and A_1 (phylloquinone acceptor) in the intact PS I complexes isolated from cyanobacteria *Synechocystis* sp. PCC 6803 embedded in dry trehalose matrix at different temperatures. In the ESEEM signals, the frequency of the dipole-dipole interaction of a radical pair is observed. From the definition of this frequency, we were able to determine the distance between P_{700}^+ and A_1. A decrease of the modulation frequency of the ESEEM of the P_{700}^+ cofactor of PS I embedded into glassy trehalose matrix upon temperature increase from 150 K to room temperature was observed. It was concluded that the decrease of modulation frequency with rising temperature can be fully attributed to the influence of accelerated spin-lattice relaxation of A_1. It was suggested that the terminal iron-sulphur clusters F_A and F_B can affect the rate of spin-lattice relaxation. To confirm this model, we studied the temperature dependence of ESEEM frequency for PS I lacking iron-sulfur clusters F_A/F_B and extrinsic protein subunit PsaC (F_X-core complexes). It was shown that in contrast to the intact PS I, the ESEEM frequency for F_X-core complexes does not change as the temperature rises from 150 K to room temperature.

The obtained results allow us to suggest a model of the protective effect of trehalose matrix on the electron transfer in the reaction center of PS I that is based on different hydrogen-bond networks between trehalose, local water, and protein.

This work was supported by the Russian Foundation for Basic Research (project no. 18-43-160017) and Presidium RAS Program No. 5.

Poster

Bacteriochlorophyll *c* Q-/B-band hyperchromism/hypochromism as a tool for investigation of the oligomeric structure of the *Chloroflexus aurantiacus* chlorosomes

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Chlorosomes of green photosynthetic bacteria are the most amazing example of long-range ordered natural light harvesting antennae. Chlorosomes are the largest among all known photosynthetic light-harvesting structures (~10^4 – 10^5 pigments in the aggregated state). The chlorosomal bacteriochlorophyll (BChl) *c*/*d*/*e* molecules are organized via self-assembly and do not require proteins to provide a scaffold for efficient light harvesting. Despite numerous investigations, a consensus regarding the spatial structure of chlorosomal antennae has not yet been reached. In the present work, we studied hyperchromism/hypochromism in the chlorosomal BChl *c* Q/B absorption bands of the green photosynthetic bacterium *Chloroflexus* (*Cfx.*) *aurantiacus*. These chlorosomes were isolated from cells grown under different light intensities and therefore had different sizes of BChl *c* antennae and their unit building blocks. We showed experimentally that the Q/B-band hyperchromism/hypochromism is proportional to the size of the chlorosomal antenna. We explained theoretically these findings in terms of excitonic intensity borrowing between Q and B bands for the J-/H-aggregates of the BChls. The theory (D. Gülen (2006) *Photosynth. Res.* 87: 205–214) predicted a dependence of the Q-/B-band hyperchromism/hypochromism on 3D structure of the aggregates. For the model of exciton-coupled BChl *c* linear chains within the unit building block, the theory predicted an increase in the hyperchromism/hypochromism with a number of molecules per chain and a decrease in it with a number of chains. It was previously showed that this model ensured a good fit of spectroscopy experiments and approximated the BChl *c* low packing density *in vivo*. The presented experimental and theoretical studies of the Q-/B-band hyperchromism/hypochromism permitted us to conclude that the unit building block of *Cfx. aurantiacus* chlorosomes comprises of several short BChl *c* chains. This conclusion is in accordance with previous studies.

Section 1.2: Structure, Function and Biogenesis of the Photosynthetic Apparatus

Lecture

Structural biological studies on photosynthetic systems

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Light energy conversion and water-splitting reactions in oxygenic photosynthetic organisms take place in two large membrane-protein complexes photosystem I and II (PS I and PS II). We have been using modern structural biological approaches, including X-ray crystallography with both synchrotron X-rays (SR) and femtosecond X-ray free electron lasers (XFEL), time-resolved structural analysis using the pump-probe method, as well as cryo-electron (cryo-EM) single particle analysis, to solve the structures of both PS I and PS II and their super-complexes with light-harvesting antenna systems, from various photosynthetic organisms. In particular, we have solved the high-resolution [1] as well as radiation damage-free structure [2] of cyanobacterial PS II by SR and XFEL respectively, and further solved one of its reaction intermediate structure, the S3-state, by a pump-probe approach [3]. These studies provided the basis for elucidating the mechanism of photosynthetic water oxidation. We also solved the crystal structure of a super-complex between the reaction center (RC) and its surrounding light-harvesting 1 (LH1) complex from a purple photosynthetic bacteria [4], which is the ancestor of PS II. Furthermore, we solved the cryo-EM structure of PSI-LHCI from a red alga, which revealed some unique features of this super-complex in the primitive eukaryotic photosynthetic alga [5], as well as the crystal structure of a fucoxanthin-chlorophyll *c*-bind protein (FCP) from a diatom. In this talk, I will introduce these structures and discuss their functional and evolutionary implications.

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Lecture

Structure-Function of the Cytochrome *b6f* Complex: (i) Lipid-Protein Interactions; (ii) Structure-Based Control of the Electron Transport Rate; (iii) Arabidopsis

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(1) Lipid-Protein Interactions. The high resolution structure (2.5 Å) of the cytochrome b_6f complex from the cyanobacterium *Nostoc* sp. PCC 7120 revealed the presence of 23 lipid binding sites per b_6f monomer. Although the structure of the cytochrome b_6f from a plant source has not been solved, the identity of lipids present in spinach b_6f complex has been determined: MGDG, DGDG, PG, and SQDG are the predominant lipid species. Effects of lipid on structural/functional integrity of b_6f complex: (i) phospholipid tail groups provide structural stabilization; (ii) SQDG has a major role in conferring dimer stability; (iii) nanodiscs and bicelles stabilize the structure of the complex; (iv) removal of phospholipids by phospholipase A2 deactivates the complex, which (v) can be significantly re-activated by addition of PG. **(2) Genetic Alteration of the Rate of Electron Transfer.** A rate limitation of oxygenic photosynthesis, reduction of the cytochrome b_6f complex by plastoquinol, was chosen as a target for genetic modification based on information derived from the three-dimensional crystal structure of the cytochrome complex alone and in complex with a quinone-analogue inhibitor. Site-directed mutagenesis was applied to a site in the cytochrome complex inferred to be involved in the rate-limiting electron transport step associated with (a) translocation of the plastoquinol/-quinone, and (b) its oxidation and deprotonation by the high potential iron-sulfur (2Fe-2S) protein and cytochrome *f* subunit of the b_6f complex. From the high resolution crystal structure of the complex and a structure containing the quinone analog inhibitor, tridecyl-stigmatellin, bound in an 11 Å long portal on the electrochemically positive side of the membrane, which provides access to the iron-sulfur protein, the entry/exit of plastoquinol-quinone was inferred to include passage through the p-side portal. Additional structure information implies that the approach of plastoquinol to the p-side portal and access to the iron-sulfur protein is also limited by passage of the quinol through a channel in the b_6f complex bounded by two conserved proline residues in trans-membrane F-helix of subunit IV in the b_6f complex. Change of the two prolines to alanine in the cyanobacterium *Synechococcus* sp. PCC 7002 caused a two-fold decrease in the rate of (i) cell culture growth, (ii) O_2 evolution, and (iii) plastoquinol-mediated reduction of cytochrome *f*. **(3)** The b_6f Complex from *Arabidopsis* has been isolated to allow genetic analysis. Research support from U.S. Dept. of Energy (DOE DE-SC0018238).

Lecture

Vibrational dynamics and excitation energy transfer in LHC II

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Dynamics-function correlations are usually inferred when molecular mobility and protein function are simultaneously impaired at characteristic temperatures or hydration levels. In this sense, excitation energy transfer in the photosynthetic light-harvesting complex II (LHC II) is an untypical example, because it remains fully functional even at cryogenic temperatures relying mainly on interactions of electronic states with protein vibrations. Here, we study the influence of vibrational and conformational protein dynamics of monomeric and trimeric light-harvesting complex II (LHC II) from spinach using a combination of absorption spectroscopy [1], line-narrowing spectroscopy [2] and inelastic neutron scattering [3] in the temperature range between 20 and 305 K. INS spectra of trimeric LHC II reveal a distinct vibrational peak at ~2.4 meV. At temperatures above ~160 K, however, the inelastic peak shifts towards lower energies, which is attributed to vibrational anharmonicity. A more drastic shift is observed at about 240 K, which is interpreted in terms of a "softening" of the protein matrix along with the dynamical transition.

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Lecture

Modification of light-harvesting and energy-transfer processes in diatoms under different light conditions

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Primary processes of photosynthesis respond to changes in environmental conditions. Pigment compositions and energy transfer change depending on light quality and light quantity. In cyanobacteria, the content ratio of the membrane-anchored light-harvesting complex, phycobilisome, to chlorophyll *a* changes under different light conditions [1, 2], and the energy-transfer processes involving photosystem and phycobilisome also alter [2]. Diatoms possess unique light-harvesting antenna, fucoxanthin-chlorophyll *a*/*c* protein complex, embedded in thylakoid membranes. We recently reported effects of light intensity on pigment composition and energy transfer in diatoms *Chaetoceros gracilis* and *Phaeodactylum tricornutum* [3, 4]. In the present study, we will discuss differences in light-harvesting and energy-transfer processes in diatoms *C. gracilis* and *P. tricornutum* cells grown under different light qualities, by means of time-resolved fluorescence spectroscopy.

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Lecture

Features of bacteriochlorophylls axial ligation in the photosynthetic reaction center of purple bacteria

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Chlorophylls and bacteriochlorophylls (BChls), the most common photosynthetic pigments, are essential components of photosynthetic complexes in bacteria, algae, and plants. These pigments are responsible for absorption and transfer of solar energy in light harvesting antennae and act as electron carriers in photosynthetic reaction centers. An overwhelming majority of natural (B)Chls are Mg porphyrins. Central Mg atom in (B)Chl forms four coordination bonds with pyrrole's nitrogens. In addition, at least one axial position of the Mg atom is always occupied by an electron donor group of a solvent or a protein. The maximal coordination number of Mg is 6; however, in natural pigment-protein complexes, (B)Chl magnesium atom is most often penta-coordinated, and the cases of its complete saturation are quite rare. The π-electron system of (B)Chl is sensitive to changes in the partial charge density of the central metal; therefore, the nature and the location of nucleophilic groups in the axial positions of Mg atom largely determine spectral and redox properties of this pigment. The work will be focused on recent experimental data obtained by site-directed mutagenesis of the reaction center from purple nonsulfur bacteria. The role of (B)Chl axial ligation in the regulation of spectral and redox properties of this pigment, as well as correlation between the structure of chromophores and nature of their ligands, will be discussed. Cofactor ligation in various types of reaction centers will be compared, and possible reasons for observed differences will be examined in the light of modern ideas on the evolution of photosynthesis.

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Lecture

Photosynthetic reaction centers – Robustness with increased complexity

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The ability of photosynthetic organisms to use the sun's light as a sole source of energy sustains life on our planet. Photosystems I (PS I) and II (PS II) are large, multi-subunit, pigment-protein complexes that enable photosynthesis, but this intriguing process remains to be explained fully. Plant PS I is one of the most intricate membrane complexes in Nature. It is comprised of two complexes, a reaction center and light-harvesting LHCI.

Recently we solved the structure of trimeric PS I from *Synechocystis* at 2.5 Å resolution. Several differences between the mesophilic and thermophilic PS I were revealed and the position of lipids between the monomers was determined. The structure of green and red algae PS I was solved at intermediary resolution. The mechanistic and evolutionary implications will be discussed.

An operon encoding PS I was identified in cyanobacterial marine viruses. We generated a PS I 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 PS I with subunit composition similar to the viral PS I.

The perspective of robustness versus complexity of this highly important bioenergetic complex is discussed. From our studies, and several other laboratories, it has become apparent that advancements in evolution have resulted in increased complexity, and also increased sensitivity to structural alterations, without compromising on the robustness of the system.

Recently we joined the structural revolution through the cryo-EM technique. We solved two kinds of PS I structures that open up the possibility to follow stress-induced structural alterations.

Lecture

Employing barley mutants to dissect chlorophyll biosynthesis and chloroplast development

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In a collection of Scandinavian barley mutants (www.nordgen.org/bgs) consisting of more than 12,000 accessions, 357 mutants (representing 105 loci) have been assigned to be deficient in chlorophyll biosynthesis and chloroplast development. The mutants were sorted in six groups according to their phenotype: xantha mutants are yellow since they can make carotenoids but not chlorophyll, albina mutants are white since they do not make carotenoids nor chlorophyll, tigrina mutants are transversally striped mutants, striata mutants are longitudinally striped mutants, viridis and chlorina mutants are light green mutants. The mutants are lethal except the chlorina mutants. The lethal mutants need to be kept in heterozygous stocks. Still the lethal mutants can be obtained in relatively large amounts because the large barley kernel can support the growth of a homozygous mutant for approximately two weeks until the energy reserves are depleted. In our research we identify the genes that are deficient in the various lethal mutants by different approaches like map-based cloning, candidate gene sequencing and genomic sequencing of allelic mutants. We further explore the mutants to obtain information about the corresponding proteins and enzymatic mechanisms.

Poster

Role of specific Photosystem II complexes in the cyanobacterial chlorophyll *a* biosynthesis

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Photosystem I and II (PS I and PS II) are complicated membrane complexes consisting of a number of proteins, chlorophylls, carotenes, and other cofactors. The biogenesis of PS II involves stepwise assembly (so called modular model) of pre-assembled modules, each consisting of a large chlorophyll-binding protein (D1, D2, CP43, and CP47), associated small subunits and pigments. This consecutive process is regulated by a number of auxiliary proteins/factors that are not components of the final active PS II but were identified as components of PS II assembly complexes or modules. However, our recent results showed that many of these factors are also needed for the efficient chlorophyll (Chl) biosynthesis and related accumulation of PS I.

Recently, we found out that the Flag-tagged version of the PS II assembly factor Pam68 expressed in strain unable to assemble PS II due to the missing D2 protein, co-purifies with newly synthesized CP47 and with POR, the light-dependent protochlorophyllide oxidoreductase enzyme involved in the biosynthesis of Chl. Moreover, the mutant lacking CP47 is remarkably Chl deficient and the His-tagged version of another PS II assembly factor Psb28 is specifically co-isolated with PS II core complexes lacking CP43 (RC47) which also binds POR and Mg-protoporphyrin methyl ester cyclase, another Chl biosynthesis enzyme. Based on these results we propose that CP47 and especially the RC47 assembly intermediate with bound specific factors (Pam68, Psb28) represent a specific subpopulation of PS II assembly intermediates that fulfill a specific function in the biosynthesis of Chl *a*. They bind enzymes of Chl biosynthesis pathway and markedly increase the availability of Chl for the efficient biogenesis of PS II and especially PS I, which represents the main primary sink for the newly synthesized Chl a molecules.

Poster

Lipid polymorphism of plant thylakoid membranes. Reversible changes induced by low pH and elevated temperature

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The thylakoid membranes of oxygenic photosynthetic organisms are capable of utilizing the light-induced electrochemical potential gradient for ATP synthesis due to organization of their lipids into a bilayer. However, their major lipid species, the monogalactosyl-diacylglycerol, which constitutes approximately half of their total lipid content, is a non-bilayer forming lipid. Earlier ^{31}P-NMR experiments have revealed that fully functional thylakoid membranes contain three non-bilayer lipid phases, two isotropic and a H_{II} phase, in addition to the bilayer phase, and that these phases undergo substantial variations upon changes in the physicochemical environment of the membranes [1]. We have also shown that increased intensities in the resonances arising from the isotropic phases are associated with increased membrane permeability – suggesting that the non-bilayer lipid phases tune the basal flux of ions across the membrane [2].

In this work, by using ^{31}P-NMR on isolated spinach thylakoid membranes, we show that two important factors influencing the photosynthetic functions, namely variations of the pH of the medium and the sample temperature, cause largely reversible changes in the lipid-phase behaviour of thylakoids. These data further strengthen our earlier conclusion that non-bilayer lipid phases, co-existing and interacting with the bilayer phase, play important roles in the structural dynamics of thylakoid membranes.

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Poster

Probing the effects of mutation of PsbV-Tyr137 in the thermophilic cyanobacterium *Thermosynechococcus vulcanus*

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Photosynthetic water-oxidation is catalyzed by a Mn_4CaO_5-cluster bound to the protein matrix of photosystem (PS II). The high-resolution structure of PS II has revealed several well-defined H-bond networks leading from the Mn_4CaO_5 cluster to the lumenal bulk solution, which may be important for the transport of protons out of its reaction site. PsbV-Tyr137 is located in the exit of one such H-bond network starting from the D1-Try161-His190 residue pair, and located in the interface between the D1, CP43, and PsbV (cyt c-550) subunits. In order to probe the function of PsbV-Y137, four mutants, PsbV-Y137A, PsbV-Y137F, PsbV-Y137G, and PsbV-Y137W, were generated with *Thermosynechococcus vulcanus* (*T. vulcanus*). These mutants showed slightly slower growth rates than that of the wide type (WT*) strain; however, their oxygen-evolving activity was apparently different. At pH 6.5, the oxygen evolution rates of Y137F and Y137W were almost identical with that of WT*, whereas the oxygen evolution rates of the Y137A, Y137G mutants were 63% and 61% of the WT*, respectively. Furthermore, the thylakoid membranes isolated from the PsbV-Y137A, PsbV-Y137G mutants lost the oxygen-evolving activity almost completely, which was found to be caused by the release of PsbV. In addition, PS II-complexes purified from the PsbV-Y137F and PsbV-Y137W mutants contained two PS II fractions, one of which lacks the three extrinsic proteins PsbO, PsbV and PsbU and showed almost no oxygen-evolving activity, whereas the other one contained the three extrinsic proteins and showed a lower oxygen-evolving activity than that of the WT-PS II. These results suggested that the PsbV-Y137 residue is required for the stable binding of the extrinsic proteins to PS II and also has a role in maintaining the oxygen-evolving activity.

Poster

Impairment of Photosystem II assembly and acceptor side electron transfer following mutation of Thr243 and Lys264 of D2 in cyanobacteria

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The D2 protein, together with D1, forms the polypeptide core of the Photosystem (PS) II reaction center. D2-Thr243 and D2-Lys264 have been hypothesized to participate in stabilization of a hydrogen-bond network for delivering protons via a bound HCO_3^- to the $Q_B^{2-}(H^+)$ form of the secondary plastoquinone electron acceptor of PS II [1, 2]. The HCO_3^- ion is a bidentate ligand to a non-heme iron (NHI) positioned between Q_A, the primary plastoquinone electron acceptor, and Q_B. Stabilization of HCO_3^- is supported by hydrogen bonds from D2-Thr243 and D2-Lys264 via the water molecule W582 (PDB 4UB6). The D2-Lys264 residue also participates in stabilization of the NHI through a hydrogen bond with D2-His268. We have created the T243A and K264A mutants and also introduced a Glu at D2-Lys264 to produce the K264E mutant. The T243A and K264A strains exhibited a photoautotrophic doubling time similar to the control whereas the K264E mutant had retarded growth. All three mutants accumulated an unassembled pre-complex containing the chlorophyll-binding core antenna CP43 protein and reduction in their PS II:PS I ratio. The assembled PS II centers in these mutants exhibited impaired oxygen evolution and were susceptible to high-light-induced photodamage in the presence of PS II-specific electron acceptors. In addition, unlike the T243A and K264A strains, the K264E mutant was unable to acclimate to high light when HCO_3^- was added to support whole chain electron transport. In all mutants the decay of variable chlorophyll fluorescence following a single actinic flash was impaired. Furthermore, the chlorophyll fluorescence decay kinetics in the K264E strain were insensitive to addition of either formate or HCO_3^- whereas HCO_3^--reversible formate-induced inhibition in the T243A and K264A mutants was observed. Our data indicate that disruption of the hydrogen-bond network associated with the HCO_3^--binding environment leads to impairment in both PS II assembly and electron transfer through the Q_A-Fe-Q_B acceptor complex of the photosystem.

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Poster

Characterization of the Photosystem II reaction centre assembly intermediate in the cyanobacterium *Synechocystis* PCC 6803

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Photosystem II (PS II) is a multi-component pigment-protein complex embedded in the thylakoid membranes of cyanobacteria and chloroplasts. The PS II biogenesis occurs in a step-wise manner by assembling smaller sub-complexes, and requires precise coordination of protein biosynthesis with the insertion of numerous pigments and other cofactors. Using a *Synechocystis* PCC 6803 strain expressing the His-tagged D2 protein in the absence of the CP47 antenna we purified a preparation of the early PS II reaction centre assembly intermediate (RCII). It contained the size-distinguished RCIIa and RCII* complexes, but also a complex of the RCIIa and the monomeric Photosystem I. RCIIs consisted basically of D1-D2 heterodimer, PsbI, cytochrome b-559 subunits PsbE/F, and the Ycf48 assembly factor. RCII* also bound an already described complex of the Ycf39 and Hlips. The ratio of chlorophyll (Chl), pheophytin, beta-carotene and heme-b in the RCIIa, which does not contain any auxiliary pigment-binding factors, was close to that expected from the PS II crystal structure. The preparation was able to photoaccumulate oxidized primary donor ($P680^+$) and reduced pheophytin ($Pheo^-$). Overall these results show that the RCII assembly intermediate forms a preassembled pigment-protein complex *in vivo*, already capable of the primary charge separation.

Among the assembly factors optimizing the RCII assembly, the Ycf48 plays a crucial role. The Ycf48-null strain is PS II- and Chl-deficient and shows reduced autotrophic growth. The Ycf39-knock out mutant has a wild type phenotype under mild conditions, but it is high light sensitive. The additional deletion of the Ycf39 in the Ycf48-less background caused a synergic effect resulting in further reduction of the cellular Chl content, fatally impaired PS II biosynthesis and the loss of autotrophy. However, this effect could be overcome by an improved Chl supply after the application of a specific inhibitor of ferrochelatase, which restored formation of the active PS II complexes. This result supports the key importance of the sufficient Chl supply for the PS II biogenesis.

Poster

Chlorophyll *f* is synthesized by a modified non-oxygen-evolving photosystem II complex

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Certain cyanobacterial species are able to synthesize a special chlorophyll (Chl) molecule termed Chl *f*. Its biosynthesis requires the expression of a gene encoding a distant relative of the D1 subunit of oxygen-evolving photosystem II (PS II). It has been suggested that this so called super-rogue D1 (srD1) functions as a Chl *f* synthase and acts as a homodimer [1]. Here we show that in a *Synechocystis* sp. PCC 6803 strain heterologously expressing the gene encoding srD1 from *Chroococcidiopsis thermalis*, this protein behaves like a regular D1 protein and forms a monomeric non-oxygen-evolving PS II core complex lacking the lumenal proteins stabilizing the oxygen-evolving Mn_4O_5Ca cluster (PsbO, PsbV and PsbU) but containing Psb27. The complex has a very similar pigment composition to oxygen-evolving PS II complexes but contains a small amount of Chl *f* (about one molecule of Chl *f* per three PS II complexes), which shows a typical 77 K Chl fluorescence peak at 717 nm. We did not find any evidence for formation of a srD1 homodimer; instead srD1 has a high affinity for D2 and the srD1/D2 heterodimer is highly resistant to SDS treatment. The synthesis of Chl *f* is inhibited in mutants expressing srD1 but unable to assemble the complete PS II core complex and also in a strain lacking the secondary PS II electron donor, TyrZ. Remarkably, when two residues in the second transmembrane helix typical for srD1 are introduced into the regular D1 protein of *Synechocystis*, the PS II complex containing this mutated D1 also produces Chl *f*. Our work has identified a new type of PS II complex with a physiological role in pigment biosynthesis rather than water-splitting.

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Poster

Structural basis of energy harvesting and dissipation in diatom PSII-FCPII complexes revealed by cryo-electron microscopy

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Oxygenic photosynthetic organisms have developed light-harvesting complexes (LHCs) to capture the solar energy, which is subsequently transferred to the reaction center of the two photosystems (PS I and PS II) to initiate charge-separation reactions. Although the core components of photosystems are well conserved among photosynthetic organisms, LHCs have a wide variety of pigment compositions such as chlorophylls (Chls) and carotenoids, and of protein structures. From their large diversity, LHCs are mainly divided into two branches, namely green and red lineages, during evolution. In the red lineages, diatoms have unique light-harvesting fucoxanthin chlorophyll *a*/*c*-binding proteins (FCPs) that are associated with both PS I and PS II. We have so far shown the biochemical and spectroscopic characterizations of diatom PSII-FCPII complexes, whereas their structural organizations are still missing. In this study, we solved an overall structure of the PSII-FCPII supercomplexes isolated from a diatom, *Chaetoceros gracilis*, by cryo-electron microscopic single particle analysis. The PSII-FCPII forms a homodimeric structure in which two FCP homo-tetramers and three FCP monomers are associated with one PS II core monomer. The structure reveals the existence of a huge and sophisticated pigment-protein network in FCPII that are different from green-type light-harvesting complexes. These findings provide novel and important structural insights into the excitation-energy-transfer and dissipation mechanisms in the diatom PSII-FCPII.

Poster

Lipid rafts in membranes of chloroplasts and mitochondria of halophyte plants

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Specific areas of cell membranes are called lipid rafts [1]. In comparison with the rest of the membrane sites, they are resistant to the action of detergents and notable for their high stability and packing density due to the special composition of lipids. Lipid rafts are enriched with sterols (Ster) and sphingolipids/cerebrosides (Cer). On the ground of experimental data, it has been established that rafts can affect the biological activity of cellular and subcellular membranes. Hence, they can affect the biological activity of the whole cell [2].

In this work, we have studied the composition of lipid rafts in chloroplast and mitochondrial membranes – organelles that provide photosynthesis and respiration in a plant cell. Halophyte plants *Salicornia perenns* Willd., *Suaeda salsa* (L.) Pall., *Halocnemum strobilaceum* (Pall.) M. Bieb. and *Artemisia santonica* L., which are able to effectively protect the photosynthetic apparatus and promote photosynthesis under the conditions of soil salinization, were the object of this research [3].

In the composition of membrane lipids rafts in chloroplasts of halophytes *S. perenns*, *H. strobilaceum* and *A. santonica*, the following order was determined: glycolipids (GL) (46–53%) > Ster + Cer (37–27%) > phospholipids (PL) (17–20%). In mitochondrial rafts, Ster + Cer is the first (89–79% of the total lipids), followed by GL and PL.

The effect of 1000 mM NaCl under controlled conditions of temperature and illumination led to an increase in the proportion of Cer in mitochondrial membranes of *S. salsa* and in chloroplast membranes of *S. perennans*. Thus, the composition of lipid rafts is determined by species characteristics of plants, degree of soil salinity, and membrane structure of organelles from which they are isolated.

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Poster

Sterols component of chloroplast membranes of halophyte plants

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Sterols (ST) are important components of most eukaryotic membranes [1]. Intermolecular interactions between ST and membrane glycero- and sphingolipids modulate the physical condition of the membranes limiting the mobility of acyl chains, thereby regulate the fluidity and permeability [2].

At the moment the most studied ST are those which included in the content of plasma membrane. In particular, the role of ST in the organization and functioning of plasmalemma microdomains (lipid rafts) was demonstrated. The data is received on the participation of ST in the formation of endomembranes, including EPR membranes, mitochondria and chloroplasts [3]. It is known that ST participate in the lateral separation of pigment-protein complexes of the photosynthetic apparatus, ensure the mobility of electron carriers in the electron-transport chain and connected to movement of the light-harvesting complexes of PS I and II [4]. The present work has studied the composition of the chloroplast ST in halophyte plants, which are differ by salt tolerance strategy, in paticular eugalophyte *Salicornia perennans* Willd. (salt accumulating type) and glycogalophyte *Artemisia santonica* L. (saltproof type). The CT analysis of total lipid extract in chloroplast fraction was carried out using the GC-MS method after the silylation procedure.

Among the ST of chloroplast membranes, cholesterol, β-sitosterol, stigmatrol, campesterol are identified. The greatest amount was ethyl-containing ST – β-sitosterol and stigmasterol (79–91% of sum of ST). The amount of campesterol did not exceed 4%. The content of ST with a saturated cyclopentan perhydrophenanthrene core (sitostanol, 5,6-dihydro-stigmasterol) varied in the range of 3–9%. Eugalophyte *S. perennans* is characterized by a higher content of cholesterol and sitostanol compared with glycohalophyte *A. santonica*. Significantly greater contribution of ethyl-containing ST was found in *A. santonica* than in *S. perennans* (91 and 79%, respectively). The results obtained suggest that a change in the composition of ST membranes of chloroplasts is one of the factors for the adaptation of halophytes to salinization.

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Poster

Embryogenesis of *Fucus vesiculosus* is characterized by significant changes in photosynthetic performance, hydrogen peroxide production and haloperoxidase activity

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Embryogenesis of fucoid algae is a generally applied model system for studying numerous aspects of algal physiology. Fucoid zygotes and embryos develop independently of maternal tissues. After fertilization they undergo a series of complicated physiological processes (polarization, cell wall formation, attachment to the substratum, germination and organogenesis) accompanied with dramatic changes of cells' metabolism. Here we study the dynamics of photosynthetic performance, H_2O_2 content and haloperoxidase activity during the first 9 days of *Fucus* embryogenesis.

Samples of zygotes and embryos of *Fucus vesiculosus* L. were taken in 1, 3, 6, and 12 hours and then in 1, 3, 6, and 9 days after fertilization (AF). Photosynthetic parameters were determined by microscopy-PAM fluorometry (Walz GmbH, Effeltrich, Germany). H_2O_2 content was studied with FOX method [1]. The haloperoxidase activity was determined in the reaction of thymolsulfonphthalein halogenation [2].

Hydrogen peroxide content in *Fucus* egg cells was relatively low (0.02 μM/g FW), but it began to increase immediately AF, so that 3 days old embryos contained already an order of magnitude more H_2O_2, than eggs. This H_2O_2 accumulation apparently reflects the intensification of photosynthetic processes. Relative electron transport rate of Photosystem II increased 4 times during the first 9 days of embryogenesis. We may suppose that H_2O_2 accumulation in *Fucus* zygotes is needed to provide a substrate for the reactions of phlorotannins and alginates cross-linking, catalyzed by vanadium dependent haloperoxidases. Enhanced haloperoxidase activity supports the final steps of zygote cell wall biosynthesis and bioadhesion. Thus rapid enhancement of photosynthetic performance during fucoid embryogenesis not only provides energy and assimilates for developing zygotes and embryos, but may also contribute to such critical step as attachment to the substrate.

