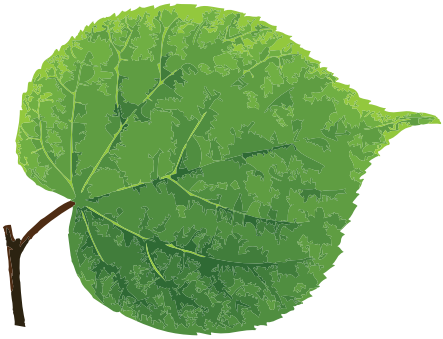

International Conference

Photosynthesis Research for Sustainability

in honor of Nathan Nelson and T. Nejat Veziroglu

June 19–25 2016
Pushchino, Russia

ABSTRACTS AND PROGRAMME

Institute of basic biological problems
Russian Academy of Sciences

International Conference

“Photosynthesis Research for Sustainability-2016”

in honor of Nathan Nelson and T. Nejat Veziroglu

June 19–25, 2016
Pushchino, Russia

Abstracts and Programme

Pushchino – 2016

International Conference "Photosynthesis Research for Sustainability-2016: in honor of Nathan Nelson and T. Nejat Veziroglu"
Eds. Suleyman Allakhverdiyev, Ilya Naydov.
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The volume contains abstracts of the lectures and poster presentations at 7th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability-2016: in honor of Nathan Nelson and T. Nejat Veziroglu" to be held in June 19–25 (2016) in Pushchino city (Biological Research Center), Moscow Region, Russia. Experimental and theoretical works covering a wide range of photosynthetic and biohydrogen topics, from the primary processes of electron transfer and energy bioconversion to the physiological aspects of photosynthesis and applied aspects of hydrogen production are discussed at the conference. Considerable attention is paid to discussion of structural organization of photosynthetic reaction centers and mechanisms of hydrogen production. The book will be of interest to researchers and students involved in the study of photosynthesis and biohydrogen production.

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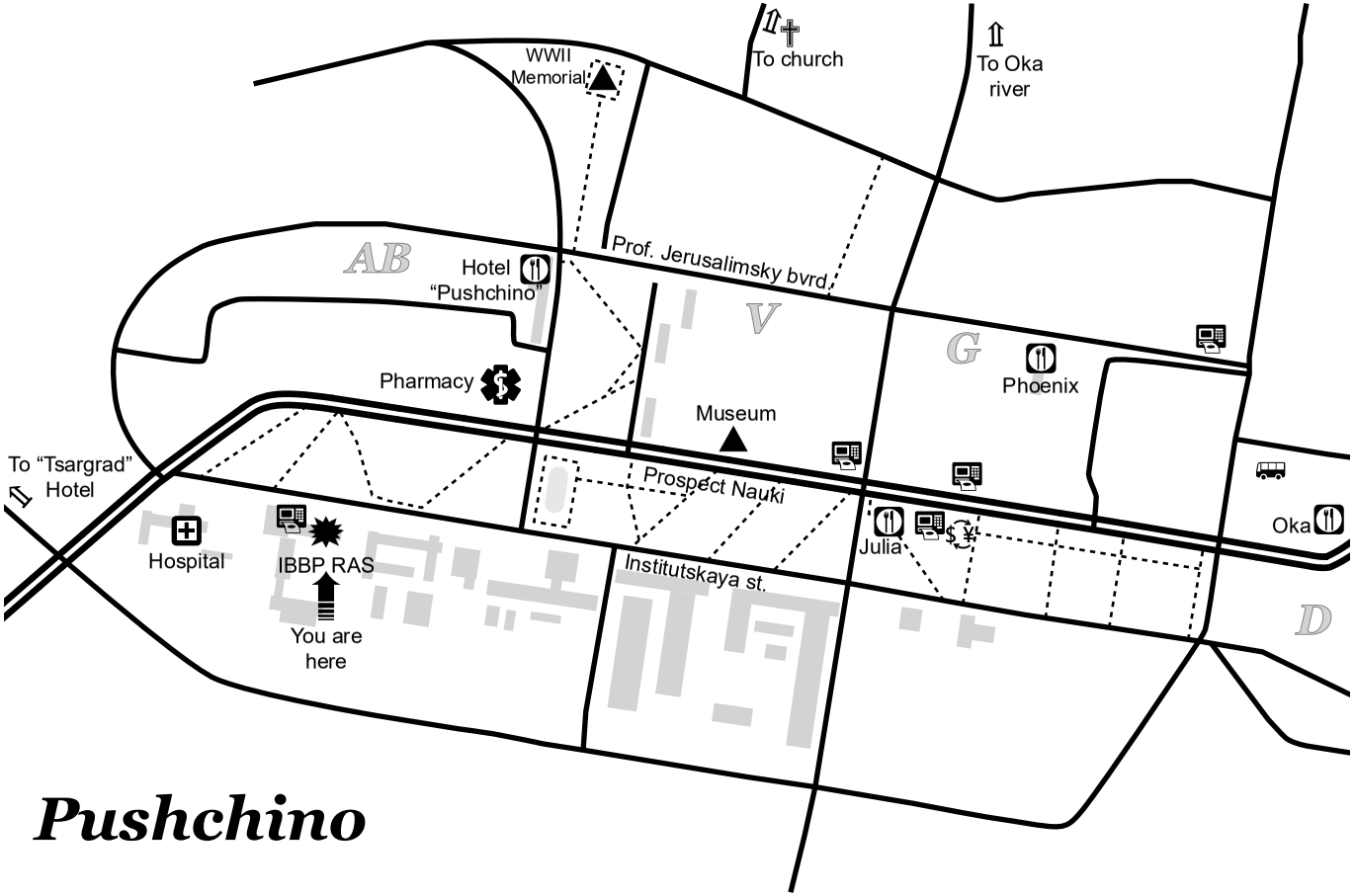
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Pushchino