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Poster

Protein composition and spectral characteristics of thylakoid membranes of the maize mesophyll and bundle sheath chloroplasts

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The light reactions of oxygenic photosynthesis are carried out by the four large enzymatic complexes located in the thylakoid membranes of higher plants and green algae. These complexes contain a large number of polypeptide subunits encoded either in the nuclear or in the plastid genome. The protein complexes are unevenly distributed in thylakoids of higher plants. A characteristics feature of C4 plants is the differentiation of the photosynthetic leaf tissue into two distinct cell types, mesophyll (M) and bundle sheath (BS) cells. In this study, polypeptide pattern and spectral characteristics of mesophyll and bundle sheath thylakoids of isolated maize (*Zea mays* L.) chloroplasts have been analyzed. Thylakoid membrane proteins were analyzed according to Laemmli using a 10 to 25% (w/v) linear gradient polyacrylamide gel in the presence of SDS. It is shown that the protein composition of mesophyll thylakoids is similar to that of chloroplast of typical higher plants (C3 plants). The amount of the PS I core apoprotein with molecular mass (Mr) of 68 kDa is greater in bundle sheath thylakoids compared with mesophyll cells. α- and β- subunits with molecular masses of 55 kDa and 52 kDa of CF1 domain of the H^+-ATP synthase complex were present in both subcellular fractions. The protein of 45 kDa belonging to the PS II core antenna is more intensive in mesophyll thylakoids. Polypeptides with Mr of 28–24 kDa of the light-harvesting complex of PS II (LHCII) are observed in both types of thylakoids, but their amounts are reduced in bundle sheat cells. The analysis of low-temperature (77 K) fluorescence spectra of chlorophyll in mesophyll chloroplasts showed the presence of three maxima, characteristic of light-harvesting complex at 686 nm, a PS II complex at 695 nm, and a PS I complex at 735 nm. However, in the fluorescence spectrum of agranal plastids, there are almost traces of the band at 695 nm. The absence of this fluorescence band pertaining to the chlorophyll-protein complex of PS II is considered as a violation in its antenna chlorophyll. In this case, the low level of 695 nm band in the fluorescence spectra is suggested to be associated with a small amount of light- harvesting complex.

Poster

Photosynthetic function during plastid development in barley

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The photosynthetic apparatus of plants undergoes significant structural and functional changes during plastid development. There are several models for assessing such changes – a leaf in different periods of ontogenesis, greening of etiolated seedlings, first barley leaf from the base to the top. These three models were used to characterize the functional characteristics of the photosynthetic apparatus: the rate of CO_2 uptake, the content of photosynthetic pigments, the chlorophyll fluorescence parameters, in order to identify the traits of proplastid – chloroplast and etioplast – chloroplast transition. Studies were performed on 7-day old etiolated and green barley seedlings. During greening of etiolated plants the gas exchange was biphasic: at the first 6 h of illumination CO_2 was emitted during respiration, and after 6 h CO_2 was assimilated. Already after 1 h of illumination chl *a*, chl *b* and carotenoids were identified in the leaf, by 8 h of illumination their amount increased 3 times, and 24 h later – 7–8 times. The ratio of chl *a*/chl *b* has increased from 1.3 to 2.4. The rate of photosynthesis depended on the light intensity during greening: plants start to assimilate CO_2 only at light intensity of 500 μE $m^{-2}s^{-1}$. The quantum yield of photosynthesis has reached positive values starting from 5–6 h of illumination and increased over time, reaching maximum by 12 h. The Fv/Fm index decreased in the range of 1–2 h of illumination, then increased and reached maximum by 8 h. A similar trend was observed for the Fv/Fo. The RC/ABS characterizing the concentration of reaction centers in the total pool of chlorophylls rapidly decreased in the first 4 h of illumination and remained at a constant level of 0.3–0.32. In 5-day old green seedlings CO_2 assimilation rate increased almost 3 times compared to the 3-day old leaf, whereas from the 5 to 7 day – only 1.5 times. Fv/Fm, Fv/Fo, RC/ABS rates increased by 35%, 20% and 65%, respectively. Similar changes were observed in leaf sections of different age: from meristem cells containing proplastids to differentiated cells having mature chloroplasts. So each model presents the similar results: photosynthetic rate increased during plastid development not only by accumulation of pigments but by the functional changes in pigment system.

Section 1.3: Photosystem I and Bacterial Photosynthesis

Lecture

Stromatolites – old and new

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Stromatolites used to be a common feature of the Early Earth and are believed to be the oldest biological macrostructures from this time. Fossil stromatolites occur in a number of locations around the world but they are rare because the normal processes of subduction in most locations around the world means that on a geological time scale these structures are returned to the Earth's lower mantle where the structures are broken down. The Pilbara region of Western Australia is just one of a few locations where a craton exists, which rotates horizontally, thus maintaining the integrity of stromatolites, albeit as fossils, for billions of years, without subduction. Given the right tools the organisms that created these stromatolites can be inferred, and cyanobacteria are prime candidates.

Today living stromatolites occur in a few locations around the world. And not far from the Pilbara region of WA, living stromatolites occur at the closed end of the massive embayment known as Shark Bay. These stromatolites are being extensively studied as to 1) how they are formed today and what organisms they harbour, 2) what they can tell us about the formation of the oldest fossil stromatolites and 3) the likely effect of global climate change. Already, it has been shown that there are novel cyanobacteria in the living stromatolites, which possess the recently discovered Chlorophyll *f*. This chlorophyll is present in the sublayer (~5 mm) of the surface mats where most of the surface visible light does not penetrate, so that these cyanobacteria survive mainly on near infra-red radiation, which penetrates deeper than the visible light. However, we have discovered that there are also Chlorophyll *d*-containing cyanobacteria present and these must compete with the Chl *f* organisms in the deeper layers.

Our studies have shown that there are a wealth of new organisms to be discovered in the mats of the stromatolites. How far back in time these novel cyanobacteria extend is unknown. However, our studies using phylogenetic analyses and molecular clock techniques indicate that precursors of the Cyanobacteria with some kind of water splitting and oxygen evolution go back 3.5 billion years – and maybe even further back than this.

Lecture

The role of oxygen in evolution of photosystem I

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The emergence of oxygenic photosynthesis led to the rise of O_2 in atmosphere, which have become a key factor governing the evolution of photosynthetic reaction centers including photosystem I (PS I). From the one hand, the efficient interactions of PS I components with O_2 would reduce the quantum yield of photosynthesis and produce deleterious reactive oxygen species such as singlet O_2, superoxide radicals, and H_2O_2. From the another hand, O_2 represents an alternative acceptor of electrons from PS I cofactors and can release surplus electrons generated under stress conditions, and the stable product of the reaction, H_2O_2, is an important signaling messenger. In this work, the recent data concerning mechanisms of PS I interaction with O_2 are summarized and analyzed in terms of evolution of PS I. The hypothesis about a controlled electron flow from phylloquinones at the quinone-binding sites of PS I to O_2 is proposed.

Lecture

Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer

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Photosynthetic complex I enables cyclic electron flow around photosystem I, a regulatory mechanism for photosynthetic energy conversion. We report a 3.3 Å resolution cryo-EM structure of photosynthetic complex I from the cyanobacterium *Thermosynechococcus elongatus*. The model reveals structural adaptations that facilitate binding and electron transfer from the photosynthetic electron carrier ferredoxin. By mimicking cyclic electron flow with isolated components *in vitro*, we demonstrate that ferredoxin directly mediates electron transfer between photosystem I and complex I, instead of using intermediates such as NADPH. A large rate constant for association of ferredoxin to complex I indicates efficient recognition, with the protein subunit NdhS being the key component in this process.

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Lecture

Room temperature immobilization of photosynthetic reaction centers in amorphous matrices: testing the role of protein dynamics in electron transfer

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Trehalose glassy matrices exhibit an exceptional efficacy in protecting hosted proteins against denaturation induced by freezing, heating and drying. This property is exploited *in vivo* by anhydrobiotic organisms which can withstand extreme draught and high temperatures for a very long time [1]. Thermal stabilization of proteins embedded into dry trehalose matrices stems from the dramatic hindrance of internal protein dynamics. Protein-trehalose glasses represent therefore an attractive system to test the role of dynamics in protein functions. Protein conformational dynamics can in fact be retarded by many orders of magnitude at room temperature by reducing the hydration level of the embedding amorphous matrix. We used such an approach to examine how conformational dynamics control electron transfer processes catalyzed by photosynthetic reaction center complexes. An overview will be presented of optical and high-field EPR spectroscopic studies performed in trehalose-coated bacterial reaction center, in the absence [2] and presence of light-harvesting complex 1, and photosystem I [3]. In all complexes, the incorporation in soft glasses inhibits electron transfer to the terminal acceptors; only extensive dehydration hinders protein relaxations and electron transfer processes localized in inner regions of the protein complexes, mimicking at room temperature effects observed in hydrated systems at cryogenic temperatures. Trehalose-coated reaction center complexes retain their native structure for months at temperature as high as 40°C. We propose that the protein-matrix dynamical coupling is governed by a network of multiple H bonds connecting surface protein residues, sugar, and residual water molecules at the protein-matrix interface (anchorage model) [4]. Such a network, at low water contents, tightly locks the protein surface to the solid matrix.

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Poster

Carotenoid-induced formation of LH1 light-harvesting complexes from B820 subunits of sulfur photosynthetic bacterium *Ectothiorhodospira haloalkaliphila*

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Using chromatography on DEAE-sepharose, the B820 subunit has been obtained from the sulfur bacterium *Ectothiorhodospira haloalkaliphila* with inhibited synthesis of carotenoids (Crts). This subunit had a strong affinity for Crts, similar to the B820 subunit of the non-sulfur photosynthetic bacterium *Rsp. rubrum* [1]. Adding Crts in the form of a concentrated solution in an organic solvent (acetone) to a buffer system containing B820 in 1% β-octyl glucoside (β-OG) resulted in the rapid formation of spectral forms with the Qy absorption band shifted to 889–890 nm. During this process, Crts were incorporated into the structure of the newly formed complexes. The complexes were purified by polyacrylamide gel-electrophoresis. The absorption bands of Crts in these complexes were also shifted to the red with respect to the absorption bands of Crts in acetone or in 1% β-OG, which did not contain peptides of the LH1 complex. The resulting complexes were reconstructed with different C40 Crts, differing both in side functional groups and in the number of conjugated double bonds, increasing from 9 to 13. Together with an increase in the conjugation system, the absorption bands of Crts gradually shifted to the long-wavelength region. The color of these complexes was completely determined by the incorporated Crts. The circular dichroism and fluorescence excitation spectra indicated that the environment and interaction of the cofactors of the reconstructed and native LH1 complexes closely matched each other. A set of these complexes, differing only in the type of embedded Crts associated with the same bacteriochlorophyll-protein matrix, is a promising model system for studying the interaction between Crts and the functioning pigment-protein apparatus of the complexes. We have obtained complexes both with complete filling of the binding sites of Crts and intermediate complexes with a reduced content of Crts. The latter complexes are able to accept an additional amount of Crts.

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Poster

Structure of photosystem I from halotolerant green algae *Dunaliella salina*

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Solar energy harnessed by oxygenic photosynthesis the most abundant energy-storing and life-supporting process on earth, it is employed by cyanobacteria, algae and plants to power themselves for growing and proliferating. The light reactions of photosynthesis rely on the co-operative interplay of two large multisubunit protein-pigment complexes, designated photosystem I (PS I) and photosystem II (PS II), that are embedded in the thylakoid membrane of eukaryotic subcellular organelles called chloroplast. PS I is highly efficient nanomolecular machine, exhibiting a quantum efficiency close to 100%. PS I functions as a plastocyanin-ferredoxin oxidoreductase that accepts and transfers electrons from the luminal donor plastocyanin/Cyt c6 to ferredoxin at the stromal side via a chain of electron carriers. The multisubunit supercomplex PS I can be divided into two parts: the reaction centre where the charge separation occurs, and the peripheral light-harvesting antennas which enlarge the absorption cross-section of the core. Core complexes have been well conserved during evolution, whereas the light-harvesting antennas divers among photosynthetic organisms considerably from cyanobacteria to higher plants. The structure of plant PS I comprises core and light-harvesting (LHCI) complexes, which together form PS I-LHCI. Studies indicated that green algal PS I-LHCI is more versatile than plant. Here, we report the structure of PS I–LHCI from halotolerant green alga *Dunaliella salina* at 3.5 Å resolution, obtained by single-particle cryo-electron microscopy, revealing 13 core subunits and 6 light-harvesting complex a (Lhca) antennas, including a novel four-transmembrane-helix Lhca. Overall, the data indicate that DsPSI–LHCI represents a modular organization for photosynthetic acclimation and provide important information for reconstructing the plasticity and evolution of PS I.

Poster

High-potential quinone in the A_1-binding site of photosystem I can reduce molecular oxygen after flash-induced charge separation

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Flash-induced reduction of the primary electron donor P_{700}^+ in photosystem I (PS I) from *menB* mutant of *Synechocystis* sp. PCC 6803, with quinone in the A_1 binding site substituted with high-potential 2,3-dichloro-naphthoquinone (Cl_2NQ), was studied in the presence of varying concentration of external electron acceptors [1]. Under these conditions electron transfer to the iron-sulfur clusters did not occur. Kinetic modelling of P_{700}^+ reduction revealed the existence of electron escape from the secondary electron acceptor A_1 even in the absence of artificial acceptors, which was interpreted as A_1 reaction with molecular oxygen in the surrounding medium.

Analysis of the PS I crystallographic structure revealed the presence of two water-filled cavities within 1 nm of A_1 binding sites connected with the external water, which may be engaged by molecular oxygen during its reaction with A_1. Molecular dynamics modelling of PS I was used to analyze the oxygen binding to the intraprotein water pocket. Free energy profile of the interaction between molecular oxygen and the protein complex (potential of mean force) was calculated by molecular dynamics simulation of oxygen transfer along 1.5 nm channel from inner-most part of the water pocket to the surrounding water solution. Total molecular dynamics simulation time exceeded 100 ns. The potential of mean force revealed a deep (>20 kJ) minimum in the proximal to A_1 hydrophobic part of the channel, where the nonpolar oxygen molecule can be captured owing to substitution of polar water molecules (hydrophobic effect).

Interaction of water-dissolved oxygen with quinone in the A_1-binding site can lead to the side production of superoxide radical by PS I (Mehler reaction), comprising over 0.3% of the total electron flow in the intact PS I complexes. The existence of highly efficient electron transfer to the iron-sulfur clusters in PS I may serve as an evolutionary implementation against such bypassing.

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Poster

Kinetic analysis of the reaction between $NADP^+/H$ and ferredoxin-$NAD(P)^+$ reductase from green sulfur bacterium in the presence of ferredoxin

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Ferredoxin-$NADP^+$ oxidoreductase (FNR, [EC 1.18.1.2]) is a flavoprotein catalyzing the reversible redox reaction between $NADP^+/H$ 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, which is distinctive from plant-type FNR. In our previous work, *Ct*FNR promotes rapid NADPH oxidation, but reduction of $NADP^+$ by reduced *Ct*FNR was somewhat slow [1]. In this work, we examined the reaction of *Ct*FNR with $NADP^+/H$ in the presence of ferredoxin using a stopped-flow spectrophotometry.

*Ct*Fd was expressed in the *E. coli* cells and purified to homogeneity. UV-vis absorption spectrum of the purified *Ct*Fd was typical of a bacteria-type Fd with the A385/A280 ratio of > 0.7. EPR spectrum indicated that purified *Ct*Fd contains [3Fe-4S] type iron sulfur cluster. Reconstitution of the iron sulfur cluster under the anaerobic conditions successively gave the spectrum of [4Fe-4S] type one, and utilized for the measurement. Mixing $CtFNR_{ox}$ with NADPH resulted in a rapid formation of CT complex followed by a rapid reduction of *Ct*FNR ($k_{obs} > 300\ s^{-1}$). The presence of $CtFd_{ox}$ hardly affected the observed rate for the oxidation of NADPH. At the equilibrium, most *Ct*Fd remained in oxidized state, indicating redox potential of *Ct*Fd is low as previously reported [2]. When $CtFNR_{red}$ was mixed with $NADP^+$, the presence of Fd hardly affected the observed rate. The reaction mechanism of $NADP^+$ reduction catalyzed by *Ct*FNR with reduced *Ct*Fd as donor will discussed.

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Section 1.4: Photosystem II and Water Oxidation Mechanism

Lecture

Hydrophobic interactions between the D1 and PsbT subunits of Photosystem II stabilize the iron-quinone acceptor complex

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In Photosystem II, the iron-quinone acceptor complex contains the primary plastoquinone electron acceptors Q_A and Q_B, a non-heme iron (NHI) and a bicarbonate ligand to the NHI. The loop region connecting the D and E α-helices of the reaction center D1 subunit in Photosystem II (the DE loop), lies directly above the iron-quinone acceptor complex, and is the site of frequent light-induced damage. The DE loop appears to interact with the PsbT subunit of Photosystem II through hydrophobic interactions between the Phe-239 residue of D1 and the Pro-27 and Ile-29 residues of PsbT. To investigate the importance of this interaction, mutants were generated in *Synechocystis* sp. PCC 6803 to disrupt the interaction between the DE loop and PsbT. Two mutants (F239A, F239L) were generated at the Phe-239 position in D1, along with a mutant lacking the PsbT subunit (ΔPsbT). The physiology of the mutants was characterized using chlorophyll *a* variable fluorescence induction and decay measurements, oxygen evolution assays and low-temperature fluorescence spectroscopy. In addition, radiolabeled pulse-chase experiments were performed and analyzed using blue-native polyacrylamide gel electrophoresis. When grown under 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ light, the F239A mutant displayed considerably slower rates of oxygen evolution and chlorophyll *a* variable fluorescence decay compared to the control, while the F239L mutant showed a similar phenotype to the control. In contrast, following high light treatments, both the F239A and F239L mutants displayed increased high light sensitivity, in comparison to the control; although, both the F239A and F239L mutants were more tolerant to high light that the ΔPsbT mutant. This indicates that the interaction between the DE loop and PsbT plays a role in efficient acceptor side electron transport and photoprotection. During growth under 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ light, it appears that this interaction is disrupted in the F239A mutant, but not the F239L mutant. In contrast, high light conditions (e.g., 200 $\mu E \cdot m^{-2} \cdot s^{-1}$) appear to be capable of disrupting this interaction in both mutants. Since the DE loop contains residues that bind the bicarbonate, the underlying cause of the observed phenotypes associated with the different mutants has been putatively assigned to the dissociation of bicarbonate from the NHI. Consequently we hypothesize that increased DE loop flexibility, through the disruption of the interaction between the DE loop and PsbT, is likely to facilitate bicarbonate dissociation.

Lecture

Mutation of D1 and D2 residues associated with bicarbonate and bound waters on the acceptor side of PS II impair the quinone-Fe-acceptor complex

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The bicarbonate co-factor of the quinone-Fe-acceptor complex of Photosystem II (PS II) is a bidentate ligand to the non-heme iron found between the primary (Q_A) and secondary (Q_B) plastoquinone electron acceptors. It has been hypothesized that the protonation of Q_B proceeds first through D1-His252 and D1-Ser264 to give $Q_B^-(H^+)$ followed by the second proton (resulting in Q_BH_2) being delivered via the D1-His272 and D1-His215 ligands of the non-heme iron [1, 2]. The pathway for the second proton is hypothesized to include two waters that are designated as W582 and W622 in PDB 4UB6. W622 has hydrogen bonds to both bicarbonate and W582, and protons are suggested to pass via this route to D1-His272 and D1-His215. We have introduced mutations at D1-Glu244 and D1-Tyr246 that hydrogen bond to W582 and W622, respectively, and D1-Tyr246 is also within hydrogen-bonding distance from bicarbonate. The W582 water is also hydrogen-bonded to D2-Thr243: additionally, D2-Glu242 interacts with D1-Glu244 and is also hydrogen-bonded to D2-Lys264, potentially stabilizing the hydrogen-bond network around W582. We have also introduced mutations at D2-Glu242, D2-Thr243 and D2-Lys264. In our mutants, which assemble near wild-type levels of PS II, Q_A to Q_B electron transfer is substantially slowed. Moreover, D2-Tyr244 is hydrogen-bonded to bicarbonate and targeting this residue also slows Q_A to Q_B electron transfer, but, in addition, PS II assembly is impaired and the back reaction with the S2 state of the oxygen-evolving complex is also disrupted. Our results indicate that D2 plays an equal role to D1 in supporting the putative pathway of protons from the cytosol to Q_B via bicarbonate. In addition, our results support the interpretation that bicarbonate and the W582 and W622 waters are required for the normal operation of the quinone-Fe-acceptor complex even after a single turnover of the reaction center.

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Lecture

FTIR study on the water oxidation reaction in photosystem II microcrystals

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Photosystem II (PS II) performs water oxidation through a light-driven cycle of intermediates called S states (S_0-S_4) at the water oxidizing center (WOC). To clarify the molecular mechanism of the water oxidation reaction, time-resolved serial femtosecond crystallography (SFX) has been applied to the microcrystals of PS II core complexes [1]. However, it is crucial to know whether the reactions efficiently proceed and the intermediates retain their native structures in PS II crystals. In this study, we investigated the water oxidation reactions in PS II microcrystals using flash-induced Fourier transform infrared (FTIR) difference spectroscopy, which can monitor the enzymatic reactions detecting even very subtle structural changes. FTIR difference spectra were measured using the PS II microcrystals from *Thermosynechococcus vulcanus* deposited on the surface of a silicon prism of the attenuated total reflectance (ATR) accessory. ATR-FTIR difference spectra upon 4 successive flashes to the microcrystals showed very similar features to those of PS II in solution. It was thus shown that the structures of all the intermediates were virtually unchanged by crystallization [2]. It was also found that the efficiencies of the S-state transitions were kept relatively high, although those of the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions were slightly lowered in the microcrystals [2]. We further analyzed the S-state cycle in a single PS II microcrystal using FTIR microspectroscopy. Microscopic FTIR difference spectra of a single microcrystal, which were measured using a transmission method, showed features virtually identical to the spectra of numerous PS II microcrystals obtained with the ATR method. This result indicates that the S-state transitions proceeded with relatively high efficiencies at the WOC retaining native structures in the entire inside of the PS II microcrystal.

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Lecture

How different wavelengths of light change energy transfer and trapping in far red light adapted cells of the Chl *f* containing cyanobacterium *Halomicronema hongdechloris*

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The phototrophic cyanobacterium *Halomicronema hongdechloris* contains chlorophyll *a* and *f* in photosystem II. While *H. hongdechloris* has only very low amounts of Chl *f* in white-light culture conditions the ratio of Chl *f* to Chl *a* is reversibly changed up to 1:8 under illumination with far red light (720–730 nm) (FRL). The phycobilisomes (PBS) exhibit highly efficient excitation energy transfer (EET) to Chl *a* and from there to Chl *f* within 200 ps apparent transfer time if *H. hongdechloris* grown under FRL is illuminated with 630 nm laser radiation which is absorbed by PBS. Our simulations and thermodynamic considerations suggest that the time- and wavelength-resolved ps fluorescence data can be explained assuming light-induced far red-shifted traps of excitation energy localized on Chl *f* in the light harvesting antenna while the large majority of Chl *a* is strongly coupled to these Chl *f* traps driving an uphill EET from Chl *f* to Chl *a* by an entropy effect. Chl *a* might possibly represent the primary donor in photosystem II (PS II); however, energy would still migrate from far red antenna Chl *f* traps to the special pair and finally drive water oxidation [1].

After adaption to FRL the PBS of *H. hongdechloris* are localized in separated clusters of the cell. In parallel, high contents of carotenoids are found when *H. hongdechloris* grows on far red light. Short illumination with blue light (405 nm) and red light (630 nm) leads to a mobilization of the PBS on the time scales of seconds. The PBS shortly appear completely decoupled from PS II for several seconds and subsequently recouple to PS II with efficient excitation energy transfer from PBS to PS II. Such light-triggered PBS mobilization is not observed upon illumination of the cells with green light (530 nm). From quantitative analysis of the PBS mobility in dependence of the applied light intensity and wavelength we assume that production of reactive oxygen species after excitation of Chl *a* leads to mobilization and recoupling of the PBP antenna complexes after the cells had been adapted to FRL.

H. hongdechloris appears as a highly adaptive organism that can grow under FRL conditions and develop in ecological niches not favorable for other organisms, but it is still capable to carry out efficient light harvesting, EET and, finally, growth in white light radiation by quick PBS mobilization, protective levels of carotenoids and efficient EET from PBS to the special pair that establishes within seconds when white light is offered.

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Lecture

Characterisation of a phylogenetically distinct group of D1 proteins from cyanobacteria

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The *psbA* gene family in cyanobacteria encodes different forms of the Photosystem II reaction centre D1 protein. Previous phylogenetic analyses have shown that D1 proteins separate into four distinct groups from G1 to G4 [1,2] and we have expanded this approach to include 206 cyanobacterial strains. Our extended analysis identified a collection of sequences representing a D1 grouping that is intermediate between G3 and G4 and that separates into two sub-groups. One of these sub-groups ($D1^{int}$) is usually present in the genomes of nitrogen-fixing heterocystous cyanobacteria and is located in close proximity to either genes encoding urease subunits or an NADP-dependent malic enzyme. The second sub-group ($D1^{FR}$) is associated with the far-red light photoacclimation gene cluster of cyanobacteria [3,4]. We will describe these groups of the D1 protein and their distribution across cyanobacteria. In addition, using a triple *psbA*-deletion strain of the non-nitrogen-fixing *Synechocystis* sp. PCC 6803, we have investigated how a $D1^{int}$ from *Nostoc punctiforme* ATCC 29133 (NpF1022) alters photosynthetic performance when introduced under the *psbA2* promoter. Furthermore, we have investigated the role of signature amino acid changes associated with $D1^{FR}$ following their introduction into the Group 4 D1 protein encoded by *psbA2*.

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Lecture

Relationship between pigment-protein structure and dynamics of the energy transfer and trapping in cyanobacterial PS I

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Cyanobacterial photosystem I (PS I) constitutes monomeric and trimeric pigment-protein complexes [1], the optical properties of which are marked by the presence of long-wavelength absorption bands. In spite of numerous experimental studies, the nature of these bands is still under debate and requires intensive theoretical analysis. Collecting together the data of linear spectroscopy, single molecule spectroscopy and pump-probe measurements of PS I from *Arthrospira platensis*, we performed the quantum modeling of optical response based on the molecular exciton theory and the multimode Brownian oscillator model [2–4]. Within the framework of our PS I exciton model, the long-wavelength bands of the spectra are attributed to so-called antenna red chlorophylls. Discussion of exciton model micro parameters of these chlorophylls such as site energies, interaction between pigment molecules, the electron-phonon coupling (Huang-Rhys factors), and also the red chlorophyll localization in PS I monomer and trimer are the subjects of our presentation.

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Poster

Bicarbonate dehydration activity of Photosystem II, is it a myth or a reality?

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As it is known, bicarbonate ion is needed for optimal photosystem II (PS II) function. Therefore, this complex should be supplied by bicarbonate using a high rate of interconversions $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. Actually, this activity was found in PS II and was termed as carbonic anhydrase activity (CA-activity). The direction of the reaction from left to right named as hydration, the opposite direction – dehydration.

The phenomenon of PS II CA-activity has been studied over the past few decades. The hydration activity was low (about 12–15 un. W-A/mg of Chlorophyll), but the measurement always were precise and reliable. Attempts in dehydration activity measurements were made several times by different methods. But the results were defined as not precise. In addition, they were presented in units of activity that did not allow to compare their amount with the level of activity for known carbonic anhydrases. In a number of works on PS II was shown that the level of activity was lower in dehydration direction then in hydration one. Thereby some researchers had doubts about enzymatic nature of CA-activity in PS II and its significance for water oxidizing. In our recent paper, we showed the significance of CA-activity for revealing of maximal electron transfer rate in PS II.

In this work, we optimized one of the methods of dehydration activity registration (published by Shingles and Moroney) for measurements in PS II. This procedure based on quick registration of pH change using Stop-flow technic. The dehydration activity was determined with high accuracy (80±5 un. W-A/mg Chl). The rate was 6 times higher than hydration in the same PS II preparation. This fact may indicate on a significance of dehydration reaction as a main function of CA-activity for PS II. The dependence of the activity from substrate concentration, matched the Michaelis-Menten equation. This finding allowed us to determine K_m=2.7 mM, V_{max}=1.2 rel.un.fl./sec (0.0054 dpH/sec), k_{cat}=2.1 x 10^5 sec^{-1}, k_{cat}/K_m=7.6 x 10^7 $M^{-1}\cdot sec^{-1}$ for the first time for PS II.

Showed above parameters were measured also for known and high active carbonic anhydrase II from bovine erythrocytes (Sigma, USA, Cat. № C3934). Obtained results (K_m=25.7 mM, V_{max}=25.8 rel.un.fl./sec (0.142 dpH/sec), k_{cat}=8.2 x 10^6 sec^{-1}, k_{cat}/K_m=3.2 x 10^8 $M^{-1}\cdot sec^{-1}$) were in agreement with literature data and testify about applicability of optimized method for determination of high rate of carbonic anhydrase reaction. The turnover number (k_{cat}) and rate constant (k_{cat}/K_m) for CA-activity in PS II differed no more than an order of magnitude from ones for erythrocyte carbonic anhydrase. Thus, we show that PS II CA-activity has an enzymatic nature and reveals high efficiency of catalysis.

Poster

Quantitative assessment of the photosystem II oxygen-evolving complex inactivation by OJIP-curves

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To assess the activity of the photosystem II (PS II), fluorescence transients called OJIP-curves are traditionally used. The kinetics of chlorophyll *a* fluorescence is a multiphase curve. The phases of the curve are usually related to the stages of electron transfer in PS II. The initial part of the curve, the so-called K-phase, is usually related to the state of the oxygen-evolving complex (OEC) located on the donor side of PS II. To visualize the K-phase, a method of comparing induction curves based on subtracting control OJIP-curve from those recorded under different conditions is often used. It is believed that the appearance of the K-phase (maximum on the difference curve at about 300 μs) indicates the inactivation of the OEC in the sample. However, this method provides only a qualitative description of the OEC inactivation. We hypothesized that a change in the magnitude of the K-phase maximum (we called it as Δ_{max}) may indicate a fraction of photosystems with inactivated OEC. Confirmation of this hypothesis would allow to quantify the degree of inactivation of OEC using OJIP-curves. To test this hypothesis, we conducted the following experiment to assess the degree of inactivation of OEC. We used photosystem II prepared from spinach leaves and cell suspension of *Chlorella* algae as model systems. At first, the samples were heated to a certain temperature in the range from 25°C to 50°C, then cooled, and the rate of oxygen evolution was measured, and fluorescence induction curves were recorded as well. The temperature dependences for the rate of oxygen evolution (V_{O_2}) and for the Δ_{max} parameter, calculated from the induction curves, were obtained. The correlation coefficient between the temperature curves for V_{O_2} and for Δ_{max} measured on PS II samples was 0.99, whereas for *Chlorella* cells it was 0.95. The high correlation between the curves allows us to offer Δ_{max} as a parameter that quantitatively characterizes the OEC inactivation degree.