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Additional information is available
on our website:
http://photosynthesis2016.cellreg.org

WELCOME!

You are most welcome to the 7th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability-2016: in honor of Nathan Nelson and T. Nejat Veziroglu" held in Pushchino city (Biological Research Center), Moscow Region, Russia. This Meeting is organized by Institute of Basic Biological Problems RAS in 50th year of its history.

This Meeting is 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 Conference provides a forum for students, postdoctoral fellows, and scientists from different countries to deepen and exchange their knowledge and understanding, widen professional contacts as well as create new opportunities for new joint projects and informal collaboration. The topics of this conference range widely and separated by two main subjects: photosynthesis and biohydrogen. Photosynthetic section includes primary processes of photosytnhesis, structure, function, and biogenesis of the photosynthetic apparatus, photosystem I and II, as well as water oxidation mechanism, artificial photosynthesis, regulation of photosynthesis and environmental stress, applied aspects of photosynthsis and emerging techniques. Biohydrogen section includes biological hydrogen production, hydrogenases, artificial photosynthesis for hydrogen energy, hydrogen purification and storage, hydrogen economy, hydrogen energy education as well as emerging techniques for studying hydrogen energy.

The multidisciplinary nature of this conference follows from the list of topics and presented lectures. In total, more than 160 lectures and posters will be presented.

Together with all of you, we look forward to a most interesting week with fascinated presentations and inspiring discussions within all scientific topics of the Meeting.

James Barber,
Suleyman Allakhverdiev,
Anatoly Tsygankov

Schedule: Photosynthesis Research for Sustainability-2016

JUNE 18 (SATURDAY)

Arrival and accomodation

JUNE 19 (SUNDAY – 1ST DAY)

9:00–10:00 Registration

10:00–10:30 Opening Ceremony

Acad. Anatoly I. Miroshnikov, Chairman of the Pushchino Research Center;
Dr. Ivan Savintsev, The head of the Pushchino city;
Dr. Anatoly Tsygankov, President of Russian Society of Photobiology.

Pushchino Readings: 50 years of Institute of Biological Problems RAS (former Institute of Photosynthesis AS USSR)

Chairpersons: James Barber (UK), Govindjee (USA), Julian J. Eaton-Rye (New Zealand), Suleyman Allakhverdiev (Russia)

10:30–10:50 **Vladimir A. Shuvalov** *(Academician, Director of the Institute of Basic Biological Problems, RAS).* On the history of the Institute of Basic Biological Problems RAS and Pushchino Readings on Photosynthesis

10:50–11:30 **Andrey B. Rubin** *(Corr. Member of RAS, Department of biophysics, Biological Faculty, Lomonosov Moscow State University, Moscow).* Problems of biophysics and mechanisms of primary photosynthetic reactions

Events in honor of Prof. Nathan Nelson and T. Nejat Veziroglu

11:30–12:00 **Govindjee**. In honor of Prof. Nathan Nelson and T. Nejat Veziroglu

12:00–13:30 (90 min) Lunch

Chairpersons: Ada Yonath (Israel), John H. Golbeck (USA), Leslie Dutton (USA), Kimiyuki Satoh (Japan), Norio Murata (Japan)

13:30–14:00 **Ada Yonath** *(Nobel Laureate in Chemistry, 2009; Department of Structural Biology, Weizmann Institute, Rehovot, Israel)*. Key issues in contemporary medicine, the microbiome and environmental sustainability

14:00–14:30 **Nathan Nelson** *(Department of Biochemistry and Molecular Biology, Tel Aviv University, Tel Aviv, Israel)*. Half century of scientific wandering – Freedom, Serendipity and Joy

14:30–15:00 **William A. Cramer** *(Department of Biological Sciences, Purdue University, USA).* On the mechanism of state transitions: redox- and structure- dependent interaction *in vitro* between Stt7 kinase and the cytochrome b_6f complex

15:00–15:30 **Rachel Nechushtai** *(The Alexander Silberman Institute of Life Sciences and the Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel)*. Photosystem I: From protein composition to photo-bio-nano-electronics, a personal prespective dedicated to prof. Nathan Nelson

15:30–15:50 (20 min) Coffee break

Chairpersons: Norio Murata (Japan), Govindjee (USA), Arvi Freiberg (Estonia)

15:50–16:20 **John H. Golbeck** *(Department of Biochemistry and Molecular Biology, The Pennsylvania State University, PA, USA).* Charge separation in *Heliobacterium modesticaldum*: An exemplar of an early homodimeric type I photosynthetic reaction center

16:20–16:50 **Arvi Freiberg** *(Institute of physics and institute of molecular and cell biology, University of Tartu, Tartu, Estonia).* Structural constraints for excitation energy migration and trapping in photosynthetic bacteria

16:50–17:20 **Michael Hippler** *(IBBP, WWU Münster, Germany).* Calredoxin – a novel calcium-dependent sensor-responder connected to regulation of photosynthesis

Chairpersons: Kintake Sonoike (Japan), Marián Brestic (Slovakia), Marek Živčák (Slovakia), Kentaro Ifuku (Japan), Olaf Kruse (Germany), Gadi Schuster (Israel), Iftach Yacoby (Israel), Seiji Akimoto (Japan), Eugene Maksimov (Russia), Rajagopal Subramanyam (India), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan)

17:20–20:00 Poster viewing (Library on the first floor)

JUNE 20 (MONDAY – 2ND DAY)

Chairpersons: James Barber (UK), Julian J. Eaton-Rye (New-Zealand), Barry Bruce (USA)

8:00–8:30 **Govindjee** *(University of Illinois at Urbana-Champaign, Urbana, USA).* Towards efficient photosynthesis: Overexpression of C4 enzymes in C3 plants: A case study

8:30–9:00 **Norio Murata** *(National Institute for Basic Biology, Okazaki, Japan).* ATP is the driving force of the repair of photosystem II during photoinhibition: Important role of cyclic electron transport

9:00–9:30 **Leslie Dutton** *(The Johnson Research Foundation, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA).* First principles design of water-soluble photochemical proteins engineered for solar energy conversion in living cells

9:30–9:55 **Julian J. Eaton-Rye** *(Department of Biochemistry, University of Otago, Dunedin, New Zealand).* Bicarbonate-reversible inhibition of the iron-quinone acceptor complex of photosystem II lacking low-molecular-weight proteins or with targeted mutations to the D1 protein

9:55–10:15 (20 min) Coffee break

Chairpersons: Hiroshi Ishikita (Japan), Galina Riznichenko (Russia), Alexey Semenov (Russia)

10:15–10:45 **James Barber** *(Department of Life Sciences, Sir Ernst Chain Building, South Kensington Campus, Imperial College London, UK).* The Mn_4Ca-catalyst of the photosynthetic oxygen evolving centre: structure, function and evolution.