Poster

Influence of vibrations of conjugated bonds of lutein on the energy transfer rate in LHCII

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LHCII is the primary light-harvesting complex in higher plants photosystem II. These complexes are widely studied both experimentally and theoretically. In particular, the phenomenon called non-photochemical quenching of fluorescence (NPQ) is of great interest since this is one of the photoprotecting mechanisms of higher plants [1].

The process of NPQ appears at high intensity of light. The essence of this phenomenon is that the efficiency of energy transfer from chlorophylls to the dark state of the carotenoids increases [2]. As a result, excitation energy can disappear only through dissipation and quantum yield of fluorescence decreases.

Non-empirical all-pigment model was proposed to describe the excited states of the LHCII by means of the excitonic Hamiltonian approach. Linear adsorption and circular dichroism spectra which are in a good agreement with the experimental data were obtained. It was shown that the most effective transition takes place between chlorophyll *a* and lutein (CLA612 and LUT620 according to 1rwt PDB structure). Six vibrational normal modes for LUT620 were singled out for which locations of PES minima of the ground and excited states differs significantly. The vibrational structure of lutein adsorption spectra was modeled using excitation energies scans along these modes and excitation energy gradients.

For lutein geometries displaced along these normal modes rate constants of energy transfer to lutein molecule were calculated. It was shown that such displacements has a great impact on the energy transfer rate and thus it should be taken into account when modeling of NPQ is performed.

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Poster

Interaction of trivalent metal cations with the high affinity Mn-binding site of photosystem II oxygen-evolving complex

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The catalytic center of the photosystem II (PS II) oxygen-evolving complex (OEC) consists of 4 cations of Mn and one cation of Ca connected by oxygen bridges (Mn_4CaO_5). Extraction of Mn cations from OEC is accompanied by appearance of only one high-affinity (HA) Mn-binding site. The Mn cation connected with this site is oxidized by the tyrosine Y_Z. HA site binds along with Mn^{2+} and Fe^{2+} cation which is oxidized by Y_Z under illumination. Formed Fe^{3+} cation is effectively bound by HA site and blocks it, preventing the ligation of exogenous Mn cation at this site [1]. Thus, it means that actually not the divalent metal cation, but trivalent cation binds to the HA Mn-binding site with high affinity. It should be noted that the same situation also takes place in the case of Mn cations in the photoactivation process, for example. Thus, these results demonstrate that in the binding of a metal cation to the HA site, its valency is a decisive factor. Therefore, in the presented work the interaction of some other trivalent metal cations, such as Tb^{3+}, La^{3+}, Al^{3+}, Cr^{3+} with the HA Mn-binding site was investigated. We revealed for the first time that not only cations of Fe^{3+}, but also cations of other trivalent metals effectively bind to the HA site, by showing the inhibitory effect of these cations on the interaction of Mn^{2+} with it. The blocking of HA Mn-binding site by bound trivalent cations inhibit the transfer of an electron to the oxidized Y_Z by the exogenous electron donors – a donor system [Mn^{2+} + H_2O_2] and 1,5-diphenilcarbazide. In the case of a donor system [Mn_{2+} + H_2O_2] the effect of full blocking of electron transport to Y_Z is observed at the same concentration of lanthanoids and Cr^{3+} cations – 50 μM. Al^{3+} ions block the electron transport from the same donor up to 75% in the concentration range 0.05 – 10 mM. Blocking of the HA Mn-binding site by Fe^{3+} cations provides 50% inhibition of electron transfer via PS II when 1,5-diphenilcarbazide was used as electron donor [1]. The same effect (50% inhibition in the concentration 200 – 500 μM) was also observed in the case of trivalent cations studied in our work. The blocking of electron transport from electron donor via the HA site to Y_Z is supported by measurement of fluorescence induction kinetic in the Mn-depleted PS II membranes in the presence of electron donors and terbium cations. The obtained data demonstrates that Mn cation connected with this site in native PS II is in a trivalent state in the PS II membranes adapted to darkness.

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Poster

Light-induced conformational changes and rate-limiting steps in Photosystem II revealed by FTIR, transient absorption and fluorescence measurements

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Photosystem II (PS II) is the redox-active pigment-protein complex embedded in the thylakoid membrane that catalyzes the oxidation of water and the reduction of plastoquinone. Despite the detailed structural information and the wealth of functional data, our understanding of the processes associated with or accompanying the functioning of the reaction center of PS II is incomplete. We investigated chlorophyll *a* fluorescence transients, elicited by trains of single-turnover saturating flashes (STSFs), of dark-adapted cyanobacterial cells, isolated plant thylakoid membranes and PS II core complexes in the presence of DCMU [1]. Our measurements performed between 170 and 280 K showed that the first STSF raised the fluorescence yield from F_o to F_1 (F_o-F_1) and further flashes were required to reach the maximum level F_m (F_1-F_m). However, the F_1-F_m increment could only be reached with a sufficiently long waiting time between the excitations – suggesting the existence of a rate limiting step in the dark-to-light transition of PS II. Further, while the F_1 level remained stable in the absence of charge recombination, the F_m level decayed at all temperatures. These data and the nanosecond absorption transients at 819 nm indicate that the F_o-F_1 rise and the F_1-F_m increment are generated by different physical mechanisms, the stable charge separation involving the reduction of Q_A, and P^+Pheo^- charge separation followed by rapid recombination, respectively [2]. Differences between single and multiple excitations were also revealed by rapid-scan FTIR measurements. These findings are interpreted in terms of conformational changes/dielectric relaxation processes due to local electric fields in the reaction center complex.

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Poster

Trehalose matrix effects on charge-transfer reactions in Photosystem II at different dehydration levels

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In nature, the bioprotective action of trehalose and other disaccharide glasses is used by several organisms able to survive for long periods (up to years) of extreme draught and high temperatures by preserving the structural integrity of their cellular structures, while reversibly arresting their metabolism (anhydrobiosis) [1]. Here, matrix effects on long-range electron transfer were studied in Mn-depleted PS II complexes, embedded into trehalose glasses at different hydration levels. In dry trehalose glasses at room temperature, PS II was stable for months. Measurements of the absorption changes at 830 nm using optical spectroscopy showed that the kinetics (2–40 μs) in hydrated PS II-trehalose glasses mostly reflected the reduction of the photooxidized primary donor $P_{680}^{\bullet+}$ by the redox active tyrosine Y_Z (tyrosine 161 of the D1 subunit). Continuous distributions of lifetimes were extracted from the kinetics by a maximum entropy method (MemExp program). The obtained results revealed that the contribution of the fast (2–10 μs) component, which mostly correspond to the kinetics in the solution (also in the presence of a high – 1.2 M trehalose concentration) was at least halved in the glasses at $11\% < r < 53\%$. In parallel at least two additional slower components appeared in the glasses with lifetime in the 100 μs and 1 ms time scale. The fastest phase decreased in amplitude upon dehydration, and its lifetime tended to increase. The intermediate phase was approximately constant in amplitude, but its lifetime increased. The amplitude of the slow millisecond phase increased upon dehydration, while its lifetime was more or less constant or slightly decreased. The study presented for PS II provides new insights into the crucial issue of protein-matrix interactions for protein functionality controlled by hydrogen-bond networks of the hydration shell.

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Poster

Synthetic Cu(II) complexes affect reaction center components of photosystem II

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As a process crucial for plant development, photosynthesis is an attractive target for numerous synthetic compounds aimed to be suppressors of various metabolic pathways in plant organism. The studies are carried out in order to create the inhibitory compound capable of multiple suppression of several key reactions to obtain new means of plant growth control.

Here, new synthetic Cu(II) complexes were characterized as potent inhibitors of photosynthetic activity related to photosystem II (PS II). First, we found that the agents inhibit photoinduced oxygen evolution on the thylakoids mainly from PS I in the presence of hydrophilic artificial electron acceptor FeCy, i.e. they suppress electron transfer from water through whole ETC. To test a possibility that complexes impair the interaction between photosystems, we compared the effect of Cu(II) complexes on photoinduced changes of fluorescence yield with the action of DCMU that blocks electron transfer at Q_A/Q_B site and DBMIB that disturbs the interaction between photosystems at Q_0 site of Cyt b_6f. However, it was revealed that Cu(II) complexes affect PSII-PSI interaction to a rather more less extent than DCMU and DBMIB. Therefore, we checked effects of Cu(II) complexes on ET through PS II as: (1) photoinduced O_2 evolution by thylakoids but in the presence of electron acceptor mainly from PS II (lipophilic DCBQ + FeCy); and (2) photoinduced changes of PS II chlorophyll fluorescence yield. It was found that Cu(II) complexes inhibit photosynthetic O_2 evolution of PS II, and this effect is nearly comparable with the decreased O_2 evolution throughout the whole ETC. Furthermore, the agents decrease the ΔF value in concentration-dependent manner, but do not change F_0 level and do not have other effects typical for DCMU and DBMIB. As the addition of electron donors does not change the result in the presence of Cu(II) complexes, it can be concluded that the possible site of their action is at the level of the components of PS II reaction center.

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Poster

Quantification of bound bicarbonate in photosystem II

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Over twenty years ago professor V. V. Klimov with his co-workers at the Institute of basic biological problems RAS revealed stimulating effects of bicarbonate ions on various events at the donor side of photosystem II (PS II). An explanation of those effects implied bicarbonate binding to components of the water-oxidizing complex [1]. To test that explanation, an overall content of CO_2/bicarbonate in PS II membrane preparations and in the isolated PsbO protein was measured in the present work. We applied a new approach based on a combination of membrane-inlet mass spectrometry and ^{18}O-labelling [2]. The vacuum of the mass spectrometer provided full extraction of CO_2, and as a result no interchangeable bicarbonate ions could be retained in the preparations, in contrast to the formerly used replacement of bicarbonate by formate. Before the measurements, the preparations were equilibrated with air to saturate all physiological binding sites with ambient bicarbonate. With this approach, we determined that in spinach PS II membrane fragments 1.1 ± 0.1 HCO_3^- are bound per PS II reaction centre, while none was bound to isolated PsbO protein. We suppose that this one HCO_3^- molecule is delivered from the non-heme iron complex of PS II, while unbound HCO_3^- optimizes the water-splitting reactions by acting as a mobile proton shuttle.

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Poster

Function of PsbO-Asp158 in Photosystem II: Evidence from mutagenesis studies in the thermophilic cyanobacterium *Thermosynechococcus vulcanus*

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PsbO is one of the extrinsic proteins of photosystem II (PS II) and functions in maintaining binding of the manganese cluster. PsbO participates in the formation of extended hydrogen-bonding networks that starts from the vicinity of manganese cluster and extends to the lumen. PsbO-D158 (cyanobacterial numbering) is a highly conserved residue and participates in one of the hydrogen-bonding networks. In order to examine the role of PsbO-D158, we replaced this residue with E, N or K in *Thermosynechococcus vulcanus* (*T. vulcanus*) and characterized the photosynthetic properties of the mutants obtained. The photoautotrophic growth of these three mutants was found to be almost the same as that of the wild type, whereas their oxygen evolving activities were approximately 60–64% of the wild type. Chlorophyll fluorescence relaxation kinetics showed that the mutations did not change the electron transfer rate from Q_A to Q_B, but slightly affected the donor side of PS II. Moreover, all of the three mutant cells were more sensitive to high light and became slower to recover from photoinhibition. In the isolated thylakoid membranes from the three mutants, the PsbU subunit was lost and the relative oxygen evolving activities were reduced to a lower level. PS II core complexes isolated from these mutants showed no oxygen-evolving activity, which was found to be due to the loss of PsbO, PsbV and PsbU during purification. Moreover, PS II core lacking the PsbO, PsbV and PsbU subunits contained Psb27, an assembly co-factor for the mature PS II. These results suggest that the PsbO-D158 residue is required for the functional binding of the three extrinsic proteins to PS II and plays an important role in maintaining the optimal oxygen-evolving activity, and its mutation caused incomplete assembly of the PS II complex.

Poster

Investigation of the of O_2-dependent photoinactivation of isolated D1/D2/cytochrome b559 photosystem 2 complex upon photoaccumulation of a long-lived state with the reduced pheophytin ($P680Pheo^-$)

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The photoinactivation of the D1-D2-cyt b559 photosystem 2 (PS2) reaction center (RC) was investigated. A new type of photoinhibition of the D1-D2-cyt b559 RC associated with the oxidative transformations of reduced pheophytin with participation of molecular oxygen was revealed. It was shown that the revealed O_2-dependent photoinactivation of D1-D2-cyt b559 RC is accompanied by damage to the pigments in D1-D2-cyt b559 RC.

Section 1.6: Plant Development and Growth Regulation

Lecture

The increase in the activity of AOS enzymes is an indicator of abnormal growth of woody plants, which differ in the heartwood/sapwood ratio

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Xylogenesis of plants is epigenetic process. Its implementation is influenced by both genetic factors and changes in environmental conditions. Different scenarios of xylogenesis are reflected in the normal or abnormal growth of woody plants, which results in a change in the composition of cell walls in the ratio of cellulose/lignin, which can be accompanied by the formation of unusual (patterned, spiral grain) wood, and accordingly changes its physical and chemical properties. On the example of woody plants that differ in the heartwood/sapwood ratio, such as Karelian birch (*Betula pendula* Roth var. carelica (Mercl.) Hämet-Ahti), Scots pine (*Pinus sylvestris* L.) and European spruce (*Picea abies* (L.) H. KARST.), the relationship between the cascade work of the AOS enzymes and the degree of implementation of alternative scenarios of xylogenesis, which can often be observed even within the trunk of a single tree, is revealed. The metabolic reason for the strengthening of the role of the AOS enzymes (SOD, CAT, POD, PPO) is the invertase pathway of sucrose metabolism, which comes from the photosynthetic donor leaves to the tissues of the trunk – acceptors. The increasing content of free hexoses is realized along the way of synthesis of ROS and phenols, which, as well as being signal molecules, trigger a chain of enzymes of AOS – diagnostic indicators of the abnormal wood formation. In addition, the higher activity of SOD, POD and CAT in abnormal tissues involves superoxide radical and hydrogen peroxide in the lignin biosynthesis, causing a more rigid structure of abnormal tissues. It is interesting that the activity of PPO – as one of the key enzymes of secondary metabolism, in addition to indicating the degree of development of abnormal wood, may also indirectly reflect the heartwood/sapwood ratio in the studied woody plants. Thus, the activity of the AOS enzymes is a diagnostic sign of the implementation of different programs of xylogenesis and the degree of manifestation of abnormal growth of woody plants.

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Poster

Effects of plant growth regulators on the composition of phenolic compounds in Kale *Brassica oleracea* var. sabellica

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One of the most important metabolites of plants are phenolic compounds (PCs), the proportion of which can reach 30% of their biomass [1]. The functional role of PCs is associated with the processes of photosynthesis, respiration, growth, development, and also the protection of cells and tissues from stressful influences [2]. Flavonoids (FLs) are one of the largest classes of PCs, They play an important role in ultraviolet (UV) protection since UV-B responsive flavonoids can reduce the risk of reactive oxygen species (ROS) generation and thereby prevent oxidative damage [3]. Therefore, the impact of flavonoids on the human body after food consumption as well as their effect as pharmaceutical supplements was discussed in several reviews [4].

Brassica oleracea is an important source of secondary plant metabolites, especially phenolic compounds, flavonoids and other polyphenols [5]. We investigated the effect of growth regulators – natural phytohormones (gibberellic acid and brassinolide) and commercial growth stimulant (novosil) on the content of phenolic compounds and flavonoids of *Brassica oleracea* var. sabellica in different growing conditions.

Planting of *B. oleracea* var. sabellica was performed in June 2018, plants were treated with growth regulators by soaking the seeds for 24 hours in the following concentrations: Gibberellic acid 10^{-6} M, novosil 5 g/ha and brassinolide 10^{-6} M. Samples for analysis (leaves) were taken in 85, 115 and 145 days after planting at +8°C, 0°C and −6°C, respectively.

Shown, that all studied growth regulators stimulated the formation of PCs and FLs. The most effective was brassinolide, and its stimulating effect was significantly enhanced after the action of negative temperatures.

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Poster

Comparison of the activity of exogenous entomocidal metabolites of ss-ET and EM *Bacillus thuringiensis*

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β-exotoxin as a main entomocide component of commercial formula produced by *B. thuring* during industrial fermentation. Efimtsev and co-authors studied details of its biotech outcome. They experimentally demonstrated that the decrease of the β-exotoxin (β-ET) content in cultural media caused by its aggregation with another unknown product (EM) of the biotech fermentation [1, 2]. They proved that the optimisation of pH value of cultural media with the aid of interactve aggregation of β-ET and EM is a fundamental adaptive mechanism for bacterial survival and growth. They isolated EM from cultural media and experimentally detected some important features of the EM. EM induced pH shift to alkaline range, revealed entomocide activity against *Musca domestica*, and didn't interact with Ba2+. Its molecular structure was Uracyl-like but not identical to that of the β-ET. EM but not β-ET were also isolated from other commercial entomocide formula like dendrobacyllin (*B.t. dendrolimus*) and lepidocyde (*B.t. kurstaki*) [3]. Imitating their contents in commercial formulas the differential and combined influence of β-ET and EM on seedlings of *Larix gmelinii* from Russia and China were studied experimentally. In this work *Pisum sativum* was used as a biotest system also. It was shown that suppressed influenses of differential and combined application of β-ET and EM had significant but different effect on growth and development of the plants (table 1) and caused by inhibition of chlorophyll synthesis (table 2). EM revealed greater inhibitory activity then β-ET. Combined EM+β-ET action was less then EM alone inhibitory action on the plants. This effect proved to be an antagonistic action of EM and β-ET. It has been determined that the process of photosynthesis in larch and pea sprouts is inhibited by β-ET, EM, and their combination. The inhibition extent is estimated by the changes in the form and level of kinetic and light graph curves of oxygen release (Fig. 1).The difference of exotoxins influence on light-independent stages of photosynthesis has been demonstrated. β-ET induces the mechanism of light-independent chlorophyll synthesis in etiolated larch sprouts, forming photosystems I and II. EM partially initiates the process of chlorophyll synthesis, forming only photosystem II (Fig. 2). It has been proved that β-ET, EM and their total have negative influence on the sprouts, which directly depends on the toxin type, its concentration, and type of plants.

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Poster

The lack of Chl *b* in a barley *chlorina f2* mutant affects the lipid composition of chloroplast membranes, particularly the phosphatidylglycerols

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Glycerolipids of the chloroplast envelope and thylakoids include monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG), sulfoquinovosyldiacylglycerols (SQDG) and phosphatidylglycerols (PG). The chloroplast lipidome plays an essential role in stable functioning of the photosynthetic machinery, and is expected to change together with the parameters of pigment-protein complexes. Established in the course of plant evolution, chloroplast lipid profiles exhibit a certain flexibility in the MGDG/DGDG and galactoglycerolipid/phosphoglycerolipid ratios in response to environmental changes. In particular, PG are important for chloroplast biogenesis and for the functioning of PS II, whereas 16:1t/18:3 PG molecular species is sufficient to prevent the assembly of the LHCII trimer [1]. We studied the lipid profiles of the photosynthetic membranes in the barley *chlorina f2* 3613 mutant completely lacking Chl *b*.

Total lipids were extracted from the leaves of the wild-type (WT) plants and Chl *b*-less barley mutant plants grown in the field and analyzed by HPTLC (lipid classes) and GC-MS methods (fatty acids). In chloroplasts of the *chlorina* mutant, the amounts of MGDG, DGDG, and SQDG were increased by 20–30%, while the content of PG was increased more than twice, as compared to the WT. The molecular composition of these lipids had also changed. While changes of MGDG and SQDG were minor, the content of C18:3 fatty acid in the DGDG fraction decreased by 10% which was accompanied by an increase of the proportion of C16:0, 18:0, and monoenic fatty acids, respectively. The most significant changes occurred in the PG fraction. The proportion of 16:1t and C18:3 fatty acids which are essential for the functions of PG decreased by 15%. Intriguingly, the resulting increase in the proportion of saturated fatty acids and the decrease in unsaturated fatty acids were accompanied by an increase, not a decrease, in the mobility of the lipid phase of thylakoid membranes as shown by analyses of the rates of lipid diffusion by means of FRAP (Fluorescence recovery after photobleaching). We propose that the increased saturation of the chloroplast lipids in the Chl *b*-less barley mutant is an adaptive response to changed supramolecular organization of the thylakoid membranes in this mutant associated with a switch in lateral mobility of the membrane components [2]. A change in the levels and composition of PG might be a reason for impaired function of PS II in this mutant.

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Section 1.7: Carbon Fixation (C3 and C4) and Photorespiration

Poster

The role of apoplast invertase in the regulation of assimilate transport and plant production process

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The development of an original technique for studying the biochemistry of the extracellular space of the leaf, based on the use of a pressure chamber (Dixson, 1914), designed for measuring the water potentials of the leaf in combination with labeled carbon ^{14}C, allowed us to obtain new data on the important regulatory role of apoplast invertase in the regulation of photosynthesis. Firstly, the use of fiber-flax as an object of study allowed us to establish that labeled assimilates in their transport by phloem stem went out into the apoplast and were carried away by water transpiration stream. Being on the top these assimilates were not absorbed in the leaves, they reloaded back in the sink-leaf phloem and re-exported down. This was repeated many times on all leaves tiers. As a result, a generalized apoplast pool of photosynthesis products was created in the plant and probably performed the regulatory (signal) function of the photoassimilates distribution between sink-source organs.

Many researchers observed that the decrease of labeled ^{14}C sucrose/hexose apoplast ratio occured several times more intensive than in mesophyll cells under the action of negative factors, being defined by the function of apoplast invertase, which controled the sucrose export from the leaf, thereby participating in the regulation of photosynthesis. This conclusion was confirmed in the experiments with the genetically transformed apoplast invertase plant. The introduction of an additional gene of yeast apoplast invertase led to a decrease in photosynthesis, as well as to an increased death of test-tube plants because of the less sugar transport to the roots [2]. On the contrary, blocking the gene of apoplast invertase stimulated photosynthesis, but only in normal conditions of plant mineral nutrition. The photosynthesis of transformed plants was suppressed more in nutrition deficit. The gas exchange measurements of plants with suppressed apoplastic invertase, showed an unusual transpiration response of the leaf to the reduced illumination conditions. If photosynthesis and transpiration of untransformed plants were decreased under the two times decrease of illumination, then photosynthesis was reduced (slightly), and transpiration was increased in plants with the suppressed invertase [2]. This indicated that the non-working invertase, continuing sucrose loading to phloem, contributed to a decrease in the aqueous medium osmosis of intercellular space and to an increase of turgor of the closing stomata cells (their opening). Thus, a regulatory relationship of assimilate transport with chloroplasts and leaves stomata occured. The effect on invertase by changing pH of the apoplastic aqueous medium (for example, an aqueous solution of ammonia) can stimulate the assimilates transport and plant productivity [1, 3].

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Poster

Registration of gas exchange response of maize and sunflower leaves to fluctuations in light intensity and appearance of gaseous HCl or NH_3

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Changes in transpiration and CO_2 gas-exchange were measured simultaneously every 7 seconds in sunflower (*Helianthus annuus* L.) (C3 plant) and maize (*Zea mays* L.) (C4 plant) using an infrared spectrometer IR-Affinity (Shimadzu) with custom made cuvette. To ensure stability of air passing through the leaf chamber a polyethylene gas-holder of 150 dm^3 was used. Plants were grown under artificial illumination (luminescent lamps, 10 klux). During the experiment plants were lit with halogen lamp (maximal illumination up to 100 klux). Differences in reactions of sunflower and maize plants to switching off the light were revealed. In sunflower plants, transpiration after a small and short-term initial decrease, was maintained at a rather high level (about 80–90% of the level in light), while in maize, the transpiration quickly decreased to about 10% of its level in light. These differences could be explained by different stomata structure in cereals and dicotyledonous plants. For example, stomatal conductance in plants having elliptical or grass-type stomata is known to respond differently to changes in light spectral composition [1]. Sunflower and maize were found to respond oppositely to changes in acidity of external gas medium when 10 µl of aqueous (25%) ammonium (20 ppm) or 10 µl of concentrated (38%) hydrochloric acid (18.5 ppm) was injected into the gas-holder. In sunflower, no changes were observed in the kinetics of leaf gas-exchange after NH_3 exposure, but a response to appearance of HCl vapours was found. Oppositely, maize plants reacted to appearance of NH_3 vapours, but almost no changes were found in their leaf gas-exchange kinetics after HCl addition except for a little decrease of photosynthesis after switching on the light. In the presence of NH_3 (but not HCl) no typical reaction of CO_2-gas exchange to two-fold decrease of illumination (decrease in CO_2 uptake) was observed in maize. Suflower plants did not demonstrate a typical decrease of CO_2 uptake after two-fold decrease of illumination in the presence of HCl, but not of NH_3. This opposite response to HCl and NH_3 vapours may be explained by different ways of CO_2 assimilation in C3 and C4 plants. In C3 plants gaseous CO_2 is fixed by Rubisco, while in C4 plants CO_2 is initially fixed as HCO_3^- by PEPC. Then, differences in the apoplastic pH levels between maize and sunflower plants could not be excluded.

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Poster

Dynamics of some carbon and nitrogen metabolism enzymes in the flag leaves of various wheat genotypes

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Phosphoenolpyruvate carboxylase (PEPC) is involved in the production of Carbon skeletons for the synthesis of amino acids and other metabolites that are derived from the tricarboxylic acid cycle. Aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT) are involved in the exchange of amino groups between different organic acids and central amino acids that act as amino donors during amino acid metabolism [1]. The parallel changes of the activities of PEPC and NADP-malate dehydrogenase (NADP-MDH) in ontogenesis of flag leaves of the studied genotypes allow confirming the participation of both enzymes in the malate synthesis in chloroplasts. Similar change patterns of NAD- malate dehydrogenase (NAD-MDH) localized in mitochondria and activities of AspAT and AlaAT playing an important role in the biosynthesis of amino acids were observed in flag leaves of the studied genotypes during ontogenesis. Mitochondrial NAD-MDH implements oxidation of NADH reduced in the reaction of glycine decarboxylation during photosynthesis and intensifies biosynthesis of alpha ketoglutarate playing the role of the carbon skeleton in the synthesis of amino acids. The activities of the studied enzymes changed differently during the light period of the day. In both variants of the durum wheat genotypes (Barakatli 95 and Garagylchyg 2) the highest PEPC activity was observed at 8:00. Whereas, in the bread wheat genotypes (Gobustan and Tale 38) the highest PEPC activity was found at 17:00 in flag leaves. There was no significant changes in the enzyme activity during the morning and afternoon hours.

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Section 1.8: Artificial and Applied aspects of Photosynthesis

Lecture

Temperature sensor derived from the photoactive Orange Carotenoid Protein

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Photoactive proteins occupy a crucial position in science due to the potential to serve as scaffolds for the construction of novel sensors, signalling cascades and light-triggered photoswitches for fundamental and applied research. The cyanobacterial Orange Carotenoid Protein (OCP) is one of the examples of such photoactive proteins within a rapidly developing field of science. Initial investigations in this area have been recently reported in Nature, PNAS, Science and other top-rated journals. Until now, the mechanisms of OCP functioning were investigated in the context of its physiological role as a quencher of excitation energy, which protects photosynthetic antennae. But the actual mechanism of energy dissipation by OCP is poorly understood yet. The problem is that cyanobacterial antennae emit at wavelengths (~660-680 nm) where absorption of OCP in any of its spectroscopically distinct forms is very low. Thus, the overlap between the emission of the excitation energy donor and the absorption of the energy acceptor is small and cannot afford a sufficiently high energy transfer rate to compete with the energy transfer from the antenna to the chlorophylls of the photosystems. Also, the structure of the OCP-antenna complex is unknown due to complexity of huge antennas consisting of hundreds of pigments. So, any kind of estimations of excitation energy transfer in such complex systems are speculative and full of assumptions. Thus, it is reasonable to assume that a simple (binary) model of antenna-quencher may be useful for the study of energy dissipation and photoprotection.

A few years ago, we realized that the carotenoid in OCP acts as a polyspecific quencher, not only for cyanobacterial phycobilisomes, since it is able to quench fluorescence of (non)-covalently bound organic dyes and, notably, the intrinsic fluorescence of Trp. In principle, such a reduction of excited states upon the interaction with OCP is similar to quenching of photosynthetic antennas. Considering the fact that OCP can be used as a molecular thermometer, since its photocycling strongly depends on temperature, these observations inspired us to construct a genetically encoded temperature sensor, with a fluorescence readout. In this work we were focused on explanation of excitation energy transfer which occurs between the chromophore of fluorescent proteins and the carotenoid of OCP in a single chimeric construction. We report that such artificial systems, which mimic donor-acceptor interactions in the native OCP-antenna complexes, are photoactive and could be used for temperature measurements in biological systems. Future directions of OCP-based sensor improvement will be discussed.

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Lecture

On finding ways to make plants, especially rice, more efficient: A perspective

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Almost all life on earth depends upon oxygenic photosynthesis – the process by which sunlight is used to convert water and CO_2 into food, biofuel and O_2, needed for our survival [1]. It is clear that by 2050 the projected global food production capacity will not meet the needs of our growing population. Thus, there is an urgent need to find ways, in a rather short time, to make plants much more efficient than they are today. Our focus is on rice since it is the major food crop both in China and India. Efforts are being made around the world – and some are in lime-light: see e.g., (a) Johannes Kromdijk et al. from Steve Long's lab [2] showed that transgenic tobacco plants, engineered to have a faster recovery of photosynthetic efficiency after high light exposure, were more efficient and more productive than their wild type counterparts; and (b) South et al. from Don Ort's lab [3] showed that introduction of alternate pathways of photorespiration in tobacco led to large increases in biomass. These methods are now being transferred to food crops as the critical test of their efficacy. In this presentation, we will focus on efforts being made in the laboratories of two of us.