10:45–11:10 **Thomas Friedrich** *(Technical University of Berlin, Institute of Chemistry, Berlin, Germany).* From bacteriophytochromes to iRFP: Optimization of fluorescence by "local" and "remote" amino acid substitutions

11:10–11:35 **Avigdor Scherz** *(Department of Plant and Environmental Sciences, The Weizmann Institute of Science, Rehovot, Israel).* Small residues control protein-gated electron transfer in photosynthetic reaction centers

11:35–12:00 **Alexander Tikhonov** *(Department of Biophysics, Physical Faculty, Moscow State University).* Electron and proton transport coupled to ATP synthesis in chloroplasts: Lateral heterogeneity of the proton potential

12:00–13:30 (90 min) Lunch

Chairpersons: Kintake Sonoike (Japan), Thomas Friedrich (Germany), Avigdor Scherz (Israel)

13:30–13:55 **Peter Hegemann** *(Humboldt-Universität zu Berlin, Germany)* Optogenetics, a technology with Russian Scientific roots: Channelrhodopsin and new inhibitory optogenetic approaches

13:55–14:20 **Győző Garab** *(Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary).* Roles of non-bilayer lipids and non-lamellar lipid phases in the assembly and structural dynamics of thylakoid membranes

14:20–14:45 **Hiroshi Ishikita** *(Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan).* Energetics of proton release on the first oxidation step in the water oxidizing enzyme

14:45–15:10 **Alexey Semenov** *(Belozersky Institute of Physical-Chemical Biology, Moscow State University, Moscow, Russia).* Charge recombination in photosystem I under conditions of restricted protein mobility

15:10–15:35 **Barry Bruce** *(University of Tennessee at Knoxville, Knoxville, TN, USA).* Characterization of a non-detergent isolated form of a cyanobacterial trimeric photosystem I using styrene-maleic acid co-polymers

15:35–15:50 (15 min) Coffee break

Chairpersons: Iftach Yacoby (Israel), Tatsuya Tomo (Japan), Kimiyuki Satoh (Japan)

15:50–16:15 **Agepati S. Raghavendra** *(University of Hyderabad, Hyderabad, India).* Biochemical and molecular basis of modulation by oxidative stress of photorespiratory components

16:15–16:40 **Olaf Kruse** *(Bielefeld University, Faculty of Biology, Center for Biotechnology (CeBiTec), Bielefeld, Germany).* Elucidating the regulatory network of light harvesting in photoheterotrophic microalgae

Chairpersons: Anatoly Tsygankov (Russia), Olaf Kruse (Germany), Martin Winkler (Germany)

16:40–17:10 **Rick L. Garcia** *(LI-COR Biosciences USA).* Sponsor's presentation. Exploring the power of parallel measurements of electron transport, CO_2 and H_2O flux in plant leaves

17:10–19:05 **Brent Claassen and Rick L. Garcia** *(LI-COR Biosciences USA).* Practical work-shop from the sponsor: Further dialogues concerning the new LI-COR LI-6800 portable photosynthesis system and the power of parallel measurements of electron transport, CO_2 and H_2O flux in plant leaves. Room 114, follow arrows on the wall

Chairpersons: Kintake Sonoike (Japan), Marián Brestic (Slovakia), Marek Živčák (Slovakia), Kentaro Ifuku (Japan), Olaf Kruse (Germany), Gadi Schuster (Israel), Iftach Yacoby (Israel) Seiji Akimoto (Japan), Eugene Maksimov (Russia), Rajagopal Subramanyam (India), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan)

17:10–18:30 Poster viewing (Library on the first floor)

19:05–21:00 Get together evening

JUNE 21 (TUESDAY – 3RD DAY)

Tours

Moscow, Yasnaya Polyana

JUNE, 22 (WEDNESDAY – 4TH DAY)

Section: Neutron Scattering in Photosynthesis Research

Chairpersons: Jörg Pieper (Estonia), Chris Garvey (Australia), Gergely Nagy (Switzerland)

8:00–8:25 **Jörg Pieper** *(Institute of Physics, University of Tartu, Tartu, Estonia)*. Excitonic coupling and protein dynamics in the water-soluble chlorophyll protein (WSCP)

8:25–8:50 **Christopher Garvey** *(Australian Nuclear Science and Technology Organisation, Lucas Heights, NSW, Australia)*. Deuteration as a tool to understand photosynthetic membrane organisation in the cyanobacterium *Halomicronema hongdechloris*

8:50–9:15 **Robert Corkery** *(Royal Institute of Technology, Stockholm, Sweden. Australian National University, Canberra, Australia)*. Small angle neutron scattering studies of photosynthetic membrane structures in cyanobacteria