They are: (i) Neelam Soda et al. from Ashwani Pareek's lab [4] established that increased abundance of Oryza sativa Intermediate Filament (OsIF) protein led to a significant improvement in growth of rice plants, particularly under salinity and high temperature in which proline and trehalose had increased; (ii) Saber Hamdani et al. from Xinguang Zhu's lab [5] showed the importance of quality of light on growth of rice finding that growth under white light, in contrast to red and blue light produced greater antioxidants and more biomass. (iii) Further, Hamdani et al. [6] showed, in rice, an exciting correlation between the variation of glucosidase content with the maximum quantum yield of Photosystem II that needs to be fully understood. (iv) Lastly, by the use of 'allele mining' (cf. [7]) from a wild rice (Pokkali), grown by farmers in coastal areas in India, and an extreme xerohalophyte, *Suaeda fruticosa*, Pareek's team showed an improvement of photosynthesis efficiency and ultimately the yield in both tobacco and rice [4, 8]. Thus, to reach our goal of making plants more efficient to feed the increasing human population, we need not only to do genetic engineering, but also to manage growth conditions. Govindjee thanks Don Ort for on-going discussion on the topic of improving plants for our future needs.

Let there be Light, and Let there be Hope!

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Lecture

Photosynthetic electron transport *in silico*

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We have developed a computer model to describe electron transport processes in photosynthetic organisms. Besides the thylakoid bound electron transport components the model contains also soluble electron carriers. The model also includes binding and releasing of PQ and inhibitors to the Q_B site and provides an excellent tool to simulate the kinetics of electron transport processes in a wide range of conditions, and can be used to perform *in silico* experiments with special interest in the function of Photosystem II, Photosystem I, the NDH-1 complex, cyclic electron transport, etc. The model was used to describe the period-four oscillation of flash-induced O_2 evolution yield, a wave phenomenon in the relaxation of flash-induced Chl fluorescence yield in microalgae, the protective role of non-radiative charge recombination against photodamage, the interaction of photosynthetic and metabolic electron transport with special emphasis on the kinetic changes of the NADPH pool under light-dark transitions, as well as to describe light induced changes in the amount of $P700^+$.

Lecture

Synechococcus sp. PCC7942: A cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions

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Sucrose, a compatible osmolyte in cyanobacteria, functions both as an energy reserve and as osmoprotectant. Sugars are the most common substrates used by microorganisms to produce hydrogen (H_2) by means of anaerobic dark fermentation. Cells of the unicellular, non-nitrogen fixing, freshwater cyanobacterium *Synechococcus* sp. PCC7942 accumulate sucrose under salt stress. In the present work, we used this cyanobacterium and a genetically engineered strain of it (known as PAMCOD) in order to investigate the optimal conditions for (a) cell proliferation and (b) sucrose accumulation, which are necessary for H_2 production via anaerobic dark fermentation of the accumulated sucrose. PAMCOD [1] contains the gene codA that codes for choline oxidase, the enzyme which converts choline to the zwitterion glycine betaine. Glycine betaine is a compatible osmolyte which increases the salt tolerance of *Synechococcus* sp. PCC7942. Furthermore, glycine betaine maintains cell proliferation under salt stress and results in increased sucrose accumulation. In the present study we examine the environmental factors, such as the NaCl concentration and the pH of the culture medium, as well as the carbon dioxide content of the air bubbled through it. At optimal conditions, sucrose accumulated in the cyanobacteria cells up to 13.5 moles per mole Chl *a*. Overall, genetically engineered *Synechococcus* sp. PCC7942 produces sucrose in sufficient quantities such that it may be a viable alternative (a) to sugar synthesis, and (b) to H_2 formation via anaerobic dark fermentation.

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Lecture

Artificial photosynthesis with electron acceptor/photosensitizer-aptamer conjugates

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Our recent efforts to artificial photosynthesis [1] centered on the use of photosensitizer-modified aptamer conjugates as functional scaffolds to organize supramolecular diads, mimicking photosynthesis by guiding photo-sensitized electron transfer to drive catalytic and biocatalytic transformations. We describe the synthesis of Ru(II)-*tris*-bipyridine ($Ru(bpy)_3^{2+}$)-anti-tyrosinamide aptamer conjugates as functional supramolecular scaffolds for controlling photo-induced electron transfer (ET) processes that are coupled to the light-induced biocatalyzed generation of NADPH or to the photochemically-induced evolution of hydrogen (H_2). Prof. Willner's recently reported method to design "nucleoapzymes" [2] can be adapted to introduce a versatile paradigm to assemble artificial photosynthetic model systems.Supermolecular complexes consisting of the photosensitizer, Ru(II)-*tris*-bipyridine, conjugated to the anti-tyrosinamide aptamer, and N-methyl-N'-propyl-(aminotyrosinamide)-4,4'-bipyridinium, TA-MV2+, an electron acceptor ligand provide artificial photosynthetic model systems. Four different photosensitizer-aptamer conjugates are described and these include the photosensitizer linked to the 5'- or 3'-end of the aptamer, and conjugates that include the photosensitizer tethered to the 5'- or 3'-end of the aptamer through four-thymidine bridges. The photosensitizer-aptamer/electron acceptor complexes stimulate the photosensitized ferredoxin-$NADP^+$ reductase, FNR, catalyzed synthesis of the NADPH cofactor or the photosensitized hydrogen evolution in the presence of Pt nanoparticle catalysts. The yields of the H_2-evolution processes depend on the complex configuration. Steady-state fluorescence and life-time quenching experiments reveal that static intra-complex electron transfer quenching of the Ru(II)-*tris*-bipyridine photosensitizer within the photosensitizer-aptamer/TA-MV2+ complexes control the photocatalyzed biotransformation and the light-induced H_2-evolution processes.

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Poster

Towards an *ab initio* modeling of low-lying singlet excited states of carotenoids

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Various carotenoids play a number of important roles in photosynthesis including absorption of light in blue range of visible spectrum followed by excitation energy transfer to chlorophylls and dissipation of excessive energy which prevents photodamage of photosynthetic apparatus caused by highly reactive species formed under excessive light illumination. Two excited states of carotenoids are most likely responsible for these processes: the optically bright $1B_u^+$ state and the dark $2A_g^-$ state, respectively. Adiabatic relaxation of both excited states is accompanied with a significant change of bond length alternation (BLA) in the conjugated π-system which is responsible for pronounced vibrational structure in absorption spectra and impedes assessment of quality of computational results usually relying on vertical transitions for rather large molecules.

Excitation energies of adiabatic and vertical transitions were modeled for a series of polyenes with the number of conjugated double bonds ranging from 8 to 13 which corresponds to the majority of natural carotenoids. Excited state geometries were optimized using TDDFT/CAMB3LYP for $1B_u^+$ and MCSCF in the entire space of π-orbitals, energies of excited states were computed using DMRGSCF with NEVPT2 correction accounting for dynamic correlation. This approach provided qualitative agreement with experimental data for $1A_g^- \rightarrow 2A_g^-$ excitation energies and good agreement for $1A_g^- \rightarrow 1B_u^+$. The difference between vertical and adiabatic excitation energies for $1A_g^- \rightarrow 2A_g^-$ transition is approximately −1.1 eV which is due to the strong impact of BLA on energy levels of carotenoids. The aforementioned agreement with experimental data shows that DFT/MRCI and AM1/CASCI methods widely used for modeling of non-photochemical quenching significantly underestimate excitation energies since they produce vertical energies which are close to the experimental ones. While in this work the unsubstituted linear polyenes were studied (thus the effect of substituents has been neglected), this approach can be relatively easily extended to natural polyenes provided that two computational bottlenecks are addressed: geometry optimization of the $2A_g^-$ state requiring seminumerical MCSCF gradients and limited performance of NEVPT2 in current implementation.

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Poster

In vitro reconstitution of a photosystem II-phycobilisome super-complex for enhanced light harvesting in bio-photovoltaics

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Photosystem II (PS II) is the only known protein-complex that catalyses the light-driven oxidation of water in nature. Isolated PS II complexes can be applied in the anodic half-cell of bio-photovoltaic devices [1, 2]. For this, PS II is embedded in redox-active hydrogels which transfer high energy electrons from the terminal PS II redox cofactors Q_A and Q_B towards an electrode surface. However, the activity of isolated PS II is limited to the blue- and red-light region of visible light due to the absorption properties of chlorophyll, the major pigment of the PS II core complex. Cyanobacteria evolved special light-harvesting protein complexes named phycobilisomes (PBS), which allow for light utilization in the green gap of chlorophyll.

Here we describe the first functional *in vitro* interaction of isolated PS II from the thermophilic cyanobacterium *Thermosynechococcus elongatus* and PBS from *Acaryochloris marina*, *Synechocystis* sp. PCC 6803, and *Mastigocladus laminosus*. The interaction is stabilized by a crosslinking procedure and the super-complexes were characterized by fluorescence spectroscopy and electrochemical measurements, particularly by action spectra of PBS-PS II embedded in an Os-complex-modified hydrogel. We could demonstrate the formation of a functional super-complex for enhanced light harvesting within the PS II containing anodic half-cell. Immobilized on nano-structured indium tin oxide electrodes (NS-ITO) absolute external quantum efficiencies up to 10.9% at single wavelengths were achieved.

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Poster

Unsupervised neural network model helps to attribute particular stages of chlorophyll *a* fluorescence transient

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Analysis of chlorophyll *a* fluorescence transient upon illumination after dark adaptation is widely used to assess the activity of primary photosynthetic processes. Modern fluorometric equipment allows detailed recording of induction curves with time resolution of microseconds. For green algae and higher plants fast fluorescence rise kinetics is generally described as three-step (O-J-I-P), however comparison of transients recorded under nutrient surplus and deficiency reveal sequence of much more bands (O-L-K-J-I-H-G-P) [1]. It is commonly accepted that individual phases of the induction curve depict various stages of the electron transfer in the electron transport chain (or exciton transfer between Photosystem II reaction centers); however attribution of the phases to specific processes is still under discussion. Construction of a robust method for quantitative description of the transient in terms of characteristic times and amplitudes of phases is essential for this task. Notion of photosystem as a huge complex containing several redox centers acting in chain implies that individual phases should be considered as exponential functions, each of which represent some set of processes (mostly related to electron transfer). Characteristic times of these exponents may be rather close to each other, and they vary with temperature, light intensity, and physiological state of the organism, making unequivocal determination of numeric parameters from experimental data a sophisticated task. To deal with this ambiguity, we suggest a mathematical method for simultaneous analysis of a set of fluorescence transients recorded under varying conditions. The use of an unsupervised neural network model allows finding minimal set of exponents suitable to describe a given set of experimental data. It was shown that L-band (see [1]) related to energetic coupling of PS2 RCs can be identified as a distinct exponential component with characteristic time of about 100 μs or below, having negative contribution to the fluorescence rise under normal conditions (thus leading to ‘sigmoidity’ of the early stage of the transient), and positive contribution in stress conditions (i.e., nitrogen or sulfur deficiency, high illumination, etc.). *This work was partially supported by RFBR project №17-04-00676.*

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Poster

Conjugates among photosystem complexes and graphene oxide for a photosensitive device

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Photosynthesis converts light energy into the chemical energy. The quantum yield of photosynthetic energy and electron transfer is nearly 100% by the forces of natural selections. Exploiting this photovoltaic abilities of photosystem (PS) for biohybrid device is one of the key research themes for sustainable energy. Carbon materials are also potential candidates for the next-generation devices. Among them, graphene is a new material for its remarkable electronic properties. However, graphene is insoluble in common solvents. Therefore, we used graphene oxide (GO) and reduced graphene oxide (rGO) in this study, because they are soluble in water. The redox potential of GO is between PS II and PS I. Therefore, GO acts as a good electron transfer mediator.

In this study, we used PS I and PS II complexes from *Thermosynechococcus elongatus*. When GO was used as an electron acceptor for PS II, the oxygen evolving activity was recovered in isolated PS II. When GO was used as an electron donor for PS I, the oxygen consumption was increased in isolated PS I (Mehler reaction). This reaction was enhanced by addition of Cyt c_6 complex. Conjugates among PS I, PS II complexes and graphene oxide, we observed linear electron flow. This indicates that the GO worked as a good electron mediator for PS I and II. We will discuss the hydrogen evolution by this complex.

Poster

The interaction between various redox species, widely used in the study of photosynthesis

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The careful selection of electron mediators is important for the optimal functioning of liquid-based solar cell sensitized by photosynthetic apparatus components. The mediators are the redox species which should efficiently transfer an electron either from terminal electron acceptor of the photosynthetic electron transfer chain (ETC) to the electrode or from another electrode to the terminal electron donor of the ETC. The use of electron mediators in a solar cell offers several advantages. The redox species protect the photosynthetic apparatus from both donor and acceptor photoinhibition mechanisms, caused by intense actinic light. They also manifest themselves in antioxidant activity. Photoinhibition lead to a decrease in the electron transport rate, destruction of photosynthetic macromolecules and lipid bilayers, degradation of the D1 protein followed by suppression of the photochemical activity of the photosynthetic apparatus components serving as a photosensitizer. Thus, carefully selected redox species can protect the sensitizer and extend the lifetime of the solar cell. The design of solar cells with the electron mediators in general does not require immobilization techniques for fixation of the pigment-protein complexes on the inorganic substrate. So, the exploitation of these species can help avoid aggressive effect of an electrode material on the photosynthetic complexes. This fact allows us to investigate more substances as an electrode material. For the simultaneous use of both donor and acceptor exogenous species in the solar cell, we need evidence that these species do not react with each other. There is no accurate information about the possible chemical interaction between various redox agents that can be used as electron mediators in a solar cell.

In this work, possible direct interaction between these species was investigated by registration of absorbance spectra alteration of one species in the presence of another one. We used double beam spectrophotometer Shimadzu UV1800. New experimental results were obtained on the possible direct chemical interaction between different pairs of the most used redox agents.

This work was supported by grant from RSF (No: 19-14-00118).

Section 1.9: Regulation of Photosynthesis and Environmental Stress

Lecture

Phosphorus toxicity decreases both electron sink activity and anti-oxidative activity in rice leaves

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Phosphorus is one of the essential macronutrients for land plants. Land plants acquire phosphorus from the rhizosphere through their root as an inorganic orthophosphate (Pi). In general, the Pi content is the soil is critically low (<10 μM), therefore land plants have multiple strategies to absorb Pi to maintain their growth. However, the excess amount of Pi acquisition is critically dangerous for land plants because excess Pi accumulation in leaves stimulates their withering, and declines plant growth. This phenomenon is known as phosphorus toxicity in land plants. Phosphorus toxicity has been reported in 1918 [1], and many studies observed the phosphorus toxicity symptoms. However, the detailed molecular mechanisms of phosphorus toxicity have remained to be clarified. In this study, we aimed to reveal the molecular mechanisms of phosphorus toxicity in land plants by using rice plants. Subsequently, we found that under phosphorus toxic conditions, Rubisco activation was significantly decreased due to the decrease in Rubisco activase content. Under such conditions, chloroplastic ascorbate peroxidase activity was significantly enhanced, but Cu/Zn-superoxide dismutase (SOD) activity was drastically suppressed in leaves. These results suggest that, under phosphorus toxic conditions, chloroplasts cause oxidative stress by the limitation of electron sink and the suppression of ROS scavenging activity. Here, we discuss the molecular mechanisms of phosphorus toxicity from the aspect of oxidative stress triggered by photosynthesis.

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Lecture

Photosynthesis controls plasmodesmata permeability in *Arabidopsis thaliana*

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Photosynthesis regulates a plethora of cell functions via the reduction state of thioredoxins which is controlled by electron supply from PS I. At the same time, photosynthesis can serve as a source of ROS, which in small amounts act as signal molecules while at high levels can lead to cell damage. Both thioredoxins and ROS levels were shown to influence the number and permeability of plasmodesmata (PD), the tiny channels connecting plant cells and mediating cell-to-cell exchange of developmental regulators like RNA and transcription factors, as well as of water, nutrients, and hormones [1, 2].

We investigated the relationship between the functions of PS I and PS II, ROS production and PD permeability using *Arabidopsis* mutants with changed levels of Chl *b* (*ch1-3* lacking Chl *b* and *PhCAO* transgenic plants over-accumulating Chl *b*) as well as *trxm3* and *ntrc* mutants impaired in thioredoxin functions. Numbers of PD were studied by TEM and immunohistochemistry, the functions of PS II and PS I were analyzed using a DUAL-PAM 100 (Walz, Germany), ROS were detected using fluorescent probes, callose was quantified using aniline blue staining, leaf ascorbic acid contents were determined by enzymatic analyses and PD permeability was estimated using a symplastic tracer. The results showed that performance of PS I and PS II was affected depending on Chl *b*-levels and on the presence of *trxm3*. Most interestingly, stimulation of PS I with far-red light dramatically changed PD permeability in the mutants. Altogether, our results suggest that photosynthesis exerts control over PD functions not only via ROS levels and thioredoxins but also via supply of energy to leaf cells.

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Lecture

γ-aminobutyric acid confers cadmium tolerance in maize plant by concerted regulation of polyamines metabolism and antioxidant defense system

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Gamma-aminobutyric acid (GABA) accumulates in plants following exposure to heavy metals. To investigate the role of GABA in cadmium (Cd) tolerance and underlying mechanisms, GABA (25 and 50 µM) was applied on Cd-exposed maize plants. Vegetative growth parameters were improved in both Cd-exposed and control plants due to GABA application. Cd uptake and translocation were considerably inhibited by GABA. Antioxidant enzyme activities were enhanced in plants subjected to Cd; concurrently GABA caused further increase in catalase and superoxide dismutase activities, which led to significant reduction in hydrogen peroxide, superoxide anion and malondealdehyde contents under stress condition. Polyamine biosynthesis-responsive genes namely ornithine decarboxylase and spermidine synthase were induced by GABA in plants grown under Cd shock. GABA suppressed polyamine catabolism-related gene (polyamine oxidase) when plants were exposed to Cd. Consequently, different forms of polyamines were elevated in Cd-exposed plants by GABA application. Maximum quantum efficiency of photosystem II (Fv/Fm) was decreased by Cd; while, GABA recovered Fv/Fm in Cd-exposed plants to the same value in the control. These results suggest a multifaceted contribution of GABA, through regulation of Cd uptake, reactive oxygen species and polyamines metabolism in response to Cd stress.

Lecture

Evaluation of the thylakoid membrane processes in *Synechocystis* sp. PCC 6803 by modeling fluorescence induction on the time scale from microseconds to several minutes

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In cyanobacterial cells, the transmembrane charge fluxes proceed between the lumen and cytosol that are separated by the thylakoid membrane where the PQ-pool is shared both by photosynthetic and respiratory electron transport (PET and RET) [1–3]. We have compared fluorescence induction (FI) data from the wild type *Synechocystis* sp. PCC 6803 (Synechocystis) cells using the Thylakoid membrane model (TM model) elaborated before [4] and adjusted here to analyze photosynthetic membranes of cyanobacteria. FI curves were measured with actinic light of λ=630 nm, 3000 µmol photons·m^{-2}·s^{-1} in the µs-to-10 minutes time window as well as with λ=455 nm, 1000 µmol photons·m^{-2}·s^{-1} from the µs- to 2 seconds. The TM model [4] was modified in this work to reproduce the data obtained on Synechocystis cells (see, also [2,3]). For the photosystem II (PS II) reaction center (RC) of *Synechocystis* the ET parameters differ from those of higher plants [4], especially for the closed (with Q_A^-) RCs. The initial OJ phase after 10 min dark adaptation of *Synechocystis* is explained by the extent of PQH_2/PQ pool reduction in the range of 20–40%. Simulations confirmed that F_0 contains contributions from the redox silent Chl *a* fluorescence of PS I [1,2], which was varied from ~10% in pea leaves [4] to ~40% for phycobilisome (PBS)-containing cyanobacteria. The assumption of PS I:PS II:Fd= 2:1:1.25 allowed us to reproduce qualitatively the oxidoreduction transients of P700 in PS I RCs, measured as ΔA_{810} for Synechocystis in [3]. The nature of OJIPSMT curves induced by either high or moderate light was analyzed due to simulations of light-acclimative processes: state 1→2 and state 2→1 transitions [1,2].

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Lecture

The good and the bad of lacking chlorophyll *b*: photosynthesis and growth in barley *chlorina f2*^{3613} mutant

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The '*chlorina*' mutants are partially or completely devoid of chlorophyll *b* (the antenna chlorophyll) due to mutations in the gene encoding chlorophyllide-a-oxygenase (CAO). *Chlorina* mutants are known for barley, maize, pea, rice, soybean, wheat, raps etc. The photosynthetic apparatus in *chlorina* mutants is highly effective: the rates of CO_2 assimilation per chlorophyll molecule are higher in *chlorina* mutants than in wild-type plants. Potentially, *chlorina* mutants of crops should be able to show an increase in yield and biomass production. However, until now, such mutants have never been considered as potential basis for the generation of transgenic lines or for cultivars with an economically significant increase in productivity. This is due to the multiple negative side effects of the *chlorina* mutations on plant growth, flowering and yield, which nullify the potential benefits of higher efficiency of photosynthesis. We characterized the key mechanisms underlying the formation of this mutant phenotype and explained the strong pleiotropic effects of the *chlorina* mutation in the barley *chlorina f2*^{3613} mutant. Moreover, we demonstrated that the *chlorina f2*^{3613} mutant, under certain growth conditions, can form a high-yield phenotype without displaying the 'negative' side effects of the *chlorina* mutation. The underlying mechanisms and the potential practical applications will be discussed.

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Lecture

Apparent lack of stomatal growth irradiance response in four *Tradescantia* species

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Stomatal responses are among key determinants of the photosynthetic performance under volatile environmental conditions. They contribute significantly to the environmental fitness of vascular plants. We characterized the stomatal apparatus and its plasticity in response to the growth irradiance in four *Tradescantia* species belonging to contrasting ecological groups – heliophiles of deserts and semideserts (*T. sillamontana* and *T. navicularis*), and shade-tolerant plants of tropical forests (*T. fluminensis* and *T. zebrina*) to test the hypothesis if the low stomatal apparatus plasticity is related with increased flexibility of light interception by leaves in these species. In this study, we analyzed hyperspectral leaf reflectance and micromorphometry of the *Tradescantia* leaf architectonics and stomatal apparatus.

Despite their adaptation to contrasting environments, none of the studied species showed a growth irradiance dependence of the design of their stomatal apparatus which was remarkably uniform with a low stomatal index (*SI*; 7–11), low stomatal density (*SD*; 10–30 mm^{-2}), and large stomatal size (SS, 50–70 μm) guard cells. Although the studied *Tradescantia* species are hypostomatous, a single row of stomata was revealed in their adaxial leaf surface ("bordering stomata").

The lack of stomatal apparatus environmental plasticity in the studied species is in apparent disagreement with their stomatal architecture and stomatal responses of other plant species studied to date. We hypothesize that the low environmental plasticity of the stomata in these *Tradescantia* plants stems from (i) the lack of stomatal conductivity limitation of photosynthesis and (ii) very efficient chloroplast avoidance-based photoacclimation in Tradescantia plants.

The work was carried out with partial financial support of Russian Foundation for Basic Research (grant 19-016-00016).

Lecture

The communication of chloroplasts with the stomatal leaf apparatus in the regulation of photosynthesis

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The authors present the results of their own long-term research in the field of photosynthesis, photosynthetic carbon metabolism and assimilate transport in plants *in vivo* under changing conditions. The sequence of the data presented leads the listener to understanding as to the principle of photosynthesis regulation in the system of the whole plant. The essence of the proposed conception is to coordinate the light stage, during which the energy from sunlight is absorbed and converted to be stored in the form of ATP and $NADPH^+$, and the dark stage, during which (at CO_2 fixation) organic acids are formed. Sugars (which are completely exported to the organs-acceptors of photosynthesis product) are formed at a balanced state of these two stages of photosynthesis. If the conditions are changed (reduced illumination, inhibition of sugars outflow from the leaf with a reduction in the mass of the organs-acceptors or an increase in nitrate supply), the export of sugars from the leaf becomes inhibited, and the number of products of the dark stage is relatively greater than that of the light one, then: 1) acids accumulate in the mesophyll cells (including resulted from photorespiration and the formation of glycolate with its metabolic products); 2) excess acids come out of the mesophyll and acidify the aqueous medium in the apoplast, which activates apoplast invertase; 3) as a result of hydrolysis of sucrose (from the sucrose molecule → two moles of hexose) osmolality of the extracellular aqueous medium increases, which increases also due to evaporation of water when moving to the stomata; 4) stomata are closed osmotically, the diffusion of CO_2 into the leaf and the intensity of photosynthesis decreases. As a result, the disturbed ratio of dark and light processes in chloroplasts is normalized. All these processes in the leaf are changed in the opposite direction in case of an increase in illumination, demand for the products of photosynthesis with the reduction of the leaf surface or nitrate reduction. Thus, it is possible, by changing the pH of the apoplast, to regulate the activity of invertase, the export of leaf sugars and the production processes of the plant.

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Lecture

Expression of LHCSR3 induces change in supercomplexes of thylakoid membranes under PEG stress from *Chlamydomonas reinhardtii*

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Light is indispensable for photosynthesis, whereas high-light can adversely affect this process resulting in photoinhibition. Plants have evolved with diverse set of mechanisms collectively called as "non-photochemical quenching" that can harmlessly dissipate excess energy from photosynthetic complexes. In algae and mosses, expression of Light-harvesting-complex-stress-response-protein 3 (LHCSR3) is involved in energy dissipative quenching (qE) to confer photo-protection. Its expression is associated with acidification of thylakoid lumen due to increased photosynthetic electron flow under high light and acidification of thylakoid lumen is prerequisite for LHCSR3 expression. There are reports where, apart from high-light, LHCSR3 is expressed under iron, sulfur and phosphorous deficiency. The role of its expression and mechanism of its induction is not clear. In this work we grew the *C. reinhardtii* cells with PEG (Polyethylene glycol) to induce osmotic stress that also can result in nutrient deficiency by limiting water uptake by the cells. We studied the expression of LHCSR3 in *C. reinhardtii* grown under moderate light with PEG (Polyethylene glycol) induced osmotic stress. By studying supramolecular changes in thylakoid membrane complexes using BN-PAGE in wild-type and NPQ4 mutant it was found with that the expression of LHCSR3 closely correlates with the instability of the super and mega-complexes and its association is predominantly found with the fraction of LHCs that are non-isolatable with either of photosystem supercomplexes. We also suggest the down regulation of ATP synthase complex for induction of ΔpH under moderate light conditions is an essential requirement for expression of LHCSR3.

Lecture

Carbonic anhydrases in chloroplasts of C3 higher plants

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Carbonic anhydrases (CAs) are the enzymes that catalyse the reversible hydration of carbon dioxide to form bicarbonate ion, increasing greatly the rates in both directions. In higher plants of different species there are from 10 to 20 genes encoding CAs belonging to three genetic families, α, β and γ.

Our research has shown that the functioning of α-CA1, discovered in stroma of *Arabidopsis thaliana* chloroplasts [1], is coupled with photosynthetic reactions. The knockout of the gene encoding this CA has led to changes in a number of photosynthetic parameters in mutant leaves, namely, the acceleration of electron transport through PS I and PS II, the reduction of non-photochemical fluorescence quenching of chlorophyll *a*, the decrease in CO_2 assimilation rate. The other stromal CA, which is the most abundant CA in plant cell, β-CA1, is not connected directly with photosynthetic reactions. Presumably, the function(s) of this CA in stroma are different from those of α-CA1 that confirms with the data from other research groups. We have shown the heterogeneity of the properties of proteins with CA activity in thylakoids of higher plants chloroplasts. The presence of soluble CA of β-family with molecular mass of 262 kDa was found in thylakoid lumen [2]. CAs with different properties were detected in thylakoid membranes enriched with either PS I or PS II. Using mass spectrometry the presence of α-CA5 was shown in stromal thylakoids of *Arabidopsis* enriched with PS I. This CA evidently participates in the effect of stimulation of photophosphorylation by bicarbonate [3]. Our research implies that α-CA2 is located on the stromal side of thylakoid membrane. This CA may function together with the other thylakoid CA, α-CA4. The data show that the operation of these CAs is possibly involved in the regulation of the development of energy-dependent non-photochemical quenching of chlorophyll *a* fluorescence in opposite way.

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Lecture

Embryonic photochemical activity is crucial for the seed maturation

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In green plants, photosynthesis is the principal route that is supplying vegetative plants with assimilates. In many species, photosynthesis takes place not only in leaves but also in the developing embryos. However, biochemistry of embryonic photosynthesis is different from that in leaves [1]. First, its primary function is the synthesis of NAD(P)H and ATP, which are required for metabolizing sucrose to acetyl-CoA, fatty acids, and triacylglycerides. Second, the main carbon source is not an atmospheric CO_2, but the sucrose from the mother plant. Equally important, embryos generate O_2 that prevents hypoxia and supports mitochondrial respiration in developing seeds. Although the embryonic photosynthesis is known for decades, its mechanism remains unclear. It is an intriguing question, how the light reaches the embryo chloroplasts through the coat and pericarp. Here, we study the dynamics of photochemical activity in the developing embryos of yellow- and green-seeded pea (*Pisum sativum* L.) cultivars and rape (*Brassica napus* L.) by the pulse amplitude modulation (PAM) fluorometric analysis [2]. Importantly, even at the low light intensities, such as 5 μmol photons$\cdot m^{-2}\cdot s^{-1}$, the photochemical activity was detected inside of embryo. There were no significant differences in the dynamics of seed photochemical activity between yellow- and green-seeded cultivars. Embryonic plastids exhibited the developed thylakoid system with single starch grains. Termination of photochemical activity in both cultivars was accompanied by the start of seed desiccation (process accompanying the late maturation). At the late maturation, the plastids included the large starch granules wrapped with chloroplast envelope and a thin layer of stroma remains. A remarkable feature of yellow-seeded mature pea embryos was the deposition of numerous plastoglobules. In mature rape embryo, we also observed numerous plastoglobules, but starch granules gradually disappeared. Using RT-PCR, the expression of Chl-catabolism-related genes (NYC1, HCAR, MgD, PPH, PAO, RCCR) was studied. The biggest difference was observed for PAO, which is responsible for the conversion of pheophorbide a to RCC. *The work was supported by grant no. 16-16-00026 from the Russian Science Foundation.*

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Lecture

Effect of β-1,3-glucane on the structure and function of photosynthetic apparatus and oxidative status of tomato leaves under fusarium wilt

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It is known that the process of photosynthesis undergoes significant changes under stressful environmental factors, which affects the stability and productivity of plants. It's generally accepted that the parameters of PAM-fluorimetry of leaves characterize the lifetime structural and functional state of photosynthetic membranes and can serve as a criterion for estimation of the stress state in plants. However, in the literature there are only a few facts reflecting the effect of pathogens on the functional activity of photosystem II (PS II) [1]. In recent years, there has been a steady interest in drugs of a glucan nature, which are widely used in world medical practice as effective immunomodulatory agents. Studies of the mechanisms of β-glucans action on the plant began relatively recently. It is assumed that β-glucans are part of microbe-associated molecular patterns (MAMPs), but the mechanisms of their reception and signaling are mostly unknown [2].