9:15–9:40 **Henrich Frielinghaus** *(Jülich Centre for Neutron Science, Forschungszentrum Jülich GmbH, Garching, Germany)*. Wood structure during pretreatment in ionic liquids

9:40–9:55 (15 min) Coffee break

9:55–10:20 **Gergely Nagy** *(Paul Scherrer Institute, Villigen, Switzerland; Wigner Research Centre for Physics, Hungarian Academy of Sciences, Budapest, Hungary).* Structure and dynamics of photosynthetic membranes as studied by neutron scattering

10:20–10:45 **Maksym Golub** *(Institute of Physics, Tartu University, Tartu, Estonia; University of Joseph Fourier, Grenoble, France).* Combined SAXS-SANS structure study of isolated PS II core complex

Chairpersons: Tatsuya Tomo (Japan), Suleyman Allakhverdiev (Russia), Kimiyuki Satoh (Japan)

10:45–11:10 **Mariko Miyachi** *(Department of Chemistry, School of Science, The University of Tokyo, Tokyo, Japan).* Fabrication of bio-conjugate photosystems using reconstituted photosystem I and II with molecular wires

11:10–11:35 **Daisuke Nii** *(Department of Physics, Tokyo University of Science, Tokyo, Japan).* Formation of biohybrid device between photosysten I and carbon material

11:35–12:55 (80 min) Lunch

Chairpersons: Yukako Hihara (Japan), Kentaro Ifuku (Japan), Agepati S. Raghavendra (India)

12:55–13:20 **Kintake Sonoike** *(Department of Biology, Waseda University, Tokyo, Japan).* Variation of redox state of plastoquinone pool in cyanobacteria revealed by photochemical quenching and non-photochemical quenching of chlorophyll fluorescence

13:20–13:45 **Miwa Sugiura** *(Proteo-Science Research Center, Ehime University, Bunkyo-cho, Matsuyama, Ehime, Japan).* Role of D1-pro173 of photosystem II in water oxidation

13:45–14:10 **Galina Riznichenko** *(Moscow State University, Moscow, Russia).* Kinetic and computer multiparticle modeling of photosynthetic regulation

14:10–14:35 **Keisuke Saito** *(Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan).* Energetics of the proton transfer from tyrosine D in photosystem II: Comparison with tyrosine Z

14:35–15:00 **Seiji Akimoto** *(Molecular Photoscience Research Center, Kobe University, Kobe, Japan).* Changes in light-harvesting and energy-transfer processes under different growth conditions

15:00–15:15 (15 min) Coffee break

Chairpersons: Miwa Sugiura (Japan), Daisuke Seo (Japan), Franz-Josef Schmitt (Germany)

15:15–15:40 **Kentaro Ifuku** *(Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto, Japan).* Evolution and function of the OEC family proteins in chloroplasts

15:40–16:05 **Yukako Hihara** *(Graduate School of Science and Engineering, Saitama University, Saitama, Japan).* A feed-forward loop consisting of the response regulator RpaB and the small RNA PsrR1 controls light acclimation of photosystem I gene expression in *Synechocystis* sp. PCC 6803

16:05–16:30 **Hisashi Ito** *(Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan).* Chlorophyll degradation by Mg-dechelatase

16:30–16:55 **Daisuke Seo** *(Division of Material Science, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa, Japan).* Comparative study on reaction kinetics of $NADP^+$/H reduction/oxidation catalyzed by ferredoxin-NAD(P)H oxidoreductases from photosynthetic and non-photosynthetic bacteria

16:55–17:20 **Zach Adam** *(The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University, Rehovot, Israel).* DEG proteases in the thylakoid lumen and their role in response to stress

17:20–17:45 **Marc M. Nowaczyk** *(Plant Biochemistry, Ruhr University Bochum, Bochum, Germany).* Light-driven whole-cell biocatalysis with recombinant cyanobacteria

17:45 Group Photo

Chairpersons: Kintake Sonoike (Japan), Marián Brestic (Slovakia), Marek Živčák (Slovakia), Kentaro Ifuku (Japan), Olaf Kruse (Germany), Gadi Schuster (Israel), Iftach Yacoby (Israel) Seiji Akimoto (Japan), Eugene Maksimov (Russia), Rajagopal Subramanyam (India), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan)

18:00–20:00 Poster viewing/discussion (Library on the first floor)