In our experiments the leaves of the upper tier of 2-month-old tomato plants (*Lycopersicon esculentum* L.), Tamara plant variety, were treated by β-1,3-glucan from *Euglena* (Sigma-Aldrich). As control were used the plants treated by distilled water. Infection with the fungal pathogen *Fusarium oxysporum* (Schlecht.) was carried out 48 hours after treatment by immunomodulator using a suspension of the fungus spores into the aqueous medium with the roots.

Infection of tomato plants with *Fusarium oxisporum* causes activation of lipid peroxidation (LPO) processes in leaves and significant changes in the structural and functional state of photosynthetic membranes, which is reflected in a decrease in Chl *a*, Chl *a*/Chl *b* ratio, disturbances in the absorption and utilization of light energy in PS II of photosynthesis. Pretreatment of plants with β-1,3-glucan contributes to the stabilization of LPO and normalizes the course of photochemical processes in chloroplasts of infected leaves, which indicates the protective activity of the drug.

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Lecture

Comparative analysis of corticular photosynthesis in grapevine varieties contrasting in freeze tolerance

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Non-foliar photosynthesis has been studied extensively lately. It has been shown that photosynthesis within bark and stem tissues contributes to the regulation of plant carbon balance and maintenance of hydraulic functions, participates in the refilling of embolized vessels. The connection between the photosynthesis in lignified stems and plant tolerance to unfavorable environmental conditions has not been previously investigated. To study the role of corticular photosynthesis, i.e. photosynthesis in chlorenchyma tissues of the inner bark (cortex) of lignified branches, in tolerance of perennial plants to suboptimal temperatures, we compared the functional characteristics of corticular photosynthetic apparatus (CPA) of grapevine varieties differing in tolerance to freezing temperatures.

Measurements of the photosynthetic activity of CPA in the first-year lignified vines conducted in the laboratory as well as under field conditions revealed the positive correlation between grapevine freezing-tolerance and activity of the corticular photosynthesis. Vines of freezing-tolerant varieties are characterized by a better-pronounced chlorenchyma layer beneath the outer bark and higher content of photosynthetic pigments. CPA in freezing-tolerant varieties display higher activity of Photosystem II and Photosystem I under optimal temperature conditions and after freezing treatment. Moreover, CPA in freezing-tolerant varieties display higher mobility of antenna complexes within photosynthetic membranes in response to altering light intensity confirming better adaptability of the CPA to the changing environment. Collectively, obtained results testify the role of corticular photosynthesis in the tolerance of grapevine plants to suboptimal temperatures. We assume that light absorption, oxygen evolution and re-fixation of inorganic carbon by CPA is an important energetic and metabolic constituent of freezing tolerance of perennial plants.

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Lecture

Production of hydrogen peroxide within thylakoid membrane with involvement of the plastoquinone pool, and the role of this production for signalling

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The study of O_2 reduction in the photosynthetic electron transport chain (PETC) had led to discovering production of H_2O_2 within the thylakoid membrane [1]. It was found that both the percent of the superoxide radicals, the primary products of O_2 reduction in the PETC, which were involved in H_2O_2 production and the amount of H_2O_2 in thylakoid membrane increased with increase in light intensity [1, 2]. These results together with the characteristics of the plastoquinone (PQ) pool oxidation after thylakoid illumination [3] as well as the theoretical calculations led to a hypothesis that H_2O_2 in the thylakoid membrane formed in the reaction of plastohydroquinone, PQH_2, with superoxide radicals generated within the membrane [4]. Our direct experiments revealed the light-induced superoxide radical generation within thylakoid membrane [5]. The reaction of PQH_2 in thylakoid membrane with the superoxide radicals coming from xanthine-xanthine oxidase system was shown [6]. Under inhibition of the PQ pool oxidation by cytochrome complex, the electron transfer through Photosystem I to O_2 was capable to generate the superoxide radicals oxidizing PQH_2. We propose that H_2O_2 produced by the above way can be the primary messenger providing information about the PQ pool redox state for the regulatory enzymes, the operation of which was found to depend on this redox state [7].

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Lecture

Microgravity modelling by 3D-clinorotation affects actin cytoskeleton, ROS production, photosynthesis and metabolome of *Arabidopsis* plants

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Microgravity conditions are required to study plant performance in space and are modelled on the Earth's ground using 3D-clinorotation, under which growing plants are constantly rotated around two orthogonal axes [1]. We have applied the special ionic exchanger polyacrylonitrile fiber substrate Panion-110/320 to facilitate successful cultivation of *Arabidopsis* plants during the whole ontogenesis under 3D-clinorotation up to the formation of viable seeds. 3D-clinorotation caused a transient (≈ 20 h) delay in biomass growth, accompanied by a rise in total H_2O_2 content in 10–14 day old seedlings. Clinorotation also inhibited root elongation temporarily, stimulated excessive root curvature and directed root growth abnormally. Metabolite profiling of 10–14 day old seedlings showed the trend to decrease in content of the majority of metabolites under clinorotation except for malate. Pulse amplitude modulation (PAM) fluorometric analysis of photochemical activity in leaves of 3D-clinorotated seedlings was performed to understand the peculiar properties of plant cultivation in microgravity conditions. We have also studied cytoskeleton organization *in vivo* in 7 day old *Arabidopsis* seedlings using GFP-fABD2 and Lifeact-Venus transgenic lines for microfilaments (MFs), and MAP4-GFP and TUA6-GFP lines for microtubules (MTs). Under vertical growth conditions, MTs laid oblique both in hypocotyl and in root elongation zone and did not change significantly under 3D-clinorotation. In actin cytoskeleton, axial MFs dominated both in root cortex and stele in elongation zone, followed by oblique MFs, and minor transversally oriented MFs. However, microgravity modelling lowered the fraction of axial MFs by 50% in root cortex but not in stele, the total number of MFs was lower as well, and the fraction of transversal MFs was higher, contributing to overall 'randomized' image of actin. Remarkably, first in a lifetime placement of seedlings from 3D-clinorotation to a vertical position stimulated fast actin 'axialization' within 30 min. Therefore, we suggest that the 'default' organization of actin is random in the absence of gravity vector, and MF axial orientation is stimulated by gravity.

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Lecture

Novel molecular chaperones in cyanobacteria: groEL and clpB paralogs

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Molecular chaperones are involved in maintaining protein homeostasis under normal and stress conditions. GroEL and ClpB are evolutionarily conserved molecular chaperones. In general, GroEL forms a double ringed tetradecamer to assist protein folding and refolding. *E. coli* ClpB hexamer can disaggregate large aggregates.

Cyanobacteria have successfully adapted to a wide range of environments. I postulate that molecular chaperones must have been deeply involved in the adaptations to environmental stresses, and thus in the evolution of cyanobacteria. Cyanobacterial chaperones may have evolved differently than those in the model organism *E. coli* in order to support the photoautotrophic life under various environments. Thus, we have been particularly interested in paralogs of cyanobacterial chaperones. In contrast to *E. coli*, cyanobacteria contain multiple genes/paralogs encoding groEL or clpB.

There are two groEL and two clpB genes in the cyanobacterium *Synechococcus elongatus* genome. We have conducted a comparative biochemical analysis of these GroELs or ClpBs from *S. elongatus* with highly purified His-tagged recombinant proteins. Our study indicated that one of the members in each gene family, i.e., groEL1 or clpB1, codes for a chaperone whose structure and function are similar to the corresponding one in *E. coli*. On the other hand, the other member, i.e., groEL2 or clpB2, codes for a protein that is different from the other one. GroEL1 and GroEL2, or ClpB1 and ClpB2 were clearly different from each other in terms of structure and chaperone function. Cellular level studies of ours and other groups showed that GroEL1 and ClpB1 are functionally similar to *E. coli* GroEL and ClpB, respectively, whereas GroEL2 and ClpB2 are totally different from the other homologs. Based on these previous and our present studies, I postulate that groEL2 and clpB2 paralogs have acquired novel, beneficial functions under normal and/or stress conditions and become preserved by natural selection, with groEL1 and clpB1 genes retaining the functions of the corresponding ancestral genes.

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Lecture

Specificity of Cd, Cu, and Fe action on cation contents in chloroplasts and activities of PS II and PS I

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Treatment with any heavy metal (HM) induces the same dysfunctions in plants. It makes an impression that all HMs affect plant organisms in the same manner. We tested three HMs to search for their specific effects on processes in chloroplasts. Cd and Cu are the most toxic HMs; Fe is HM with low toxicity. We applied concentrations that inhibited growth of barley seedlings to the same extent: 80 μM Cd, 80 μM Cu, 1.5 mM Fe.

We revealed that Cd treatment changed contents of all cations studied in barley chloroplasts [1]. Contents of Mn and K were changed non-specifically with any HM studied; contents of the rest of the 5 cations were altered with Cd treatment specifically. Cu treatment increased Cu content in thylakoids only. Fe treatment increased Mg content in thylakoids (but not Fe content itself). We found no correlation between changes of cation contents in leaves and in chloroplasts. This suggests that cation contents in barley chloroplasts don't reflect changes of cation backgrounds in leaves; instead, they are the result of subtle regulation.

Cd had a distinct effect on chlorophyll fluorescence. In the moderate range of actinic light (200–1000 μmol photons·m^{-2}·s^{-1}) Cd treatment greatly decreased non-photochemical quenching and in this way increased level of so called closed centers of PS II. Cu and Fe treatment had no such prominent effect. In contrast, Cd treatment didn't influence "dark parameters" Fm, Fv, and Fo; however, Cu and Fe affected them.

In intact barley leaves, Cd inhibited P700 absorption more than chlorophyll fluorescence. Cd treatment decreased Pm value and Pm/Fv ratio by 30%. Cu treatment changed Pm/Fv by 18%; Fe treatment didn't influence it. Cd and Cu decreased quantum yield Y(I) of PS I; Fe not affected Y(I). We calculated quantum yield of PS II analogously to Y(I). In control, the increase of actinic light from 37 to 2000 μmol photons·m^{-2}·s^{-1} raised the ratio of quantum yields PS I:PS II from 1.25 to 4. Cu and Fe treatment modified the tendency slightly. Cd treatment blocked the increase of ratio PS I:PS II till c.a. 700 μmol photons·m^{-2}·s^{-1}; at 1000 μmol photons·m^{-2}·s^{-1} the ratio reached value 2 only.

Cu treatment specifically increased acceptor side limitation Y(NA) of PS I at low light. Y(NA) was high at 37 and 70 μmol photons·m^{-2}·s^{-1} and nearly disappeared at 150–200 μmol photons·m^{-2}·s^{-1}. Probably, Cu treatment turned some components of Calvin cycle more sensitive to light activation.

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Lecture

Model of microalgae *Chlamidomonas reinhardtii* adaptation to sulfur starvation

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Adaptation of microalgae to stress conditions is often accompanied by compensatory processes such as lipid accumulation or hydrogen evolution. The study of adaptation processes is of interest both for understanding the fundamental mechanisms of the cellular response to stress, and for developing the optimal strategy for obtaining biofuels.

The hierarchical mathematical model that combines the central metabolic pathways, electron transport chains of photosynthesis, and respiration in the microalgae cell was developed to study the processes of cells adaptation to mineral stress. The central metabolic pathways including glycolysis, the Calvin-Benson cycle, and the Krebs cycle were described in accordance to Flux Balance Analysis formalism. The sub-model of photosynthetic processes was described by sets of differential equations for multi-enzyme complexes states. NAD(P)H was considered to be the key metabolite which coupled the metabolic pathways and the electron transport chain.

As input to the model, we used experimental data on starch accumulation and on activity of Rubisco and photosystem II in *Chlamydomonas reinhardtii* cells under sulfur starvation. The combined hierarchical model allowed us to obtain the series of distributions of metabolic fluxes coupled with photosynthesis reactions. A shift in intracellular flux distribution was predicted during transition from sulfur sufficient phase to sulfur starvation phase of growth.

The model revealed the relationship between the inactivation of photosystem II, the redirection of anabolic pathways to catabolic, the activation of chlororespiration, and the hydrogen production at different stages of sulfur starvation.

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Poster

Diverse roles of structural and reserve lipids in photoprotection of microalgae

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We discuss multifaceted roles of lipids in operation and regulation of photoprotective mechanisms in photosynthetic cells using microalgal models with contrasting responses to environmental stresses. Stress-resilient microalgal species generally react to the stress by reducing their photosynthetic apparatus and redirecting the excessively photo-fixed carbon to storage subcompartments of the cells (starch grains and lipid droplets) [1]. In carotenogenic microalgae such as *Haematococcus pluvialis* this response is tightly coupled with induction of secondary carotenogenesis greatly enhancing tolerance of these organisms to adverse environmental conditions via several mechanisms [2]. Certain species exhibit a high content of long-chain polyunsaturated fatty acids (PUFA) in structural and reserve lipids with an illustrious example of the producer of arachidonic acid *Lobosphaera incisa*. Our recent studies showed that the presence of this PUFA, along with acclimatory rearrangements of lipid metabolism, cell structure and photosynthetic apparatus is conductive for stress tolerance of these organisms. Thus, PUFA are essential for swift responses of the photoprotective mechanisms e.g. NPQ to stresses such as high light and/or chilling stress on the background of nutrient deprivation [3]. We generalize on common and specific aspects of orchestration of cell photoprotective capacity and lipid metabolism responses to diverse stresses.

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Poster

Seasonal adaptation changes of photosynthetic apparatus of Scots pine in conditions of South-Eastern Siberia

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The extreme combination of high light, low humidity and low air temperature in the region under study makes the achievement of high level of vegetation a difficult task. A necessary requirement for its achievement in such conditions is the development of adaptive reactions at various levels of the organization of the photosynthetic process. To obtain a complex picture of seasonal changes in the organization of photosynthesis in pine needles, long-term observations of changes in the productivity of photosynthesis (PP) and its relationship with environmental factors, water content in pine needles, composition of pigments and fatty acids (FA), grana index (GI), the content of heat shock proteins (Hsps) and dehydrins (DG) and their intracellular localization were made. The high level of irradiance, cooling of the root system and lack of moisture in the spring have a multidirectional effect on PP and create a high probability of destructive processes. During this period, there was an increase in the content of polyunsaturated fatty acids (PUFA), a decrease in GI and a low content of Chl *a*/*b*, an increase in the content of DG with their predominant localization in the cell walls and membranes of chloroplasts, the high level of Hsp70 stress protein. In the middle of the growing season, the realization of photosynthetic function reaches a seasonal maximum. Under favorable environmental conditions, a hallmark of the organization of photosynthesis in this period is the increase in the content of Chl a/b, the growth of GI, of the water content in the needles and of the content of PUFA and Hsp17,7. In autumn, environmental factors act on PP more unidirectionally, than in spring. Together with of the gradual decrease of PP, there is another increase in the content of PUFA after the fall in August, the value of GI remains high, while there is a significant decrease in the content of Chl *a*/*b*, the accumulation of DG and Hsp 70, the predominant association of DG with chloroplast membranes. Thus, complex seasonal changes in the photosynthetic apparatus of Scots pine include changes in the water content in the needles, in the composition of chlorophylls, GI and FA composition, in the content and set of stress proteins and DG, in intracellular localization of DG. However, the relationship between changes in all studied components of the organization of the photosynthetic process remains unclear.

Poster

An insight into relationships between the changes in microalgal cell ultrastructure and their optical properties

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Algal cells are highly complex optical systems capable of dynamic rearrangement of their structure to ensure efficient light capture and its photosynthetic conversion. This capability is central to environmental stress resilience and productivity of algal cultures. Interaction of light with algal cells includes absorption by pigments, mainly chlorophylls and carotenoids, as well as refraction and scattering by cellular structures such as cell wall/cytoplasm or organelle membrane/cytoplasm. To elucidate the role of changing cell organization in determining optical properties of the cells, transmission electron microscopy images of *Chlorella vulgaris* IPPAS C1 cells were taken during selected time-points of the cell cycle. The key ultrastructural features of *C. vulgaris* IPPAS C1 acclimation to HL included an increase in the size of cell and chloroplast, accumulation of starch grains (both inter-thylakoid and those of the pyrenoid sheath) decline in the thylakoid stacking, increase in the rubisco-containing matrix of the pyrenoid and expansion of the periplasmic space. To correlate the ultrastructural changes of the cells with changes in the absorption and scattering spectra during the cell cycle, we quantified in detail the ultrastructural organization of *C. vulgaris* cells sampled at the time points of measurement of the optical spectra. The key determinants of the light scattering capacity of *C. vulgaris* cell are the interfaces between cell wall, cytoplasm and periplasm (but not cell wall thickness per se) as well as size and number of inclusions with contrasting refraction coefficients (e.g., starch grains). The gained knowledge about the relationships between ultrastructure and optical properties changes of algal suspensions would provide valuable insights for analysis and modeling of light distribution inside photobioreactors and, thus, for knowledge-based analysis and modeling of algal growth.

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Poster

Intra- and intercellular communication of chloroplasts in *Chara corallina* cells

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Photosynthesis is tightly regulated in plant cell, but the regulatory mechanisms are still under investigation. In non-uniform fluctuating light, photosynthesis has specific features because the transitions between light and darkness are associated with activation/deactivation of photosynthetic enzymes and because illuminated cell parts affect the shaded cell areas via long-distance interactions. The present study focused on long-distance communications established between immobile chloroplasts by means of cytoplasmic streaming in giant *Chara* internodes. Chlorophyll fluorescence was recently found to be a sensitive noninvasive indicator of long-distance intracellular transport of physiologically produced photometabolites in characean internodes. The present work shows that the chlorophyll microfluorometry has a potential for studying the intracellular and cell-to-cell transport of reducing substances released by local illumination of one internode and detected as the fluorescence increase in the other part of internode or even in the neighbor cell. The method is based on cyclosis-dependent redistribution of metabolites produced in brightly illuminated chloroplasts and exported to the cytoplasmic flow. The products released from chloroplasts travel with the streaming liquid and enter the chloroplasts in shaded areas. The entry of reducing substrates into the chloroplast stroma modulates photosynthetic electron flows, thus causing a transient increase in chlorophyll fluorescence due to a temporal reduction of the primary quinone acceptor Q_A in photosystem II. Triose phosphates and reduced substrates (NADPH) are presumably the substances that are transported by liquid flow and modulate the electron transport in shaded chloroplasts, resulting in Q_A reduction. We have shown that the reduction of chloroplast stroma after the delivery of reductants modulates the redox state of PQ and Q_A by two separate pathways, i.e., the photochemical path that operates under weak background lighting of the whole cell and the non-photochemical path that is sensitive to inhibition by antimycin A and involves the segments of cyclic electron flow. The same method was used to evaluate permeability of plasmodesmata to natural components released by illuminated chloroplasts. The results show that approximately one third of the amount of photometabolites released into the streaming cytoplasm during a pulse of local light permeates across the nodal complex with the characteristic time of ~10 s. The results show that the permeability of plasmodesmata to low-molecular photometabolites is subject to upregulation and downregulation.

Poster

Acclimatory reduction of the Photosystem II antenna size under salinity and drought, and the role of the PQ pool reduction state

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To investigate how drought and salinity stress conditions affect the size and function the photosynthetic apparatus of higher plants, one group of *Arabidopsis thaliana* plants was watered once in three days with 200 mM NaCl solution, another group was not watered during the experiment and the control group was watered once in three days with tap water over the course of three weeks. The performance of photosynthesis was assessed by measuring OJIP kinetics every three days. It has been found that plastoquinone pool became more reduced under both stress conditions comparing to the control plants. Western blot analysis was performed to estimate the influence of each kind of stress on the amounts of certain PS II proteins. PCR was performed to measure the expression level of the PS II antenna proteins. Hydrogen peroxide content in leaves has been also measured in control and stressed groups of plants.

Under both stress conditions, the plants have shown a substantial decrease of the expression level of *lhcb1*, *lhcb2*, *lhcb3*, *lhcb4*, *lhcb6* as well as in the Lhcb1 and Lhcb2 proteins content, in comparison with the control plants. In addition to this, the apparent size of the PS II antenna, calculated from the OJIP kinetics, decreased as well. The level of hydrogen peroxide substantially increased in the group that was watered with NaCl solution and in the group that was not watered during the experiment, in comparison with the control group. Based on the obtained data it can be suggested that the reduction of the PS II antenna size represents one of the universal mechanisms of higher plants acclimation to stress conditions.

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Poster

Expression of wild plant AMPs in potato for resistance to early blight

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BACKGROUND. The resistance of many common wild plants to pathogens comes from the low molecular weight proteins (peptides) with antimicrobial activities (AMPs). Several AMP families have been reported in plants on the basis of sequence similarity and the so-called cysteine motifs (Crit. Rev. Plant Sci. (1997) 16:297-323). METHODS. Our group isolates, characterizes and transfers AMPs to crops with the aim to improve their resistance to microbes. The peptides were acid-extracted from the wild plants, then further isolated by liquid chromatography and sequenced by the automated Edman degradation. Alternatively, the peptides were discovered by searching for their genes in assembled transcriptomes. The antimicrobial activities of peptides were measured by radial diffusion assays, microtiter plate assays, potato tuber discs assays, and light microscope observations. Then the AMP genes were cloned and introduced to crops by *Agrobacterium*-mediated transformation. The transgene expression was measured by either Northern hybridization or qRT-PCR, and the *Alternaria* resistance was evaluated by the pathogen lesion sizes on inoculated leaves. RESULTS. We isolated and characterized the highly similar pairs of defensins differing by a single amino acid residue, from black cumin *Nigella sativa* (Rogozhin et al., Plant Physiol Biochem, 2011) and chickweed *Stellaria media* (Slavokhotova et al., Biochimie, 2011). These peptides inhibit growth and development of *Phytophthora infestans in vitro* and *in planta*. The hevein-like peptides (Rogozhin et al., Biochimie., 2015) from *S. media* demonstrated activities against *Alternaria* spp. An *S. media* gene proSmAMP1 encodes two hevein-like SmAMP1.1a and SmAMP1.2a that are released from the propeptide by a specific proteolysis (Shukurov et al. Transgenic Res, 2011). The gene was transferred to Russian-bred potato varieties (number of transformed lines) Skoroplodny (19), Udacha (6), Zhukovsky rannii (34), and Yubiley Zhukova (3) that are diverse with respect to *Alternaria* resistance. In transgenic potato, however, the partially processed propeptide comprising SmAMP1.1a and SmAMP1.2a connected by a linker and devoid of N- and C-termini was detected. The transgene expression inversely correlated with the symptoms of *Alternaria*, but not *Phytophthora*. The transgene improved *Alternaria* resistance of all varieties with the greatest impact (some lines demonstrated high resistance for years) on the highly susceptible varieties. We also expressed a defensin from black cumin *Nigella sativa* in several potato varieties differing in their resistance to early blight, and our preliminary results indicate that some lines are significantly more resistant to early blight, but not to late blight.

Poster

Regulation of the phosphatase activity in thylakoid membrane of higher plants

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Experiments with whole arabidopsis plants allowed to assume that H_2O_2 regulates state transitions by influencing the activity of phosphatase. Experiments with isolated thylakoids under conditions promoting phosphorylation of the antenna proteins of PS II have shown that H_2O_2 addition into the thylakoid suspension did not affect STN7 kinase operation, since it had no effect on the light-induced accumulation of the Lhcb1-phosphorylated (Lhcb1-P) and Lhcb2-phosphorylated (Lhcb2-P) proteins. No influence of H_2O_2 on the migration of the light-harvesting antenna from PS II to PS I, that was estimated based on the measurements of the chlorophyll *a* fluorescence at 77 K, was also observed. However, in the presence of H_2O_2 no dephosphorylation of Lhcb1-P and Lhcb2-P proteins occurred after 30 min in the dark following illumination, while significant dephosphorylation of these proteins was detected after 30 min in the dark in the absence of H_2O_2. The obtained data allowed to assume that hydrogen peroxide exert the inhibitory action on phosphatase.

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Poster

Phenotyping of photosynthetic responses of wheat genetic resources to drought stress using fast noninvasive techniques

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An efficient utilization of crop genetic resources is limited by insufficient phenotypic data, including information on stress photosynthetic responses. Therefore, we focus on assessment of the methods and tools for rapid evaluation of the photosynthetic traits associated with drought tolerance. To examine the links between photosynthetic traits and drought stress responses, we have realized pot and field phenotyping experiments with a collection of 25 wheat genotypes, which broadly cover a diversity of leaf traits. Plants exposed to long-term moderate water deficit were compared with a well irrigated variant. In addition to analyses of growth traits, the measurements of fast chlorophyll fluorescence kinetics (OJIP), fluorescence excitation ratio, hyperspectral analysis in visible and near infrared spectra (VNIR) to assess the photochemical reflectance indices (PRI) and infrared imaging to assess the stomatal responses were performed in 3–4 days interval to assess the effect of drought stress on photosynthetic apparatus. The measurements were followed by the analyses of aboveground biomass and grain yield. Our results indicated variations in responses to prolonged drought on plant morphology (reduced leaf area, biomass, and chlorophyll content) as well as on photosynthetic parameters. Moreover, we identified a close relationship between the changes of leaf traits and leaf optical properties measured by the hyperspectral reflectance records. A group of drought sensitive parameters was identified and their correlation with drought indices was assessed. Our results indicated that some of the parameters based on high throughput spectral reflectance and chlorophyll fluorescence techniques can be useful to assess the drought tolerance of the genotypes.

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Poster

Photoprotective effect of nanodiamonds in green microalgae *Chlamydomonas reinhardtii*

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Nowadays, emerging nanotechnologies are intensively implemented in the various fields of applied biology. Detonation nanodiamonds (ND) are particles with size of approximately 5 nm, which have crystal structure of a diamond. In order to improve solubility in aqueous solution, ND surface is functionalized with hydrophilic functional groups that bring new physical and chemical properties. It has recently been shown that functionalized ND possess oxidase-, peroxidase-, and catalase-like activity in aqueous solutions [1], which open the possibility to exploit ND for cell protection in algal biotechnology. Nutrient deprivation is often used in biotechnology to switch cell metabolism from growth towards production of lipids (biofuel), molecular hydrogen, or carotenoids (astaxanthin). But this stimulus also causes overgeneration of reactive oxygen species thus reducing photosynthetic activity, culture viability, and the yield of valuable products.

In the present work, we investigated the protection effects of ND functionalized with carboxyl group in the model green alga *Chlamydomonas reinhardtii* (cell-wall deficient strain CC-400) exposed to the oxidative stress. In order to induce oxidative stress, cell cultures were either exposed to strong light (photoinhibition), sulfur deficiency, or treated with methyl viologen or dye Rose Bengal to generate superoxide anion radicals or singlet oxygen, respectively. Photosynthetic capacity was assessed by recording the chlorophyll fluorescence parameter F_V/F_M which refers to the maximum efficiency of PS II photochemistry. We showed that the addition of ND to algal suspension results in apparent PS II protection under oxidative stress. The highest extent of ND based protection of the PS II activity was observed under the stress induced by methyl viologen, indicating particular efficiency of ND for deactivation of the superoxide anion radical and related reactive oxygen species in the cell. The obtained results allowed us to conclude that ND can serve to improve photosynthetic stability and performance in the algal cell upon oxidative stress thus facilitating culture viability.

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Poster

Anatomical and physiological adaptations for maintaining photosynthesis efficiency in halophytes under tidal cycle conditions on the White Sea coast

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Significant fluctuations in water level due to tidal dynamics are a feature of the White Sea. Twice a day the plants are flooded and released from the water in the intertidal zone. Tidal dynamics is accompanied by changes in light, temperature, oxygen and carbon dioxide concentrations, pressure and other climatic parameters [1]. These conditions formed a unique halophytic complex of higher land plants [2], which undergo ontogenesis and are resistant to tidal dynamics. However, the reaction of plants to a change in environments, regulation and possible mechanisms of response reactions of an organism under conditions of tidal dynamics are practically not discussed. The purpose of this study is a comparative study of the dynamics of the structural and functional characteristics of the *Plantago maritima* L. and *Triglochin maritima* L. under supratidal and intertidal conditions. The quantitative indices of the anatomical structure of plants leaves did not differ in the littoral and supralittoral zones. The exception is the number of stomata in the leaves of the *Pl. maritima*. The number of stomata increased in *Pl. maritima* in the intertidal zone. The functional indicators (intensity of photosynthesis, fluorescence and stomatal conductance) of supratidal plants have circadian rhythms, while functional indicators of intertidal plants have rhythmic tidal cycles. But the two studied species differ in the mechanisms of reaction to the tidal cycle. *Pl. maritima* has the highest values of stomatal conductance, fluorescence, photosynthesis and the most open stomata during periods of full high tide and full low tide (functional adaptation). *Tr. maritima* has a high functional activity only at full low tide. At full high tide, the *Tr. maritima* closes the stomata and maintains functional activity using internal resources (structural adaptation). The hypothesis of the leading regulatory role of stomata is expressed and the structural and functional adaptation of halophytes for maintaining photosynthesis efficiency to tidal dynamics in coastal ecosystems of the White Sea are considered.

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Poster

Apparent quantum yield of photosynthesis of chilling-sensitive plants affected by a daily short-term temperature drop

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Apparent quantum yield of CO_2 assimilation (α) is a parameter determined from the initial slope of photosynthetic light-response curve. The parameter is the moles of CO_2 fixed per mole of quanta absorbed; respectively it defines the efficiency of light utilization in photosynthesis. The quantum yield has some important implications since it defines plant productivity under limited light, considering that under natural conditions the rate of photosynthesis is limited by light most of the time [1]. A series of experiments was conducted to investigate the photosynthetic response of chilling-sensitive plants (cucumber, tomato, sweet pepper, eggplant) to a daily (for 6 or 14 days) short-term (2 h) temperature drop (DROP treatment). Among all studied species cucumber plants were the most sensitive to the DROP treatments. The DROP treatment resulted in a decrease of quantum yield values and the extent of this decrease enhanced with the decrease of DROP temperature.

The stage of leaf development did not have a significant influence on the parameter α. By contrast, growth light conditions did impact on both α and its response to DROP. So, shaded plants exhibited lower values of α than their high light-grown counterparts, but plants grown at 24 h photoperiod had higher α value than plants grown at 16 h day.

Plant lighting during the DROP treatment, high growth irradiation and long photoperiod (24 h) enhanced inhibitory effect of DROP on quantum yield of plants. Light requirement of species also affected the response of quantum yield to the DROP. The decrease of the parameter α was more strong for shade-tolerant than shade-intolerant plants regardless in the light or dark the DROP occurred. On the other hand the DROP treatment affected the response of quantum yield to growth conditions. So, the DROP partly restored the efficiency of plant light utilization under 24 h photoperiod. But, the DROP did not contribute to the recovery of α value of cucumber plants infected with downy mildew. In summary, a daily short-term temperature drop may decrease apparent quantum yield of CO_2 assimilation in chilling-sensitive plants. Importantly, light is one of the factors that do affect the response of α to the DROP. We suggest that the reduction of efficiency of light energy utilization in chilling-sensitive plants treated by a temperature drop is one of primary responses protecting plants from oxidative damage.

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Poster

The role of alternative oxidase according mechano-chemiosmotic model of coupling electron tansport to ATP synthesis

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It is well known that in the organs that need large amounts of ATP, mitochondria are located rightly around the myofibrils and cristae, and these are located as "coins stack". Also, it has been found that for up to 4 days of hypoxia, the cristae of plant mitochondria become parallel packing, and during hypoxia on the fifth to seventh day after coronary artery ligationin the muscle fibers of the myocardium, it revealed a large number of mitochondria with densely packed cristae. However, in the further continuation of hypoxia, on the contrary, mitochondria swell. The function of the conformational changes occurring in the mitochondria during the synthesis of ATP remains incomprehensible. On the basis of numerous published data and our own experimental data, we have developed a mechano-chemiosmotic model of coupling electron transfer to ATP synthesis, where the electron transfer along ETC, proton transfer, transport of cations, cyclic low-amplitude swelling-shrinkage, and ATP synthesis are coupled processes. According to this model, an asymmetric contact of dimers of opposite cyt *bc*1 complexes is formed in the intracristal space during shrinkage of organelles, which is a mechanical regulator of electron transfer from [2Fe-2S] cluster to heme *c*1 and ROS (reactive oxygen species) production. The formation of ROS was experimentally demonstrated in hypoxia recently. Thus, the change in the structure of the inner membrane of mitochondria, causing swelling-shrinkage of the intracristae space, is an electron transfer regulator of ROS production and ATP synthesis. Presence of cyanide insensitive alternative oxidase (AOX) forms the striking functional difference between mitochondria of higher plants (as well as some fungi and protists) and animals, i.e., presence of two terminal oxidases, AOX and cytochrome oxidase. AOX acts by introducing a branch into ETC at the ubiquinone pool preventing excessive reduction of the downstream cyt *bc*1 and cyt *aa*3 complexes (cytochrome pathway), in case of any dysfunction, thus cutting down the single electron leakage from any of the ETC complexes to O_2, hence preventing excessive mitochondrial ROS in the swelled mitochondria, when electron transfer from [2Fe-2S] cluster to heme *c*1 is impossible.

Poster

The role of photorespiration in the photosynthesis regulation

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It is known that photorespiration is the reverse process of photosynthesis and greatly reduces its efficiency. There was an idea about the possibility of photosynthesis increasing by suppressing photorespiration. However, many years studies of photorespiration role in leaves gas exchange, point to the unsuccessful attempts to increase plant productivity by suppressing this process. At the same time, the photorespiration measurement (the Warburg effect) revealed an inverse relationship between this process and the sucrose export from the leaf under removal part of mature leaves or fruit elements. The analysis of the ^{14}C inclusion kinetics [1] in the primary products of photosynthesis showed an opposite change of the incorporation labeled carbon into glycolate during the first 10–20 s under removal of some fruit elements from the cotton plant. In the control variant (the maximum at 10 s exposure of the leaf in $^{14}CO_2$ atmosphere), the labeled glycolate maximum was observed later than in the sugar outflow inhibited variant. This indicates that in the second case, the formation of glycolate occurs from the product $^{14}CO_2$ of the primary fixation (sugar phosphate), and not in the Rubisco oxygenase reaction from the previously labeled ^{14}C-RBP.

Such a glycolate source is the transketolase reaction, for which a superoxide radical, which is formed in the Mehler reaction in the electron transport chain of chloroplasts, is required as an oxidant. Under the sugar export inhibition from the source-leaf, the glycolate pathway products (organic acids) cannot return to the Calvin cycle [2]. This leads to the accumulation of acids in the chloroplast, and then in the mesophylic cell cytoplasm, and further in the apoplast. An apoplastic fluid acidification activates the invertase and the hydrolysis of sucrose, so the osmolarity of the aquatic environment of the apoplast is increased (1 sucrose → 2 hexoses), resulting in the stomata closure and an increase of CO_2 diffusion resistance in the leaf, which reduces photosynthesis [3]. This occurs in accordance with the degree of imbalance between the light/dark reactions of photosynthesis. Thus, the light/dark reactions of photosynthesis are normalized.

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Poster

The effect of salicylic acid on photosynthetic rate and antioxidant activity of wheat plants at optimal and low temperatures

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The phytohormone salicylic acid (SA) is involved in the regulation of various physiological and biochemical processes of plants, including the increase in tolerance to adverse environmental factors such as low temperatures [1–4]. The aim of our research was to investigate the effects of exogenous SA on photosynthetic rate and antioxidant activity of wheat plants (*Triticum aestivum* L.) under optimal and low temperatures. 7-day-old wheat seedlings var. Moskovskaya 39 were placed on SA solution (100 μM) and after 1 day one part of plants continued to grow for 7 days at optimal temperature (22°C), while other part was exposed to low temperature (4°C). It was found that at 22°C treatment of seedlings with SA caused a significant decrease in photosynthetic rate and had no effect on water use efficiency (WUE). At 4°C, the rate of photosynthesis was maintained at a higher level in SA treated plants (compared to untreated seedlings), and WUE was increased. It was shown that SA increased the activity of antioxidant enzymes such as catalase, superoxide dismutase, and guaiacol-dependent peroxidase at optimal and low temperatures, as well as increased the accumulation of transcripts of genes encoding CAT and FeSOD. Activation of the antioxidant system, induced by SA, led to a reduction of oxidative stress level in plants. Along with this, exogenous SA improved the cold tolerance of wheat plants both in optimal (22°C) and low-temperature (4°C) conditions.

The obtained data suggest that under optimal and low-temperature conditions, exogenous SA causes an increase in antioxidant activity, that leads to decrease in the level of oxidative stress, promotes the stabilization of photosynthesis (under cold stress) and induces the formation of increased cold tolerance of wheat plants.

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Poster

Decrease of cell division protein FtsZ leads to enlargement of the cell under acid stress in Cyanobacterium *Synechocystis* sp. PCC6803

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Acid treatment causes an increase in cell volume in *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*). We aimed to clarify the relationship between acid stress and enlargement of cells. After synchronizing the cells under the dark condition for 20 hours, the cells under different pH (pH 8.0 or pH 6.0) were cultivated under the light condition. The volume of the cells after the light irradiation for 24 hours increased about 3 times under pH 6.0.

To reveal the effect of acid stress for cell division, we performed QRT-PCR and western blot of FtsZ, which is essential for cell division and forms Z-ring. The transcription of *ftsZ* did not increase under pH 8.0, while the amount of FtsZ increased gradually under pH 8.0. The transcription of *ftsZ* increased under pH 6.0, while the amount of FtsZ did not increase under pH 6.0. Acid stress might cause FtsZ to arrest translation or to promote degradation.

In *E. coli* and *C. crescentus*, the amount of FtsZ is degraded by ClpXP. Therefore, we investigated whether ClpXP participates in the cell enlargement under acid stress in *Synechocystis*. *Synechocystis* has three different ClpP paralogs (ClpP1, ClpP2, and ClpP3). We performed QRT-PCR to investigate mRNA expression of *clp* genes. The transcription level of *clp* genes increased about 3 times under pH 6.0 than pH 8.0 for 24 hours. These results suggest that *clp* genes relate with cell enlargement under acid stress. In this presentation, we will show that the relation between acid stress and degradation of FtsZ.

Poster

Bacterial ketones inhibit cyanobacterial photosynthesis

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Bacteria produce a wide variety of secondary metabolites with miscellaneous biological functions. These metabolites are small molecules, e.g., non-proteinogenic amino acids and peptides, aldehydes, alcohols, esters, fatty acids, hydrocarbons, ketones and their derivatives in the form of volatile organic compounds (VOCs). The first database of microbial volatiles was created recently [1]. VOCs produced in the microbial world are ideal infochemicals because their spheres of actions include 'aqueous' as well as 'atmospheric' diffusion and consequently, these volatiles can act aboveground as well as belowground. The ecological functions of microbial VOCs are still under investigation. This study for the first time demonstrated the strong inhibitory effect of bacterial ketones taken at ecologically relevant concentrations on cyanobacterial photosynthesis *in vivo* [2]. Ketones cause significant disturbances in the photosynthetic apparatus of cyanobacteria during the first hour of incubation. These effects include (1) change of the induction curve shape, (2) increase of the chlorophyll and phycobilin fluorescent intensities compared to the control, and (3) prolonged dark recovery kinetics of the PS I and PS II reaction centers oxidized by a single light flash. Depending on the time of incubation, the action of ketones may be similar to that of known artificial inhibitors of PSII. After the third hour of incubation with ketones, significant chlorophyll degradation occurred. Our experimental data indicate disruption of photosynthetic electron transport and energy transfer from phycobilins and chlorophyll in the PS II reaction centers. Apparently, ketones as metabolic products of a number of microorganisms can participate in antagonistic interactions in the environment.

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Poster

The impact of fluctuating light and salinity on photosynthesis and growth of radish plants

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In nature, changes in light intensity can occur very rapidly. Temporary shading by clouds or neighbouring plants massively affects photosynthesis by rapidly altering the amount of light energy available for electron transport and carbon fixation. Moreover, the salinization of soils represents one of the largest environmental challenges worldwide. The efficiency of photosynthetic machinery under fluctuating environmental conditions has been identified as a key target for crop improvement for overcoming stress situations. In order to assess the impact of growth light regime (continuous light, CL, and fluctuating light, FL) and salt stress (0, 150 and 300 mM NaCl) on PS II photochemistry, pigment composition, antioxidative enzymes activity, water status and biomass accumulation, we grew radish plants in Plantscreen™ phenotyping facility. We demonstrated that both light regimes as well as salt stress, significantly decreased the leaf chlorophyll content and dry mass accumulation. Using the JIP test, we identified that photochemical PS II efficiency and energy flux were stronger affected by salt stress during plant cultivation in FL condition. Increasing salinity led to higher accumulation of Q_B-non-reducing PS II reaction centres in FL than in CL. Finally, has been observed that the donor side of PS II is more affected by high salt concentration compared to the acceptor side of PS II in FL. Observed down-regulation of the primary photochemistry in salt treated plants was resulted from ineffective Na^+ extrusion and raised the ionic imbalance, mainly in FL. The results are supported by the image-based non-destructive analysis of whole plant chlorophyll fluorescence and RGB leaf area colouring. Salt stress induced significant reduction of green biomass with larger increase of portion chlorotic areas of leaves.

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Poster

Adjusting photosynthetic electron transport machinery of salt-exposed lettuce plants through rhizobacteria *Bacillus subtilis*

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Salinity is an important limiting factor for cultivation of agricultural crops. To cope with negative effects of salinity on growth, performance, and yield of crops, solutions containing minimal, or no harmful effects on the environment are desirable. Plant growth-promoting bacteria are free-living soil bacteria that can help plant growth without having pathogenic effects. In current study lettuce seedling were exposed to three concentrations of NaCl salinity (0, 40 and 80 mM). Half of the salt-treated plants were fed with rhizobacteria *Bacillus subtilis* (OD=0.6) to test whether it can reduce the negative effects of salinity on photosynthetic electron flow on lettuce plants. Polyphasic chlorophyll fluorescence transient was used to study the biophysical properties of the lettuce photosynthetic system. Maximum quantum yield of photosystem II was significantly improved by application of *B. subtilis* to the rhizosphere of salt-exposed plants. Specific energy fluxes per reaction center (RC) for energy absorption (ABS/RC), trapped energy flux (TR_0/RC), and dissipation (DI_0/RC) were decreased in plants with *B. subtilis* in their rhizosphere. While, electron transport flux per reaction center (ET_0/RC) was increased in *B. subtilis*-treated plants. Performance index on the absorption basis was increased by application of *B. subtilis* to the rhizosphere of both control and salt-exposed plants. In conclusion, application of *B. subtilis* to the rhizosphere improves efficiency of photosynthetic electron transport in lettuce plants and as a result helps plants to cope with adverse effects of salinity stress.

Poster

Repetitive light pulse-induced photoinhibition in Arabidopsis mutants deficient in energy balance

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Photosystem I (PS I) often shows a slow recovery from photoinhibition, but factors related to the inhibition have been unclarified. Here we aimed to assess effects of energy-balance mutations on PS I photoinhibition. We used two non-photochemical quenching (NPQ) mutants, *npq1* and *npq4* and two mutants of the mitochondrial respiratory chain, *aox1a*. The PsbS-deficient *npq4* has a defect in NPQ induction in photosystem II (PS II), and *npq1* is impaired in zeaxanthin-dependent NPQ. The *aox1a* mutants, *aox1a-1* and *aox1a-2*, are impaired in the alternative oxidase gene, *AOX1a*. The inactivation of PS I was caused by repetitive saturated pulses (rSPs) in the dark, which led to decrease of both quantum yields of PS I (YI) and PS II (YII). YI and YII of wild-type (WT) decreased to 0.18 and 0.42 by the severe rSP treatment, respectively. *npq4* showed lower donor-side limitation of PS II (YNPQ) and higher acceptor-side limitation of PS II (YNO), whereas *npq1* exhibited slightly lower donor-side limitation of PS I (YND) than WT during an early phase of the severe rSP treatment. Since a decrease in YNPQ by the rSP treatment was only observed for *npq4*, but not for *npq1*, PsbS protein may play an important role in the formation of NPQ by the treatment. *aox1a* had lower PS I acceptor-side limitation (YNA) and higher YI than WT during an early phase of the weak rSP treatment. After the weak rSP treatment, *aox1a* still showed lower YNA and higher YI than WT under continuous low light illumination. The AOX1a deficit may cause an increase in activity of the acceptor side of PS I, and lead to the increase in YI. Possible effects of these mutations on PS I photoinhibition are discussed.

Poster

In *Arabidopsis* plants, the chloroplast members of S2P-family have functional redundancy in regulating growth processes

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Site-2 proteases (S2P) belong to the family of intramembrane-cleaving proteases which are involved in regulated intramembrane proteolysis (RIP) influencing in many important signaling pathways. Two homologues of *Arabidopsis thaliana* S2P proteases, AraSP and AtS2P2, that are resides in the envelope membrane of chloroplast are found by bioinformatics' approach. From our study, the knockout of either *arasp* or *ats2p2* alone did not affect the growth and development of corresponding transgenic lines since both single mutant's *arasp* and *ats2p2* exhibited normal growth and development similar to wild type. The composition of the photosynthetic complexes and the photosynthetic capacity was not affected in single mutants and were similar to wild-type. However, the double mutation *arasp*/*ats2p2* caused a pleiotropic effect characterized by the altered composition of photosynthetic complexes and impaired photoprotective mechanisms, which is accompanied with pale green phenotype, lower growth characteristics and infertility. Due to the possible correlation between *AraSP* and *AtS2P2* gene expression, which was demonstrated by the single mutant RT-PCR analysis, AraSP and AtS2P2 may functionally complement each other and may be involved together at least indirectly in a wide range of processes, such as photosynthetic complexes formation or development of reproductive organs.

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Poster

Influence of OCP photoactivation on the effective absorption cross-section of PS II

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Quenching of excess excitation energy is necessary for the photoprotection of light-harvesting complexes. In cyanobacteria quenching of phycobilisomes is induced by orange carotenoid protein (OCP) which photoactivation occurs under high light conditions. This process leads to a decrease in the efficiency of energy transfer from the phycobilisomes (PBS) to chlorophyll and reduction of activity of photosystem PS II. Importance of OCP in photoprotection is generally accepted, although some cyanobacteria exist without that protein. Considering the fact that both photochemical and non-photochemical quenching have similar effects on quantum yield of PBS fluorescence, *in vivo* estimations of OCP efficiency are complicated and require alternative approach. As known cyanobacteria compensate nitrogen deficiency by degradation of PBS, thus reducing the effective absorption cross-section of PS II. In the present study we followed this process in order to compare effects of the reversible OCP-dependent quenching of PBS with a reduction of its size. Analyzing these relationships, we found that OCP-dependent quenching triggered by blue light involves less than 40% of all PBS. This observation indicates that under normal conditions efficiency of photoprotection is limited by OCP concentration which is probably lower than concentration of PBS.

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Poster

The effect of photoinduced relocation of chloroplasts on the protection against photodamage in *Tradescantia fluminensis* leaves

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Chloroplast avoidance relocation is considered to be among the most efficient photoprotective mechanisms in plants. Furthermore, in *Tradescantia* species, it was supposed to be the main (if not the only) photoprotective mechanism. It's pivotal role was suggested by (i) much higher extent of relocation-dependent leaf transparency changes as compared with other plants and (ii) by the apparent lack of other photoprotective mechanisms in *Tradescantia* plants.

The effect of the photoinduced relocation of chloroplast on the overall photoprotection efficiency might be elucidated by measuring action spectra. The relocation is governed by phototropins in higher plants hence it is induced by blue but not by red actinic light (AL). Comparison of blue and red AL effects on the photochemistry in *Tradescantia fluminesis* leaves yielded surprising conclusions. Thus, the red (620 nm) AL stronger declined the PS II photochemical activity (Φ_{PSII}) as compared to the blue (470 nm) AL of the same (high or moderate) irradiance. Clearly, lowering of excitation pressure on PS II retains a higher PS II operational activity. Surprisingly, the nonphotochemical quenching coefficient (NPQ) was only slightly higher under the red AL exposure. Moreover, we observed no sigh of photodamage to PS II after the AL exposure. The first observation may be explained by the build-up of so called "apparent NPQ" reflecting both NPQ and decrease in the light absorbance. The second effect calls for a serious consideration and might change the current paradigm presuming the dominant role of chloroplast relocation in the photoprotection of *Tradescantia*. One can further speculate that an efficient PSA reparation contributes significantly to protection against photodamage, along with chloroplast relocation in the studied model.

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Poster

Inter-relationship of plant water status with photosynthetic performance and antioxidative defense system in two contrasting field grown mulberry (*Morus.* spp) genotypes during progressive drought stress

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The present study investigate the inter-relationship of plant water status with foliar and root responses in field grown mulberry (*Morus* spp.), genotypes under progressive drought stress (PDS). Drought is a predominant environmental stress, which adversely affects the physiological traits as well as metabolic pathways, influences the distribution of plant species. PDS caused significant reduction in midday leaf water potential (Ψ_{md}), root moisture content (RMC), photosynthetic rates (P_n), stomatal conductance (g_s) and Photosystem II (PS II) efficiency (F_v/F_m and $\Delta F/ F_m'$) at 25DAS with respect to controls. Among the two genotypes, S13 showed significantly higher rates of Ψ_{md}, RMC, P_n, g_s, E, WUE_i, F_v/F_m and $\Delta F/ F_m'$, suggesting a better photosynthetic performance compared to K2 under PDS. S13 exhibited lower accumulation of H_2O_2, malondialdehyde (MDA), electrolyte leakage and higher proline content, antioxidants including ascorbic acid and total phenols at 25DAS, which indicates lesser oxidative damage. Further, S13 showed higher transcript abundance and enzyme activities of key antioxidants at 12DAS and 25DAS in both leaf as well as root. K2 showed down regulation in key antioxidant enzyme activities and transcript levels compared to S13 under PDS. Evidence from our study clearly demonstrate an inter-relationship between plant water status with photosynthetic performance and anti-oxidative defense system in both root and leaf during PDS, which could be effectively targeted towards mulberry improvement programs for drought adaptation in the future changing climate scenario.

Poster

The influence of Cl^- ions on the photosynthetic activity of photosystem II from *C. reinhardtii* in the presence and in the absence of carbonic anhydrase CAH3

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Ions of Cl^- are placed near the active center of the water-oxidizing complex (WOC) of photosystem II (PS II) [1]. It is known that their extraction reduces the photosynthetic activity of PS II [2]. High content of Cl^- in the medium could prevent their removal from sites of localization within WOC. It has been shown, that the pH optimum plateau of the O_2-evolving activity (6.2–6.5) of PS II is expanding up to the neutral pH under increasing of Cl^- concentration [2]. At the same time, a high concentration of Cl^- has a negative effect on protein binding.

We suggest that the influence of Cl^- ions on the photosynthetic activity of PS II may depend on the presence of lumenal carbonic anhydrase (CA) CrCAH3 on the donor side of PS II. The aim of the present work was to study the influence of CrCAH3 on the photosynthetic activity of PS II at different concentrations of Cl^-. Membrane preparations enriched by PS II from the wild type of *Chlamydomonas reinhardtii*, as well as from the mutant *cia3* were used. *Cia3* does not have CrCAH3 in the lumen of thylakoids. During the study, the O_2-evolving activity of PS II and the variable chlorophyll fluorescence were measured in the range 0–100 mM Cl^-.

At pH 6.5 and in the presence of 35 mM Cl^- the O_2-evolving activity of PS II was maximal both in wt and in *cia3*. Under the lowering of a Cl^- concentration, it decreased equally by ~30% in preparations from wt and *cia3*. With the increasing of Cl^- concentration the minor (no more than 10%) inhibition of the O_2-evolving activity of PS II was observed in case of wt and near 30% in case of *cia3*. At pH 7.0 and in the absence of Cl^- the O_2-evolving activity of PS II was decreased equally by ~70% in preparations from wt and *cia3*. At the same time, under the increasing of Cl^- concentration the saturation of the activity of PS II function was observed at ~35 mM Cl^- for wt and at ~5–10 mM Cl^- for *cia3*. However, it was ~90% for wt and only ~60% for *cia3* comparing to the maximal readings at pH 6.5. It could be suggested, that in the absence of the CrCAH3 protein the WOC-complex is destabilized and this results in greater sensitivity of the WOC to the increased content of Cl^- ions in case of *cia3*.

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Poster

Characterisation of pH sensitive Photosystem II Mutants in *Synechocystis* sp. PCC 6803

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Synechocystis sp. PCC 6803 grows photoautotrophically across a broad pH range; however, a number of mutants that each lack at least one Photosystem II (PS II) lumenal extrinsic protein, and carry a second PS II lumenal mutation, were able to grow photoautotrophically in BG-11 medium at pH 10.0, but not pH 7.5. We have characterised several Photosystem II mutants deficient in PS II extrinsic proteins that show impaired photoautotrophic growth and oxygen evolution at pH 7.5. In particular, a ΔPsbO:ΔPsbU strain and a ΔPsbV:ΔCyanoQ strain that exhibit this pH phenotype. There was no pH-specific changes in variable fluorescence yield from PS II centers of the wild type or the pH-dependent ΔPsbO:ΔPsbU and ΔPsbV:ΔCyanoQ strains. However, 77 K fluorescence emission spectra indicated increased coupling of the phycobilisome (PBS) antenna at pH 10.0 relative to at pH 7.5 in both mutants. DNA microarray data showed a cell-wide response to transfer from pH 10.0 to pH 7.5, including decreased mRNA levels of a number of oxidative stress-responsive transcripts. We have sequenced the genome of the ΔPsbO:ΔPsbU strain and a pseudorevertant, capable of pH 7.5 growth, and identified several SNPs unique to the pseudorevertant. Dot transformation revealed that one of these candidates, an SNP located in *pmgA* (*sll1968*), could recover growth of the ΔPsbO:ΔPsbU strain at pH 7.5 It has been shown that *pmgA* has a role in induction of carbon concentrating mechanism (CCM) components *sbtA* and *ndhF3*, as well as regulating photosystem stoichiometry; this gene is essential to enable photomixotrophic growth. Here, we show that high carbon (3% CO_2) conditions enables growth of the ΔPsbO:ΔPsbU strain at pH 7.5, suggesting that growth in the ΔPsbO:ΔPsbU pseudorevertant is due to perturbation of CCM activity associated with an altered *pmgA*.

Poster

Changes in water transport in roots of intact maize plants in response to increasing atmospheric carbon dioxide concentration

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It is known that the elevated concentration of atmospheric CO_2 (carbon dioxide) may cause an increase in the rate of photosynthesis and water use efficiency (WUE) in some plants, an increase in the growth rate of roots and shoots and mitigation of some stress effects such as water deficiency and salinization. Obviously the above effects are related to water absorption and conduction by plant roots and are presumably associated with changes in their water transport system. It was established that the hydraulic conductivity of plant roots decreased under the elevated CO_2 concentration, but more detailed information on the response of plant root water transport system to the elevated CO_2 concentration is poor. In particular, there are no data on the dynamics of water transport in roots and its correlation with gas exchange. Changes in the transmembrane and symplast pathways of water transfer in roots and what is the role of aquaporins are still obscure. In the present work the water transfer in intact maize plant roots in response to the elevation of the atmospheric CO_2 concentration to a maximum value of 1% was investigated, using the original technique based on the low-field spin-echo NMR. It was shown that the water permeability of cells in the root absorption zone decreased by approximately 1.5 times with the increase in CO_2 level to 1% that is partially associated with the decrease in the stomata conductance. The data was obtained on the dynamics of changes in the maize root water conductivity depending on the CO_2 concentration, showing that the magnitude and rate of the decrease in the root water conductivity increases with the increase in CO_2 concentration. The transmembrane pathway of water transfer through aquaporins is shown to give the largest contribution to the total decrease in the root water conductivity in response to the elevated CO_2 concentration.

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Poster

Responses of energy-transfer processes in diatoms to fluctuating light

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Light-harvesting processes of photosynthetic organisms under various environments have been actively investigated. Recently, effects of fluctuating light (FL) on photosynthesis have received much attention. For example, Grouneva et al. found that a diatom *Thalassiosira pseudonana* modifies its thylakoid protein abundance under FL [1]. In the present study, we have examined effects of FL on primary process of photosynthesis of two species of diatom, *Chaetoceros gracilis* and *Phaeodactylum tricornutum*. After these two diatom cells were precultured at 30 μmol photons m^{-2} s^{-1} (continuous light: CL) for 2 days, the cells were subcultured and grown under the CL condition for 1 day, and then grown under the FL condition for 21 hours. We prepared three FL conditions; FL1, FL2, and FL3 are 30/0 μmol photons m^{-2} s^{-1} for 300/30 s, 30/300 μmol photons m^{-2} s^{-1} for 300/30 s, and 30/300 μmol photons m^{-2} s^{-1} for 3/0.3 s, respectively. We measured optical densities at 750 nm, steady-state absorption spectra, steady-state fluorescence spectra, and time-resolved fluorescence spectra. As a remarkable difference between the two species, it was found that FL made little change to energy transfer from PS II to PS I in *C. gracilis*, but reduced this energy transfer in *P. tricornutum*.

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Poster

Carbonic anhydrase CAH3 supports the activity of photosystem II under increased pH

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The lumenal carbonic anhydrase (CA) CAH3 from green alga *Chlamydomonas reinhardtii* is the only one CA identified so far in close association with photosystem II (PS II) multi-subunit protein complex. It was proposed earlier, that CAH3 could facilitate the H^+ removal from the active center of the PS II water-oxidizing complex (WOC) under the light [1], thereby increasing its activity.

In the present work, PS II enriched membranes from a wild type (wt) of *C. reinhardtii* and a CAH3-deficient mutant *cia3* [1] were used.

We demonstrate, that the suppression of the photosynthetic activity of PS II (the O_2-evolving activity, the rate of electron transport to DCPIP) by increased pH from the optimal level (6.2–6.5) is more pronounced in preparations from the mutant as compared to wt. At pH 7.0 the mutant looses ~30% of the PS II activity, but wt – only about 10%. Experiments with CA inhibitors show that the activity of CAH3 supports the function of PS II and prevents its irreversible inactivation under light upon increased pH. It was possible to restore the photosynthetic activity of PS II from *cia3* to the wt level under increased pH if an excess of HCO_3^- was added. These findings testify that the main role of CAH3 in the vicinity of PS II is the acceleration of the HCO_3^- dehydration reaction. Additionally, measurements of the photoinduced electron transfer rate in PS II from water or from an artificial electron donor to DCPIP indicate that CAH3 has a direct influence on the WOC function.

Based on the data obtained in our work we conclude, that *in vivo* CA-activity of CAH3 may support the photosynthetic activity of PS II at neutral pH in the thylakoid lumen that can be observed under the dark to light transition, or shading.

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Poster

Investigation of PHT7, a putative ascorbate transporter in *Chlamydomonas reinhardtii*

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Ascorbate (also called vitamin C) is a multifunctional metabolite in plants. It is an essential scavenger of reactive oxygen species, cofactor for a number of enzymatic reactions and it modulates the synthesis of several signaling molecules such as abscisic acid and gibberellins. Ascorbate is an alternative electron donor to photosystem II when the oxygen evolving complex is inactive. It is a cofactor for violaxanthin de-epoxidase and participates in non-photochemical quenching.

The final step of ascorbate biosynthesis takes place in the mitochondria. From there, ascorbate has to be transported through several membrane systems for fulfilling its multiple roles in the cell. Because of its size and negative charge at physiological pH, ascorbate cannot freely diffuse through membranes. In higher plants, a single ascorbate transporter has been identified, a chloroplastic phosphate transporter with dual function, called PHT4;4 [1].

In the green alga *Chlamydomonas reinhardtii*, no ascorbate transporters have been isolated and no genes have been identified. We have found 3 homologs (PHT3, PHT4, PHT7) of AtPHT4;4 in *C. reinhardtii*, among which the gene expression of PHT3 and PHT7 responded strongly to oxidative stress and ascorbate treatments. In order to study the function of PHT7, the recently developed CRISPR/Cpf1 genome editing technique [2] was used to generate *PHT7* knockout lines. The *PHT7* mutant lines show retarded growth phenotype, altered ascorbate metabolism and decreased photosynthetic performance particularly at high light intensities. To confirm the connection between the mutant phenotype and the absence of the *PHT7*, we performed genetic complementation. Determination of the intracellular localisation of PHT7 is in progress.

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Poster

The role of *sll1558* in environmental stress tolerance in the cyanobacterium *Synechocystis* sp. PCC6803

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The molecular mechanisms underlying the sensitivity of plants to acid stress are unclear. We investigated the mechanisms responsible for acid-stress tolerance. Previously, DNA microarray analysis identified the *sll1558* gene in *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803), as upregulated following short-term acid treatment.

We focused on *sll1558* genes to clarify the molecular mechanism of acid stress tolerance in *Synechocystis* 6803. We constructed mutant cells for this gene and analyzed their phenotype. As a result, the *sll1558* partially disrupted mutant was highly sensitive to acid stress conditions at pH 6.0. In addition, the *sll1558* partially disrupted mutant was highly sensitive to various stress condition, such as high salt, high osmolality, and high/low temperature. Next, we performed QRT-PCR to exam transcriptional level of *sll1558* under these condition. The transcript of *sll1558* increased under these stresses condition. These results suggest that it may function in the synthesis of materials that affect the response to these stresses. However, it is not clear that the mechanism of stress tolerance by Sll1558.

The *sll1558* gene encodes UDP-glucose pyrophosphorylase, which catalyzes the conversion of glucose-1-phosphate to UDP-D-glucose. UDP-glucose is used for the synthesis of various materials such as lipopolysaccharide (LPS), Glycogen, and Glycolipid. First, we analyzed LPS, which contain glucose, in these cells. The band pattern of wild-type LPS differed from that of *sll1558*-mutant LPS. Second, we analyzed glycogen contents in these strains under acid stress condition. The amount of glycogen increased under acid stress condition in *sll1558* mutant.

Now, we analyze Glycolipid contents in *sll1558* mutant under acid stress.

Poster

Adaptation of light-harvesting functions of the glaucophyte *Cyanophora paradoxa* under different light conditions

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Oxygenic photosynthetic organisms regulate their light-harvesting functions of photosystems (PS I and PS II) in response to light quality and quantity. Glaucophytes, which are one of the primary symbiotic algae, contain light-harvesting antenna called phycobilisome (PBS) like cyanobacteria and red algae. Compared with cyanobacteria and red algae, how glaucophytes modify their light-harvesting functions during light adaptation is poorly understood. In this study, we examined light-harvesting and energy-transfer processes of the glaucophyte *Cyanophora paradoxa* grown under white and monochromatic (blue, green, yellow, and red) LEDs. Steady-state and time-resolved fluorescence spectra showed that the *C. paradoxa* cells grown under different light qualities modified energy transfer from PBS to both photosystems. Additionally, increased green-, yellow-, and red-LED intensity induced the suppression of energy transfer within PBS and the modification of energy transfer pathway from PBS to PS II. Delayed fluorescence spectra indicated that the contribution of energy transfer from PS II to PS I (spillover) did not clearly change depending on light quality and intensity. These responses differed from those of the cyanobacterium *Arthrospira platensis* and the red alga *Cyanidioschyzon merolae* in our previous studies [1, 2], suggesting that the glaucophyte *C. paradoxa* has developed the unique light-harvesting strategies.

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Poster

The positive effect of plant colonization by bacteria of the *Pseudomonas* genus on resistance to stress factors

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It is known that some soil microorganisms are able to stimulate growth and development of plants, to induce immune response of plants under stressful influence of environmental factors. In this regard, the use of non-pathogenic soil rhizosphere microorganisms to increase plant resistance to biotic and abiotic environmental factors, as well as to increase crop yields, is one of the most relevant and promising areas in the search for biological protection of plant organisms.

In the course of the work, the effect of the colonization of barley plants (*Hordeum vulgare*) by associative microorganisms on the adaptation to different stress factors was investigated. For the colonization of barley seeds, the *Pseudomonas putida* sp. BS3701, which are part of the consortium effectively degrading oil products was used. To study the differences in the adaptation mechanisms in control plants and plants colonized by *P. putida* BS3701 grown at moderate light intensity (100 μmol quanta/m^2s), the plants were transferred to high light intensity conditions (1000 μmol quanta/m^2s) and constant illumination or to high light and high concentration of NaCl.

A number of differences in the functioning and structure of the photosynthetic apparatus of control plants and plants colonized by *P. putida* BS3701 grown at 100 μmol quanta/m^2s were found. It was found that the leaves of plants colonized by *P. putida* BS3701 contain more of the light harvesting antenna PS II proteins: Lhcb1, Lhcb2, Lhcb6, which is a consequence of a higher level of expression of these genes. Thus, it was shown that the plants colonized by *P. putida* BS3701 have a significantly larger size of the light harvesting antenna PS II as compared to the control plants.

Comprehensive assessment of the effects of plant colonization on resistance to various stress factors was carried out. It was found that the colonization of *P. putida* BS3701 barley plants has a positive effect on the resistance of the photosynthetic apparatus to conditions of high light intensity, as well as to two abiotic stress factors acting simultaneously. Thus, in the work the strain *P. putida* BS3701 was first described as a PGPR microorganism that increases the adaptation potential of barley plants. It was shown that this strain more effectively affects the resistance of barley plants to various stress factors than some other PGPR-characterized bacteria.

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Poster

The measurement of photochemical reflectance index (PRI) as an indicator of the local and systemic plants photosynthetic response

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Plants can be affected by numerous environmental stressors with spatially heterogeneous impact. A fast systemic photosynthetic response, associated with long-distance signaling, e.g. variation potential (VP), plays an important role in the adaptation of higher plants to stress. The measurement of photochemical reflectance index (PRI) as an indicator of photosynthetic response is a promising method for upcoming photosynthesis monitoring under spatial stressors. In the present research we have used this method in order to evaluate the systemic and local photosynthetic response in 14–21 days old pea plants (*Pisum sativum* L.). VP was induced by local leaf heating and measured by registration of the surface potential. Photosynthetic parameters were measured using Dual-PAM-100 fluorescence measuring system, equipped with a measuring head Cuvette 3010-Dual (Walz, Germany). Reflected light was measured by S100 compact wide-range spectrometer (SOLAR laser systems, Belarus). In the first part of the research, we have studied the changes in PRI which develop in plants under stress conditions affecting the whole plant (high temperature). We have shown that the short-term heating of pea plants caused an increase in the absolute value of ΔPRI which correlates well with the increase in NPQ value in the plant leaf. Heating also caused a shift in PRI to the negative direction; however, this effect was not always observed and was less associated with changes in NPQ. Similar data were obtained for pumpkin and wheat. In the second part of the research we have studied changes in the PRI caused by local damage and which develop during the propagation of electrical stress signals. Electrical signals caused reversible changes in the PRI to a negative direction; and the amplitude of such shift well correlated with the amplitude of NPQ increase. The results show that changes in PRI can be used to monitor the photosynthetic response caused by local and systemic stressors.

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Poster

The adaptation of *Arabidopsis* plants with knockout of α-carbonic anhydrase 2 gene to different light intensity

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Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the reversible hydration of carbon dioxide. In thylakoids of *Arabidopsis thaliana* plants, the α-carbonic anhydrase 4 was found, which, according to our data, participates in the regulation of energy-dependent non-photochemical quenching (NPQ) and is located near photosystem II (PS II). We obtained data indicating that another CA is located in the thylakoids, α-CA2, which is important for photosynthesis.

Experiments were performed on a wild-type *A. thaliana* (WT) plants, and mutant plants with knocked out *At2g28210* gene, that encodes α-CA2. The plants were grown under 8-h photoperiod at a light intensity of 50 μmol quanta/m^2s. Then some of the plants were transferred to high light (400 μmol quanta/m^2s) for 10 days. The photosynthetic parameters of the plants were measured after 1, 5, and 10 days of adaptation to high light.

At low light intensity the chlorophyll *a*/*b* (Chl *a*/*b*) ratio in the mutant was not different from WT plants. It is known that when plants are adapted to high light, the size of the PS II antenna decreases. Over the course of 10 days of illumination with high light the Chl *a*/*b* of all plants increased, as expected, due to decrease of the Chl *b* content. However, this ratio in WT plants increased by 17%, which indicated a significant decrease in the antenna size, whereas in the mutant it increased only by 6%, i.e. antenna size decreased slightly. This difference shows that in the mutant plants the regulation of antenna size in response to an increase of light intensity is disturbed. At low light intensity the starch content in the mutant was 20–40% lower than that in the WT plants. Under high light, during the first five days of adaptation, starch content in the mutant remained lower than in WT plants, but by the tenth day, it significantly increased in the mutant and became 40% higher than in the WT plants. At low light intensity the H_2O_2 content in the mutant was 15% lower than in WT plants. Within 10 days of adaptation to high light, the H_2O_2 content in the mutant remained lower than in WT plants.

We have established earlier that energy-dependent NPQ in the mutant was higher than in WT plants, so we assumed that the absence of α-CA2 causes greater accumulation of protons in the lumen. Under high light, higher number of protons in lumen could lead to formation of extra ATP, which can be used for greater starch synthesis in the mutant.

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Poster

Influence of biotic stress caused by grapevine leafroll-assosiated virus 3 on photosynthesis in *Vitis vinifera* L.

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Grapevine (*Vitis vinifera* L.) is one of the major crops widely cultivated for the wine industry, as well as for the production of fresh and dried fruit in Azerbaijan. Vineyard surveys were conducted to determine the virus infection in the various districts of Azerbaijan during the year 2017–2018. A total of 92 samples were collected from grapevine fields and screened using rapid one-step assay AgriStrip and double-antibody sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) to detect Grape leaf curl disease (GLD). Field average cumulative percentage of these viruses affecting plants was 24%. The results revealed that tested samples were infected with grapevine leafroll-associated virus type 3 (GLRAV-3), however, no sample was found infected with other GLD virus. To amplify the CP gene of GLRaV-3 from the collected samples, 18 grapevine samples from same cultivars that showed a positive reaction with DAS-ELISA were subjected to Reverse transcription (RT)-PCR using specific primer pairs, the expected amplicon of CP (~942 bp) of GLRaV-3 genome was amplified. These results indicate that GLRaV-3 was the only most prevalent endemic viral pathogen of grapevine in the dominantly warm humid continental climate of our country. Moreover, some effects of biotic stress caused by GLRaV-3 on photosynthesis in grapevines were studied. The changes in the structure of stomata, contents of malondialdehyde, pigments, relative water content (RWC), alterations in the activities of peroxidase enzymes, activity of photosystem II (PS II) and PS II efficiency in grapevine leaves were analyzed under the influence of GLRaV-3 infection. In our experiments, RWC decreased after GLRaV-3 infection. Scanning electron microscopy examination was showed virus infection caused that stomata of all infected plants were predominantly closed. Gradual reduction in green pigments like chlorophylls (a, b and total) and in anthocyanin, carotenoids was observed in all infected species. The activities of ascorbate peroxidase (APO), benzidine peroxidase (BPO) and guaiacol peroxidase (GPO) were observed to increase in virus-infected leaves comparison with the healthy control. PS II efficiency and activity of photosystem II were significantly lower in the virus-infected leaves. The photosynthetic rate (PN) was significantly inhibited by GLRaV-3 infection compared control. Reduction of PN was caused not only by stomata closure, but also by the decrease of PS II efficiency.

Poster

Assessment of photosynthetic and leaf traits for high throughput screening for drought tolerance in wheat genotypes

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Recent development of phenotyping techniques is aimed to enable efficient exploitation of the genetic diversity of crops. In our previous program, we have created a collection of diverse winter wheat genotypes selected from hundreds of accessions of Slovak Genebank, which greatly covers a diversity of leaf traits. To examine the links between photosynthetic traits and drought tolerance, we tested a core wheat collection of 35 genotypes within a series of phenotyping experiments in field conditions as well as in controlled environment represented by the automated phenotyping facility Plantscreen at SUA Nitra, Slovakia. In drought-exposed and control plants, the imaging of plants using RGB, as well as VNIR and SWIR hyperspectral cameras were done regularly. Moreover, the manually operated measurements of chlorophyll content, PS II photochemistry (chlorophyll fluorescence), leaf temperature (IR thermal imaging) and VNIR hyperspectral records were performed to monitor the effects of the stress on photosynthetic apparatus. The obtained data were correlated with the results of yield and biomass analyses, which enabled to identify the most promising methods and procedures useful for screening of wheat both in field and controlled conditions.

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Poster

Chlorophyll fluorescence parameters, phenolic compounds and benzidine peroxidase activity in wheat genotypes subjected to drought stress followed by recovery

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The ability of plants to maintain physiological functions at low plant water status and recover quickly once the stress is removed will be important for ensuring sustainable crop production under intermittent drought events. The effect of drought and recovery on RWC, H_2O_2, photosynthetic pigments, chlorophyll fluorescence parameters, phenolic compounds, benzidine peroxidase activity and isozyme composition of 14-day-old seedlings of two contrasting genotypes of *T. aestivum* L. (Gobustan, a drought tolerant, and Tale 38, a drought susceptible) was studied. RWC was found to be higher in the Gobustan genotype compared with Tale 38 under drought. The H_2O_2 content increased compared with the control and decreased at re-watering. After re-watering, the return of H_2O_2 concentrations in drought stressed plants to the level of well-watered plants indicated that wheat plants have the ability to tolerate and recover from water stress at the cellular level. Total amounts of chl *a*, chl *b* and carotenoids decreased compared with the control and recovered during re-watering. The degree of the decrease in photosynthetic pigment contents was higher in Tale 38 as compared with Gobustan under drought. The potential quantum yield of photochemical reactions of PS II (Fv/Fm) significantly decreased in both water-stressed leaves compared with control samples and increased under re-watering. Drought increased the total amount of phenolic compounds in both genotypes. After re-watering amounts of the phenolic compounds decreased, reaching the control level in the Gobustan genotype, whereas a slight decrease occurred in Tale 38 after re-watering. The metabolism of phenolic compounds includes the action of oxidative enzymes such as peroxidases, which catalyse the oxidation of phenols to quinines. Four (BPO1, BPO2, BPO3, BPO4) and three (BPO1, BPO3, BPO4) isozymes of benzidine peroxidase were found in the control variants of the Gobustan genotype and the Tale 38 genotype, respectively. Drought stress inhibited expression of 2 isozyme bands (BPO2 and BPO3) in Gobustan and 1 (BPO3) in Tale 38, respectively. The BPO1 band was slightly reduced under drought and was enhanced again during the recovery phase in both genotypes. BPO2 and BPO3 were reduced under drought and recovered after re-watering, whereas BPO4 was persistent. The data obtained suggest that both genotypes retained the ability to recover after rehydration under soil drought.

Poster

Ultrastructure of the cell energy systems with an example of apple fruit *Malus* Mill. (Rosaceae)

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The use of electron, fluorescence and confocal microscopy methods substantially expands the characterization of structural and functional parameters of plant cells, the compartmentalization of the energy-transdusing membrane systems in particular. Comparison of the functional and structural characteristics of apple tree fruits during their development is an informative approach to investigate plant adaptation under natural environments and stress conditions. According to our data, the cells of subepidermal tissue in apple fruits contain the population of bioenergetic structures (chloroplasts) with the distuinguished features, such as the granular structure, well-developed systems of membranes, the peripheral parietal position, as well as the contacts between themselves and with other organelles. In comparison with lowlands, the chloroplasts of mountain fruits possess an altered membrane system: their grana are composed of a smaller number of tylakoids and have invaginations as a consequence of the increased size. The data obtained by fluorescence methods indicate that chloroplasts of the subepidermal zone of fruits grown in the mountains and on the plain are characterized by equally high potential photochemical activity of photosystem II. In our experiments, we used green fruits of apple *Malus* Mill. with the diameters ≥ 20–40 mm. Measurements were made using a fluorimeter PEA (Plant Efficiency Analyzer, Hansatech, England) on the fruit outer layers (peels) 2–4 mm thick, with an area of approximately 1.5 cm^2. Under actinic irradiation (flux of quanta 50 μmol/(m^2 s) the efficiency of photosynthesis in the mountains was significantly lower than darkness. In plain conditions, the change in the illumination intensity does not lead to significant changes in the rate of noncyclic electron flow. The role of intracellular membrane systems is not yet fully characterized, there are only assumptions that the interorganellar connections are a part in the regulated system of metabolic and information exchange between individual compartments. Evaluation of plant resistance to environmental factors using the methods of chlorophyll fluorescence induction and electron microscopy can be used to study the adaptive role of plastid signals during stress. In our view, the altered parameters of chloroplasts are caused by stressors of mountain environment, primarily, the high intensity of solar radiation and by the necessity of mobilizing bioenergy resources for successful completion of the program of ontogenesis.

Poster

Role of hydrogen peroxide in photosynthetic machinery response under biotic stress

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The mechanisms of pathogenesis of tomato plants after infection with *Fusarium oxysporum* were investigated. The death of plants with fusarium wilt occurred as a result of oxidative stress induced by water deficiency and *Fusarium oxysporum* toxins. Both destructive and signal functions of hydrogen peroxide were identified for this type of stress. Elevated level of hydrogen peroxide caused an accumulation of protectors and PR proteins. Root pretreatment of tomato plants H_2O_2 in low concentrations increased the level of endogenous hydrogen peroxide and increased the resistance of plants to *Fusarium oxysporum*, preventing the development of water deficiency, reducing the rate of destructive processes and activating the protector systems and the synthesis of protective proteins.

The key role of hydrogen peroxide in regulating the photosynthetic and respiratory activity of chloroplasts and mitochondria of tomato leaves during fusarium wilt was established. The suppression of oxygen evolving activity of thylakoids with simultaneous decrease in photochemical activity of photosystem II, decline in photochemical quenching of chlorophyll fluorescence and rise in non-photochemical quenching under fusarium wilt were registered. The interregulation of the level of hydrogen peroxide and the redox state of the plastoquinone pool during the development of pathogenesis and pretreatment of plants with hydrogen peroxide was show.

On the basis of the data obtained, it can be assumed that after treatment of plants with H_2O_2, the level of endogenous hydrogen peroxide increased, a cascade of transduction reactions is triggered, protective mechanisms are activated, including the redistribution of electron carriers in the electron transport chain of chloroplasts. An attack of a pathogen during this period again causes an increase in the level of endogenous hydrogen peroxide, stress signal is transmitted, and the already activated protective systems effectively prevent the development of biotic stress. Thus, hydrogen peroxide treatment induces the change of homeostasis, activation of plant protective systems and development of the systemic acquired resistance.

Poster

Effect of natural drought on chlorophyll a fluorescence of contrasting silver fir (*Abies alba* Mill.) provenances

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The knowledge about intraspecific variation of adaptive traits, resulting from genetic differentiation due to adaptation to specific environmental conditions, is crucial for selection of suitable reproductive material. Therefore, we studied performance of PS II photochemistry in 16-years-old silver fir trees using the complex parameters of chlorophyll *a* fluorescence derived from the JIP-test equations. We tested five Central European provenances differing in origin altitude (250–1300 m a.s.l.), but growing in the same experimental plot, in two dates of 2016; in the start (24th May) and in the peak of growing season (12th July). Within this period, natural drought was recorded allowing us to monitor changes in photochemistry efficiency under drought condition. Since PS II is relatively resistant to water deficit, we focused on parameters for which the negative effect of drought has been already described.

Differences among provenances in tested parameters were recorded for the both dates, whereas natural drought caused their deepening. These differences were confirmed also by altitudinal trends, while the natural drought was better tolerated by individuals originating from higher altitudes. The regression analysis indicates that PI and RC/ABS are increasing along the altitudinal gradient of provenances' origins in both cases. Since the altitude is closely correlated with rising rainfall and decreasing average temperatures in Central Europe, we noted the relationships also between PI and RC/ABS and these climatic variables.

For other parameters, we recorded formation of climatic trends after exposure to stressful conditions. Also in this case, the provenances from higher altitudes (wetter and cooler locations) showed higher photochemical performance compared to the provenances from warmer and drier areas.

Our observations confirm the assumption of higher performance and resistance of PS II to natural drought conditions in provenances from high altitudes. The results of presented study can contribute to the knowledge of adaptation and acclimation abilities of forest trees species in the extreme natural conditions with possible use for the forestry purpose.

Section 1.10: Systems Biology of Photosynthesis:

Integration of Genomic, Proteomic, Metabolomic and Bioinformatic Studies

Lecture

Self sinking capsules, a final solution to radioactive disposal

Cesare Marchetti

International Institute for Applied Systems Analysis, Austria-Italy

Poster

Using systems biology techniques for identifying resistance markers in *Prunus rossica* Erem. to abiotic stress

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Under stressful conditions in plants, the production of reactive oxygen species (ROS), which are one of the key damaging factors for living organisms, increases. The activity of the antioxidant system increases plant resistance to stress [1, 2]. Antioxidant protection of plants can increase their resistance to abiotic stresses, such as various graft-rootstock combinations. Metabolic processes of *Prunus rossica* Erem. depending on the stock used, are not well studied. Selection of the most important metabolic markers will speed up the search for the optimal strategy in the selection of combinations to improve growth, development and fruiting of *Prunus rossica* Erem plants. The purpose of our research was to identify changes in the antioxidant activity and the content of low molecular weight metabolites in the leaves of the cherry plum, depending on the stock. It was established that the antioxidant activity of the leaves of the studied varieties varied from 72 to 91% depending on the stock. A close correlation was established between the indicators of the antioxidant activity of the rootstocks and the plants grafted on them, r = 0.87. As a the result of the metabolic analysis of alcohol extracts of leaves, 56 substances belonging to various classes of organic compounds were identified. Presumably, organic acids and substances of phenolic nature make a greater contribution to the functioning of the antioxidant system of the plants studied. Due to differences in the biochemistry of plant photosynthesis of cherry plums on different stocks, the data obtained allows us to identify the most adaptive graft-rootstock combinations. It is shown that the integration of analytical methods represents a promising approach to predicting the compatibility of graft-rootstock combinations of economically valuable plants.

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Section 1.11: Plant Mineral Nutrients and Photosynthetic Capacity

Lecture

Photosynthetic activity of barley plants under zinc deficient and excess stress conditions

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In controlled conditions, the effect of deficient and excess of zinc in growth medium on the photosynthetic activity of spring barley (v. Nur) was studied. Plants were grown for 14 days on a Hoagland-Arnon nutrient solution with an optimal zinc concentration – 2 μM (control). In the variant with metal deficiency, zinc was absent, and its excess was created by adding zinc in nutrient solution at a concentration of 1000 μM.

The results showed that both a deficiency and an excess of zinc slows down the photosynthesis rate, and approximately equally – by 28 and 22% (relative to the control), respectively. In both variants, the total content of chlorophylls decreased (with respect to the control): under a zinc deficiency by 5%, and under its excess by more than 30%. However, changes in the ratio of chlorophylls (*a*/*b*), as well as in the distribution of pigments between light-harvesting complexes and photosystems, were not recorded, which indicates the ability of seedlings to adapt to these conditions. However, in the variant with a zinc excess, markedly decreased the Fv/Fm, which characterizes the potential quantum efficiency of photosystem II (PS II). This indicates on possible disturbance in PS II. Under conditions of metal deficiency in growth medium, this did not occur. The evaluation of stomatal conductance, which is an important factor in gas exchange regulating, showed that this parameter is very sensitive to the zinc content in the environment. So, under metal deficient, it decreased by 30% in relation to the control and under zinc excess – more than 70%.

Thus, in barley plants under zinc deficit and excess stress conditions, the rate of photosynthesis slows down, but the reasons for this are different in the first and second cases. Under a zinc excess, the inhibition of the process is due to a decrease of the pigments content, a disturbances in the light reactions of photosynthesis and a decline of stomatal conductance. Under a deficiency of metal, only a stomatal conductivity decreases markedly, so the slowing down of the photosynthesis rate is apparently associated with a decrease in the activity of enzymes involved in the dark reactions of this process.

The study was carried out under state order (project № 0218-2019-0074).

Lecture

Potassium and glucose as putative signals regulating processes in shoots and roots

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Photosynthesis results in the productions of sugars which can act as mobile signals coordinating shoot and root functions [1]. In the hypocotyl of *Arabidopsis* seedlings, shoot-derived glucose was shown to influence stress responsiveness and growth via the G-protein regulator RGS [1], while in roots, it conferred light responses at the level of activation of the TOR kinase pathway [2].

Potassium is the major mineral nutrient in plant cells responsible for maintenance of membrane potential and osmotic regulation. The level of potassium is emerging as the major switch between ana- and catabolism [3], and the loss of K^+ from root cells was proposed to activate autophagy and ultimately cell death during stress [4, 5]. Using *Arabidopsis* seedlings, we investigated how the levels of glucose affect root responses to either the lack of potassium in the growth medium, or to salt stress, by analyses of autophagic flux in cells of three root zones: meristematic, elongation and root hair zone. Also, we addressed the question whether low levels of K^+ in root cells can influence the transcription of the photosynthetic LHC genes in leaves of *Arabidopsis* seedlings, and thus lead to the production of a root-to-shoot signal to attenuate photosynthesis.

The results show that the levels of both constitutive and salt-stress induced autophagy were strongly affected by the lack of potassium in the growth medium. Constitutive autophagy was increased in K^+-deficient plants probably via TOR-dependent regulation and was inhibited by glucose. Glucose inhibited also stress-induced autophagy in K^+-deficient plants, suggesting TOR kinase control in addition to AMPK. Altogether, the results suggest that potassium and glucose can function as putative signals regulating processes in roots and shoots.

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Poster

Effect of zinc deficiency on the photosynthetic apparatus of barley plants at different phases of development

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The effect of zinc deficiency on the photosynthetic apparatus of spring barley plants (v. Nur) being at different phases of development – vegetative (3 leaves), early reproductive (the beginning of the stem elongation) and seed maturation – was investigated in pot experiment. For this purpose the plants were grown in vessels with sand (5 kg). Watering carried out with Hoagland-Arnon solution with 2 μM zinc sulfate (control) or without it (experiment). The effect of zinc deficiency on the photosynthetic apparatus was estimated by the change (compared to the control) of the total chlorophylls and carotenoids content, the maximum quantum yield of PS II (Fv/Fm), the rate of photosynthesis and stomatal conduction in youngest, fully expanded leaf (2-nd – for vegetative phase and 4-th – for early reproductive phase). It was revealed that at the vegetative phase of development deficiency of zinc weak effect on plants. In this case, there was only a slight decrease in chlorophylls ratio (*a*/*b*) and value of Fv/Fm. In contrast, during early reproductive phase zinc deficiency cause noticeably (by 25%) decrease of photosynthesis rate, content of chlorophylls (by 17%) and carotenoids (by 14%), and almost 2-fold drop of the stomatal conduction. At the phase of seed maturation, the intensity of photosynthesis in the 4th leaf remained at a reduced (compared to the control) level, whereas in the 5-th (subflag) and 6-th (flag) leaves – the main donors of assimilates for the developing ear, did not differ from control. Obtained data show that zinc deficiency has a strong negative effect on the activity of photosynthetic apparatus in barley plants at the early reproductive phase, when in cereals forms the main elements of the inflorescence and their demand for this micronutrient increases. This may be one of the reasons for the decline in seed yield of barley plants, experiencing zinc deficiency.

The study was carried out under state order (project № 0218-2019-0074). The research was carried out using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences.

Poster

Modeling of phosphate pools dynamics during the growth of *Chlorella vulgaris* with different availability of phosphorus in the medium

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Microalgae cells are able to accumulate phosphorus in the form of polyphosphates, which are involved in cell energy metabolism. Understanding the mechanisms of regulation and the ability to control the accumulation of polyphosphate pools can be used in solving various biotechnological problems.

A hybrid model of phosphate pools dynamics during the growth of *Chlorella vulgaris* cells under different media conditions is proposed. The model links different physiological levels of cellular processes and consists of two blocks. The first block is a system of 5 differential equations describing the phosphorus uptake from the environment, the inclusion of inorganic phosphate into biomolecules, the formation and growth of a polyphosphates pool, biomass increase during culture growth. The change in intracellular concentrations of phosphorus compounds is determined by the minimum cell quota for the phosphorus content in the inorganic form and the maximum in the form of biomacromolecules. For parameterization of the model, experimental data on the growth of the culture of *Chlorella vulgaris* were used. The second block of the model is based on stoichiometric equations of the central metabolic pathways, including glycolysis, the citric acid cycle, respiratory and photosynthetic electron transport chains. The reaction scheme takes into account ATP synthesis and consumption, the binding and release of inorganic phosphorus. A redistribution of the metabolic fluxes for the different availability of inorganic phosphorus in medium was calculated. The model allowed us to detect the growth stage of the culture, at which the maximum accumulation of polyphosphates occurs.

Poster

TOR kinase and GORK channels in sensing of cellular potassium levels during induction of autophagy in *Arabidopsis* and barley

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Autophagy is an adaptive mechanism induced during the cellular response to stress. A basal level of autophagy, called 'constitutive autophagy', is maintained in cells over the whole life span, and is needed for recycling of cellular components which become damaged or no longer necessary. Activation of autophagy includes several mechanisms, one of them being potassium leakage from the cytosol. However, this mechanism is not yet fully understood. The protein kinase TOR integrates energy-, nutrient- and stress signals in eukaryotic cells. In plants, a number of TOR-activating signals have been characterized which include glucose, nitrogen, sulfur and, for roots, auxin. Although K^+ efflux precedes autophagy, the direct relation between K^+ ions and TOR-kinase has not been studied thus far.

The loss of potassium by rhizodermis occurs via outward-rectifying potassium channels GORK. These channels were reported to constitute a part of mechanism of plants' salt resistance. A possible mechanism can involve an induction of autophagy as a cytoprotective process, from one side, as well as a change of potassium signaling, from the other side.

In the present study, we showed that the deficit of potassium in plant growth media leads to an inhibition of constitutive autophagy in root cells of *Arabidopsis thaliana*. Using a specific inhibitor of GORK channels, tetraethyl-ammonium, we confirmed the participation of these channels in the induction of autophagy in response to salt stress. The expression levels of genes encoding GORK, as well as of autophagy marker genes ATG5 and ATG7, did not change in A. thaliana grown under potassium starvation. However, an increase in the expression levels of HvGORK was observed in a salt-resistant variety Donetsky as compared to a less resistant variety Donaria, both under normal conditions and during salinity. Contrariwise, the levels of expression of HvATG6 and HvATG8 in salt-resistant Donetsky as compared to Donaria, were decreased both under normal conditions and during salinity. Thus, these genes are likely involved in pre-adaptation of plants to salinity stress. In plants grown under potassium starvation, we established a method of determination of the levels of activity of TOR kinase and analyzed how potassium starvation acts on TOR activity.

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Section 1.12: Photosynthesis Education and Emerging Techniques for Studying Photosynthesis including Neutron Scattering

Poster

Development of microfluidic chambers for investigation of green algae

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Green algae are of outstanding ecological importance and appropriate model organisms for studying different cellular processes and have an increasing importance in biotechnology. In order to better understand their life cycle processes, we aim at establishing single-cell analysis for the green alga species *Chlamydomonas reinhardtii*.

Our approach is based on microfluidics, which allows the trapping of a small number or unique algal cells for several days, during which morphological examinations can be performed by microscopy. We combined this with chlorophyll *a* fluorescence measurements that provide valuable information on photosynthetic activity. We have developed three different types of microfluidic chambers:

I. The first type is a triple chamber suitable for observing cell populations for several days, with continuous nutrient supply. We have successfully grown various nitrate-inducible amiRNA lines targeting subunits of the oxygen-evolving complex in this type of chamber and measured their photosynthetic activities. Changing the nitrogen source in the growth media from ammonium to nitrate resulted in severe loss of photosynthetic activity and cell death for the amiRNA transformants.

II. The second chamber type is comprised of "tulip" shaped traps, suitable for trapping individual cells, which allows for the observation of a single cell without being bound to a solid support surface, so that its photosynthetic activity can be continuously measured for several hours.

III. The third type of chamber consists of traps that are capable of capturing single cells, allowing the growth of progeny cells. By the aid of chlorophyll *a* fluorescence measurements valuable information will be obtained on photosynthetic changes during the cell cycle.

Part 2.
Hydrogen Energy for Sustainability

Section 2.1: Energy for the Future – Hydrogen economy

Lecture

Future prospect and challenges in production of hydrogen

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Hydrogen is an energy source and is available in chemically combined forms in water, fossil fuels, biomass etc. Climate depletion and fossil fuel scarcity are the reasons leading to hydrogen technology. Hydrogen has been widely used in chemical industries as well therefore its use in non-energy applications is likely to expand further in coming years substantially. The advantage of hydrogen is that it can be produced biologically and thermochemically in many various ways from water or organic compounds. Along with a complete classification, an overall comparison is carried out, and the results comprising both the conventional and renewable methods are presented. Hydrogen produced through the photosynthetic process is the ultimate renewable source since it directly uses inexhaustible resources: energy from sunlight and water. However, oxygen reactivity is a drawback in this process. Pure hydrogen may be obtained by electrolysis for fuel cells applications. The thermochemical pyrolysis and gasification are economically viable approaches providing the highest potential to become competitive on a large scale. Fermentative BioH_2 offers a high production rate, but poor conversion efficiency. Higher conversion efficiency can be achieved by microbial electrolysis cell, but it is not entirely developed. The status and challenges of other processes also have been presented.

Lecture

Prospects and challenges of mainstreaming hydrogen-fueled automobiles

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A variety of factors encompassing of economical, environmental and engineering and design innovation have made hydrogen energy as one of the forerunner for our primary energy resource. The hydrogen fuel cells in the past decade has made its way into multiple production cars as hydrogen internal combustion engine. Since 2007 there have been 7 major production cars which can be classified as Hydrogen internal combustion engine vehicle with the latest being Hyundai Nexo. The advantages of HICEV are well documented including no carbon emission and almost zero nitrogen oxide emission. However, hydrogen`s flammability and the significant increase (twice) in engine size for the same power output have deterred manufactures to aggressively invest and innovate in HICEV despite favorable trade policies in most of the European nations, to encourage it. This paper makes a case for hydrogen as the sole fuel powering our cars and discusses the long term challenges of making HICEV mainstream. The scale at which automobiles are used today, HICEV could be one of the first steps towards long term sustainable energy.=

Lecture

Hydrogen energy technologies: recent developments and prospects in Russia

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Share of renewable energy sources (RES) in electricity generation increases rapidly. Variable nature of RES is one of their major drawbacks, and integration of the renewables into grid raises several problems, such as the need in costly back-up capacities during low RES generation and curtailment during excess generation periods. Hydrogen is an environmentally friendly secondary energy carrier, which fits for long term and large-scale energy storage.

The role of hydrogen at national and international strategic levels relies entirely on renewable energy and energy efficiency. Hydrogen would play a crucial role in medium and long distance road and rail vehicles; in coastal and international shipping; in air transport; and for longer-term seasonal storage on electricity grids relying mainly on local renewable energy sources and feedstocks [1].

The key challenge today is to identify concrete short-term investment opportunities for RES hydrogen production, initial business cases will likely be based on producing green hydrogen and supplying it to industry and mobility ("Power-to-Hydrogen" and "Hydrogen-to-X") [2].

RES share in Russia is very low due to cheap and abundant fossil fuels; renewable generation was only 0.04% of the total electricity production in 2017. On the other hand about 2/3 of the Russia's territory is not covered by the unified grid system, and power supply of isolated regions is heavily subsidized from the federal and regional budgets and causes significant environmental damage. Renewables in isolated grids can play greater role.

Today the only region in Russia with a high share of RES is an isolated energy system of Kamchatka, where geothermal power plants produce up to 30% of electricity. Surprisingly the geothermal power in Kamchatka meets the same challenges as variable RES, and suffers from curtailment by the grid operator in favor to fossil fuels. Hydrogen production by water electrolysis is one of the ways to increase capacity factor of the Mutnovsky GeoPPs.

Other early markets for hydrogen energy technologies in Russia include biohydrogen production from organic wastes, hydrogen upgrade and purification, energy storage for small-scale applications and fuel cell power supply.

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Poster

New intelligent control strategy by robust neural network algorithm for real time detection of an optimized maximum power traking control for photovoltaic system

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To increase the power output of a PV module or a field of PV modules, an electronic controller is incorporated between the PV generator and the load, whose role and main objective is the continuous monitoring of the maximum power point of the PV generator commonly known as MPPT (Maximum Power Point Tracking) and this in general per action on a DC-DC conversion device.

The regulation and control techniques provide the impedance matching function, transferring to the load the maximum electrical power output from the PV generator whatever the temperature and sunshine conditions.

Indeed, the paper presents a new controls strategy for the photovoltaic PV, it is a command based on Neuronal Network technique. It is the first time that this technique has been introduced, and proposed by the authors in synthesizing control laws for the converters of electronic power.

The new technical algorithm based on Neural Networks, is designed to be more robust and performant with respect to tracking speed and precision.

This study, which is followed by a simulation, has enabled us to consolidate the idea that the new Neural Network controller when compared to their classical counterparts obtains the best performances.

Section 2.3: Biological hydrogen production

Lecture

Perspectives of cyanobacterial strains for biodisiel and biohydrogen production

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It is known that the technology of using cyanobacteria as fuel raw materials makes one of the central places among the approaches of modern alternative energy. To date, the possibility of using cyanobacteria in biodiesel and biohydrogen production is being considered. The usage of cyanobacteria could be a suitable alternative because they are more effective biologically producers of fatty acids on the planet, also universal renewable source of biomass [1]. In addition, hydrogen is a non-polluting source of energy, which is renewable and sufficient in the world, and cyanobacteria can use solar energy to produce biohydrogen under specific conditions [2, 3]. In this regard, the purpose of this work was to search for new strains of cyanobacteria – potential producers of biodiesel and biohydrogen, and the selection of optimal conditions for their cultivation to increase their productivities. The objects of investigation were *Cyanobacterium* sp. IPPAS B-1200, *Synechocystis* sp. PCC 6803, *Desertifilum* sp. IPPAS B-1220, *Synechococcus* sp. I12 and *Phormidium* Kützing B-26. Standard cultivation conditions were used in this work [4], fatty acids were analyzed using a gas-liquid chromatography with mass-spectrometer detector Agilent 5975S [5], and H_2 evolution was measured using a GC 3210 according the manufacturer`s instructions [6]. Strain of *Cyanobacterium* sp. IPPAS B-1200 isolated from the salty Balkhash lake, located on the south-east of Kazakhstan. Due to results, the fatty acid content of the *Cyanobacterium* IPPAS sp. B-1200: fraction of palmitic (16: 0) and palmitoleic (16: 1Δ9) acids are 40% and 10%; myristic (14: 0) and myristoleic (14: 1Δ9) acids found in cells in large quantities – 30% and 10% are matched. In this case, the 14:0 and Δ9-14: 1 fatty acids are the most promising raw material for producing biodiesel. Non-heterocystous cyanobacterial strains were used for study of hydrogenase activity, and the experiments were carried out under light and dark procedures. *Synechocystis* sp. PCC 6803 accumulated more (0.87 μl H_2 mg Chl^{-1}) H_2 under dark condition, and *Desertifilum* sp. IPPAS B-1220 was found to produce higher amounts of H_2 compared to others species under light supplement (5.257 μl H_2 mg Chl^{-1}). According to the results, *Cyanobacterium* sp. B-1200 and *Desertifilum* sp. IPPAS B-1220 strains were selected for a more detailed investigations as the most perspective biodiesel and biohydrogen producers.

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Lecture

Harnessing photosynthesis to produce electricity and hydrogen using spinach thylakoids and live cyanobacteria

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Photosynthetic organisms perform efficient solar energy conversion that can be harness for human use. Here, we describe the construction of two bio-photo-electrochemical cells (BPEC) based on either spinach thylakoids or live cyanobacteria to produce electric current and hydrogen fuel. In the first BPEC, spinach thylakoids are introduced into a BPEC cell containing buffer solution with ferricyanide. Upon solar-simulated illumination, water oxidation takes place and electrons are shuttled by the ferri/ferrocyanide redox couple from the thylakoids to a transparent electrode serving as the anode, yielding a photocurrent density of 0.5 $mA \cdot cm^{-2}$. Hydrogen evolution occurs at the cathode at a bias as low as 0.8 V. A tandem cell comprising the BPEC cell and a Si photovoltaic module achieves overall water splitting with solar to hydrogen efficiency of 0.3% [1]. In the second BEPC, we describe the harvesting of photocurrent used for hydrogen production from live cyanobacteria. A non-harmful gentle physical treatment of the cyanobacterial cells enables light-driven electron transfer by an endogenous mediator to a graphite electrode, without the addition of sacrificial electron donors or acceptors. We show that the photocurrent is derived from PS I and that the electrons originate from carbohydrates digested by the respiratory system. Finally, the current is utilized for hydrogen evolution on the cathode at a bias of 0.65 V [2]. For both systems the energy is used on the anode to produce electricity and hydrogen gas. The spinach BPEC produces high currents originating from water that decays relatively fast, but the active material can easily be replaced to maintain high rates of electron transfer. The cyanobacterial-cell based BPEC generates lower currents, yet, has a significantly longer lifetime. By studying and understanding both systems, we lay the foundation for future research to combine the advantages of both systems.

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Lecture

Photosynthetic hydrogen production as acclimation mechanism in green algae

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Among oxygenic phototrophs cyanobacteria and some green microalgae are able to perform hydrogen metabolism, but only green algae link this process to photosynthesis. For this purpose, algae possess [FeFe]-hydrogenase, a highly efficient enzyme with regard to hydrogen evolution which interacts with Fd (PetF) as redox partner and requires anaerobic conditions for the functional activity. Photosynthetic electron transport can be activated in anoxia either upon cells transition from the dark, anaerobic conditions to light or under specific treatments suppressing photosynthetic oxygen evolution. It has been well established that in green microalgae deprivation of such macronutrients as N, S, P, and Mg sustains long-term hydrogen photoproduction [1]. Nutrient deprivation inhibits photosynthesis thus promoting successively anaerobic conditions, hydrogenase activity and hydrogen evolution. In natural environment, planktonic algae are often limited by important macronutrients. Under these circumstances, hydrogen photoproduction may serve as acclimation mechanism providing a sink for the excess of reducing equivalents.

In the current work, we studied physiological role of hydrogen photoproduction in *Chlamydomonas reinhardtii* upon sulfur deprivation conditions. Cell cultures were incubated in closed glass vials under constant illumination. The dynamics of such physiological parameters as culture viability, photosynthetic activity, chlorophyll, starch, lipid, ATP content, and fermentation products were compared between hydEF mutant lacking hydrogenase activity and its parental strain CC-425. We showed that the presence of hydrogenase activity in the chloroplast facilitates stability of photosystem II, accelerates anaerobic starch degradation, alters fermentation, and improves culture viability during anaerobic phase of S deprivation. Intracellular ATP content was higher in mutant cells indicating lower metabolic demands for energy and, likely, accelerated cyclic electron flow around PS I in the absence of the hydrogenase activity. The obtained results brought us to the conclusion that the active hydrogenase provides benefits of algae acclimation to nutrient deprivation by improving redox conditions and facilitating anaerobic metabolism in the algal cell.

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Lecture

Keeping the Calvin-Benson cycle inactive is the key to sustained and photoautotrophic H_2 production in green algae

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Photobiological H_2 production has the potential of becoming a carbon-free renewable energy source, because upon the combustion of H_2, only water is produced. The [Fe-Fe]-type hydrogenases of green algae are highly active, although extremely O_2-sensitive, which has been the major limitation in exploiting algal H_2 production ever since its discovery.

We recently established a novel H_2 production protocol for the green alga *Chlamydomonas reinhardtii*, which is based on a short anaerobic incubation in the dark, followed by exposure to continuous, relatively intense illumination. Upon transferring the cultures to the light, H_2 production starts immediately and if the Calvin-Benson-Bassham cycle is kept inactive by substrate limitation (i.e. absence of CO_2 and acetate), H_2 production lasts for several days. In addition, if a simple iron-salt-based O_2 absorbent is introduced into the gas phase, several-fold increase in H_2 production is achieved. Besides the high H_2 production yield, another advantage of our protocol is that the algal cultures remain photosynthetically active and at the end of the intense H_2 production period, the cultures may readily regenerate. The H_2 production process is photoautotrophic, with the electrons feeding the hydrogenases mostly derived from water. Our method therefore demonstrates that it is possible to sustainably use algal cells as whole-cell catalysts for H_2 production [1]. Additional optimization enabled the production of about 2 ml H_2/ml culture in 6 days.

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Lecture

Understanding the mechanism of H_2 photoproduction in the pulse-illuminated green alga, *Chlamydomonas reinhardtii*

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Photosynthetic production of molecular hydrogen (H_2) by microalgae is a potential source of renewable energy. The process occurs in anaerobic, dark-adapted algae on illumination [1] and is driven by the [FeFe]-hydrogenase enzyme(s) located in algal chloroplasts [2]. The [FeFe]-hydrogenase interacts with the photosynthetic electron transport chain at the level of ferredoxin, thus linking the water-splitting reaction at photosystem II (PS II) to the reduction of protons to H_2. Since the process is O_2-sensitive, H_2 photoproduction in microalgae is difficult to sustain [3]. Recently, we introduced a new protocol for sustaining H_2 photoproduction in algal cultures, which allows efficient distribution of photosynthetic electrons to the hydrogenase instead of CO_2 fixation and production of biomass [4]. In this protocol, the sustainability is achieved by a shift of growing algal cultures from continuous illumination to a train of short (1–5 s) light pulses interrupted by longer (3–9 s) dark or low light phases. The invented protocol confirmed the presence of non-active [FeFe]-hydrogenase in aerobically grown algae and the activation of this enzyme within a few seconds after establishment of anaerobiosis. Using the membrane inlet mass spectrometry (MIMS) and ^{18}O-labeled water, we further proved that H_2 production in pulse-illuminated algae depends primarily on the direct water biophotolysis, which is compensated by the indirect pathway in the case of an insufficient electron flow from PS II (when PS II is inhibited by DCMU or in the mutants lacking the active PS II centers). Although very efficient in the beginning, the PS II independent pathway could not sustain the process as long as the direct water biophotolysis, especially with an increased duration of the light pulses in the sequence. In addition, the appearance of the labeled (*m/z* 46) CO_2 during pulse-illumination indicates on the involvement of intracellular respiration in the removal of photosynthetically-released O_2 and thus, in the protection of the [FeFe]-hydrogenase enzyme of O_2 inactivation. All these findings together show a way for further metabolic engineering and construction of efficient algal factories for production of H_2 biofuel.

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Lecture

Potential for sustained H_2 photoproduction in the *Chlamydomonas* mutant hpm91

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H_2 photoproduction by green algae such as *Chlamydomonas reinhardtii* opens promising possibilities for solar fuels. However, H_2 production by wild-type *Chlamydomonas* sustains less than a week under sulfur-deprived anaerobic condition. Thus, generation of high hydrogen-production-mutants (*hpm*) is one of crucial issues in this advanced biotechnology. In this presentation, a mutant named *hpm91* will be introduced. This mutant was recently isolated in our research laboratory which is capable of producing hydrogen for 25 days with a 30-fold yield increase compared to wild type (CC400). Genetic analysis shows that *hpm91* is a *pgr5*-deficient mutant. Comparison of H_2 photo-production under identical experimental condition shows that *hpm91* mutant displays a higher capacity of H_2 production than the original *pgr5* mutant derived from different wild type (CC124). Physiological and biochemical characterization indicates that the prolonged H_2 production of *hpm91* is most likely due to increased PS II and PS I relative to that of wild type under sulfur deprivation. Most recent data from ROS-related experiments demonstrate that ROS content in *hpm91* cells was significantly lower than wild type. Taken together, our experimental results suggest that *hpm91* is a valuable algal strain for re-engineering *Chlamydomonas* towards improving light energy efficiency in scaling-up system.

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Lecture

Remodeling of photosynthetic electron transport in *Synechocystis* PCC 6803 for future hydrogen production from water

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Photosynthetic microorganisms such as the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*) can be exploited for the light-driven synthesis of valuable compounds. Energetically, this is much more rewarding if photosynthetic electrons are branched-off at Ferredoxin (Fd), which provides electrons for a variety of fundamental metabolic pathways in the cell, with the Ferredoxin-NADP-Oxido-Reductase (FNR, PetH) being the main target. In order to re-direct electrons from Fd to another consumer, the high electron transport rate between Fd and FNR has to be weakened [1]. Based on our previous *in vitro* experiments, corresponding FNR-mutants at position FNR_K190 [2] have now been generated in *Synechocystis* cells to study their impact on the cellular metabolism and their potential for a future hydrogen producing design cell. Out of two promising candidates, mutation FNR_K190D proved to be lethal due to oxidative stress, while FNR_K190A was successfully generated and characterized: The light induced NADPH formation is clearly impaired in this mutant and it shows also major metabolic adaptations like a higher glucose metabolism as evidenced by quantitative mass spectrometric analysis. These results indicate a high potential for the future use of photosynthetic electrons in engineered design cells – for instance for hydrogen production. They also reveal substantial differences in the interaction of proteins if characterized in an *in vitro* environment [3] in comparison with the physiological conditions of whole cells which have to be considered in remodeling processes.

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Poster

Regulation of hydrogen yield in green microalga *Parachlorella kessleri* by physicochemical factors

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Molecular hydrogen (H_2) is the promising high-efficiency ecologically clean energy carrier of interest in alternative energy technology. Green microalgae are able to produce H_2 during direct biophotolysis of water using sunlight as an energy source [1, 2]. The effects of various physicochemical factors (carbon source, medium pH, illumination regimes, extremely high-frequency electromagnetic irradiation (EMI), aerobic and anaerobic conditions) on the yield of H_2 in new green microalga *Parachlorella kessleri* RA-002 from Armenia have been studied. The impact of these factors on algal growth properties was analyzed. During the cultivation of *P. kessleri* the carbon source, medium pH, temperature and light intensity were optimized: they were acetate (1 g/L), pH 7.5, 25°C and 2000 lux, respectively. The results obtained show that H_2 photoproduction by *P. kessleri* is observed in anaerobic conditions; moreover the H_2 yield is enhanced after pre-incubation of algae in darkness, which is connected with the expression of the responsible enzyme, Fe-hydrogenase. Inhibitor of PS II – diuron inhibited H_2 production by *P. kessleri* RA-002, indicating the PS II-dependent pathway of H_2 generation in this alga. The exposure of *P. kessleri* to EMI (frequency of 51.8 and 53 GHz) suppressed the H_2 yield during 72 h algal growth, but it has been restored after 96 h. Moreover, the maximal inhibitory effect has been obtained at a frequency of 53 GHz. Thus, the used physicochemical factors affect the H_2 production by *P. kessleri* in different manner. The results obtained can be used for further optimization of algal cultivation conditions for providing H_2 production.

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Section 2.4: Hydrogenases

Lecture

Hydrogen electrode with HydSL hydrogenase from *Thiocapsa roseopersicina* in fuel cell

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Fuel cells use noble metals for the catalysis of hydrogen oxidation and the oxygen reduction for electricity generation. Due to high cost of noble metals it is desirable replacing them by enzymes.

Hydrogenases are metalloenzymes that catalyze the activation of molecular hydrogen. There are three types of hydrogenases differing in the content of metals in their active site: [NiFe]-hydrogenases; [FeFe]-hydrogenases; Fe-hydrogenases with a Fe-containing cofactor. For most [NiFe]-hydrogenases, the bimetallic center was shown to be covalently bound to the protein by four cysteine residues, two of which form a connecting bridge between Ni and Fe, and the other two coordinate Ni. The Fe ion is associated with diatomic ligands, one CO- ligand and two CN- ligands.

NiFe-hydrogenase HydSL from purple sulfur bacterium *Thiocapsa roseopersicina* BBS belongs to group 1, hydrogen-uptake hydrogenases. It was shown that this enzyme conducts direct electrocatalysis with hydrogen uptake when immobilized on electrode surface. However, hydrogen electrodes based on this hydrogenase produce low current density comparing to Pt electrodes (not more than 1.5 $mA \cdot cm^{-2}$). This is the main problem preventing commercial application of this enzyme in fuel cells.

Previously we showed that hydrogen "breathing" hydrogenase electrode has high current density (Tsygankov et al., 2017). In this report we describe experiments with particular fuel with hydrogen "breathing' electrode based on HydSl hydrogenase and oxygen electrode with Pt as catalyst. Different methods of hydrogenase immobilization were explored. In optimized system fuel cell generated current up to 7.0 $mA \cdot cm^{-2}$ with maximum power generation equal to 3.0 $mW \cdot cm^{-2}$. Operational stability experiments showed that after 600 h of operation fuel cell produced current up to 3.4 $mA \cdot cm^{-2}$ with maximum power generation equal to 1.4 $mW \cdot cm^{-2}$. Possible directions for following improvement of hydrogenase based fuel cell are discussed.

Acknowledgement. This work was supported by Russian Science Foundation No 19-14-00255.

Tsygankov A, Shastik E., Zorin N. Hydrogenase electrode based on HydSL hydrogenase from Thiocapsa roseopersicina with high current density. In: "Photosynthesis and Hydrogen Energy Research for Sustainability" 8th International Conference October, 30-November 4, 2017. Abstracts. Hyderabad, India. P. 209

Poster

Modeling of interactions of proteins participating in hydrogen production

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We investigated molecular mechanisms of interactions between ferredoxin, ferredoxin:$NADP^+$-reductase (FNR) and hydrogenase using the method of multi-particle Brownian modeling developed by our group.

In this method, protein molecules are presented as rigid structures with fixed charges created by cofactors and amino acid residues. Molecules can be placed in a media imitating solution or in a structured environment imitating a chloroplast. Proteins move due to Brownian force and electrostatic forces orienting in each other's electric field and forming complexes necessary for electron transfer [1].

We have studied competition between FNR and hydrogenase for ferredoxin. It is known that during illumination chloroplast stroma becomes more alkaline, so we investigated how pH affects the rate of interactions between ferredoxin and FNR and ferredoxin and hydrogenase. At pH from 5.0 to 9.0 the rate constant of complex formation between ferredoxin and FNR does not change significantly. However, the rate of interaction between ferredoxin and hydrogenase dramatically depends on pH: at the pH range from 6.0 to 8.0 it increases three-fold. This result indicates different mechanisms of regulation of ferredoxin interaction with its protein partners.

One of the potential approaches to increasing hydrogen production is genetic engineering. Introduction of point mutations can potentially increase ferredoxin affinity to hydrogenase and decrease its affinity to FNR. We simulated several point mutations on ferredoxin and identified the most promising for increasing hydrogen yield.

The study was performed with financial support from the Russian Foundation for Basic Research, grant No. 17-04-00676A.

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Section 2.5: Proton Reduction Catalysts

Lecture

Electro-catalytic activity for H_2 evolution by a polypyridyl copper complex

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H_2 generation as clean form of energy has become a major field of research for the environmental scientist, since it can be produced through $2e^-$ reduction of $2H^+$ in a simpler route as compared to water oxidation process. Although copper complexes have been widely studied in variety of biological redox process due to its prominent biomimetic and redox chemistry [1] but only a few number of copper based electrocatalysts have been studied for hydrogen evolution reaction under organic or aqueous medium [2–4]. Herein, the talk is based on the proton and water reduction activity by a mononuclear copper complex $[Cu(tpen)]^{2+}$ (tpen = N,N,N‘,N‘-tetrakis(2-pyridylmethyl)ethylenediamine) under electrochemical conditions.

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Section 2.9: Nanomaterials for Hydrogen Production

Lecture

Najafpour-Feizi reaction under water-oxidation conditions

Mohammad Mahdi Najafpour* **and Hadi Feizi**

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Finding true catalysts during water-oxidation reaction is critical to design and synthesize new, efficient, and stable water-oxidizing catalysts [1–3]. To find out the details of the effect of the metal oxide on the metal complexes under the water-oxidation reaction, we selected some metal complexes and studied the effect of the metal oxides on the decomposition of them under the water-oxidation reaction. By the results, our experiments showed that the formed metal oxides during water oxidation not only accelerate water oxidation but also drive the decomposition of metal complexes. This self-decomposition reaction (Najafpour-Feizi reaction) is critical because it could indicate the details of the decomposition of the metal complexes in the presence of the metal oxide under the oxidation reaction. We show that such a reaction could occur for Mn, Co and Ni complexes under the water-oxidation reaction. This reaction also shows that a few amounts of formed metal oxides under the water-oxidation reaction could result in decomposition of the metal complex.

In addition to it and as a further outlook, we suggest that such a reaction may occur for other oxidation reactions such as epoxidation, alcohol or sulfide oxidation and even attributed photochemical reactions. Equivalently, we hypothesize in the reduction reactions in the presence of metal complexes, the role of the formed metal on the decomposition of metal complexes during the reaction should be investigated.

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Poster

Nickel-iron (hydr)oxides: New findings and challenges

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Water splitting to hydrogen and oxygen is a promising reaction to the storage of intermittent energy [1]. However, the water-oxidation reaction is one of the bottlenecks in water-splitting reaction at a large scale [2]. Thus, finding efficient, stable, low-cost, earth-abundant and environmentally friendly catalysts for water-oxidation reaction is a significant challenge to the storage of intermittent energies. Nickel-iron (hydr)oxides are among the best catalysts for water oxidation in basic media [3]. Boettcher's group found that increased conductivity is not sufficient to explain the increased activity in nickel oxide by iron [4]. His group investigated the proposed roles of dissolved species in water oxidation, and reported a new hypothesis to find the catalytic activity of $Ni(OH)_2$/NiOOH-based catalysts. They proposed that the $Ni(OH)_2$/NiOOH surfaces have adsorbed Fe impurities from the electrolytes, but without improving water oxidation [5]. Herein, we introduce a promising method to prepare Fe and Ni-free KOH solution, discuss the related challenges and present our progress for nickel-iron (hydr)oxides toward water oxidation. At least under our experimental conditions, soluble Ni species have no significant effect on water oxidation by Ni oxide. However, even a few amounts of Fe(III) have a significant effect on water oxidation by Ni oxide.

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Poster

Water oxidation by amorphous Ir oxide: A novel synthesizing method, toward industrial application

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Electrochemical water splitting is a promising and eco-friendly pathway for sustainable, efficient and clean H_2 production. Oxygen evolution reaction (OER) is the bottleneck of this technology due to more sluggish kinetics, which decreases the overall water splitting efficiency. Platinum group (Os, Ir, Ru, Rh, Pt, Pd) oxide-based materials are among the best candidates to use for OER in alkaline and acidic solutions [1]; among them, IrO_x is the most stable in acidic media [2].

Herein, we report on the synthesis of an amorphous IrO_x on the surface of metallic iridium with a one-step, low-cost, environmentally friendly, and easy to prepare method by operating iridium metal as the anode electrode in a two-electrode system. The Ir/IrO_x was characterized by various spectroscopic, microscopy, X-ray diffraction, and electrochemical methods. The synthesized Ir/IrO_x electrode shows high stability and activity with 10 mA cm^{-2} at <250 mV overpotential (without IR-correction) in $HClO_4$ (0.1 M). Moreover, to study the catalyst with the aim of using it in presently available polymer electrolyte membrane (PEM) electrolyzers, IrO_x was physically separated from the surface of metallic iridium and investigated.

Comparing with other similar catalysts, the catalytic performance and stability for the catalyst are among the best noble metal-based OER catalysts in acidic, alkaline and neutral environments.

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Poster

Ni-Fe oxide for water oxidation: A new strategy

Sepideh Madadkhani[1], Reza Babadi Aghakhanpour[1], Jitendra Pal Singh[2], Robabeh Bagheri[3], Keun Hwa Chae[2], Zhenlun Song[3], Mohammad Mahdi Najafpour[1*]

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Switching from fossil fuels to renewable energies is an inevitable task that should be performed in the near future to provide clean and sustainable energies for human beings. However, many renewable kinds of energy are too intermittent to be used at a large scale; thus a large capacity for energy storage is necessary1. Water splitting toward hydrogen production is a promising approach to store such energies. We report an iron/nickel/zinc mixed oxide as a catalyst for the electrochemical water oxidation. This catalyst was synthesized by a straightforward method for the synthesis of an iron/nickel/zinc mixed oxide through the calcination of a Fe/Ni/Zn organometallic compound. The calcined product contains Fe and Ni as crucial ions for water oxidation, accompanied by the presence of Zn ions. The removal of Zn ions from the mixed oxide provides more active sites on the surface of the catalyst. The composition of the compound was characterized by some common methods and found to be an efficient water-oxidizing catalyst. The catalyst on FTO at pH 13 yields a current density of 12 mA/cm^2 at 1.2 V (vs. Ag|AgCl). After 5 hours at 1.1 V, the electrode not only shows no decrease in performance but also shows an increase from 4 to 7 mA/cm^2 in the water oxidation activity.

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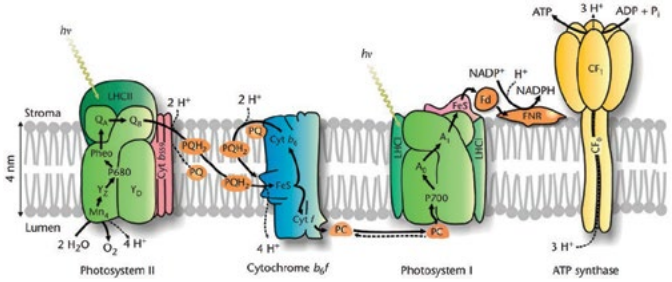

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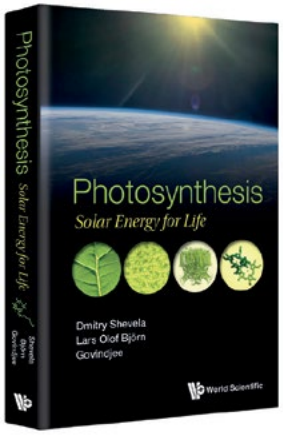

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