20:00–21:00 Chamber music

Artists: The member of Spivakov's orchestra Anastasia Kosarskaya (oboe) and laureate of international contests Vera Kryukova (piano);

Music by Johann Sebastian Bach, Robert Shuman, Camille Saint-Saëns, Ottorino Respighi, Manuel Ponce

JUNE 23 (THURSDAY – 5TH DAY)

Chairpersons: Anatoly Tsygankov (Russia), Olaf Kruse (Germany), Patrick Hallenbeck (Canada)

8:00–8:15 **Alexander Yu. Ramenskiy** *(President of Russian National Hydrogen Energy Association).* Standardization of hydrogen technologies and fuel cells in the Russian Federation

8:15–8:35 **Alexander Gusev** *(General Director STC "TATA" Limited).* Innovative technologies of the hydrogen economy and alternative energy for sustainable development

8:35–8:50 **Dmitry Dunikov** *(Joint Institute for High Temperatures, RAS, Moscow, Russia).* Russian R&D in Hydrogen Energy

8:50–9:20 **T. Nejat Veziroglu** *(President of International Association for Hydrogen Energy, Miami, FL, USA).* Global solution to global problems

9:20–9:45 **Patrick C. Hallenbeck** *(Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montréal. Canada; Life Sciences Research Center, Department of Biology United States Air Force Academy, USAF Academy, Colorado USA)*. Challenges in sustainable algal biofuels production

9:45–10:10 **Iftach Yacoby** *(Laboratory for Renewable Energy Studies. Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel)*. Continuous hydrogen production in air grown micro-algae

10:10–10:25 (15 min) Coffee break

Chairpersons: Mariko Miyachi (Japan), Tatsuya Tomo (Japan), Giuseppe Torzillo (Italy)

10:25–10:50 **Giuseppe Torzillo** *(Istituto per lo Studio degli Ecosistemi, Sesto Fiorentino, Firenze, Italy)*. Constraints in the scale-up of photobiological hydrogen production with microalgae

10:50–11:15 **Vinzenz Bayro Kaiser** *(Tel Aviv University, Tel Aviv, Israel)*. Temperature-sensitive PSII: toward a sustainable bioreactor for photosynthetic hydrogen production

11:15–11:40 **Martin Winkler** *(Department of Plant Biochemistry, Photobiotechnology group, Ruhr-University, Bochum, Germany)*. 2D-Tailoring of [FeFe]-Hydrogenases for Applications

11:40–12:05 **Evelina Slavcheva** *(Institute of Electrochemistry and Energy Systems, Bulgaria)*. Composite oxide supported catalysts for hydrogen production in anion exchange membrane electrolysis cells

12:05–13:35 (90 min) Lunch

Chairpersons: Keisuke Saito (Japan), Eugene Maksimov (Russia), Rajagopal Subramanyam (India)

13:35–13:55 **Azat V. Abdullatypov** *(Institute of Basic Biological Problems, RAS, Pushchino, Russia)*. Oxygen diffusion pathways through HydSL hydrogenase from *Thiocapsa roseopersicina*

13:55–14:15 **Zinaida Eltsova** *(Institute of Basic Biological Problems, RAS, Pushchino, Russia).* Hydrogen production by *Rhodobacter sphaeroides* mutants without LHII complex

14:15–14:35 **Alena Volgusheva** *(Moscow State University, Moscow, Russia).* Acclimation of *C. reinhardtii* to magnesium deficiency: Establishment of anoxia at high photosynthetic activity

14:35–14:50 **Alexey Kazakov** *(Joint Institute for High Temperatures, RAS, Moscow, Russia; Green Energy Development Center, Feng Chia University, Taichung, Taiwan).* Joint Russian-Taiwan project for biohydrogen production and purification

14:50–15:10 **Gadi Schuster** *(Faculty of Biology, Technion, Haifa, Israel).* Harnesing photosynthesis for solar energy conversion and hydrogen generation: Building a bio-generator

15:10–15:30 **George Sytchev** *(Joint Institute for High Temperatures of the Russian Academy of Sciences, Moscow, Russia).* Multipurpose technology of natural gas pyrolysis for the hydrogen energetics

15:30–15:50 **Franz-Josef Schmitt** *(Institute of Physical Chemistry, Technical University of Berlin, Berlin, Germany).* Fluorescent proteins as biosensors for studying the activity of hydrogenases and intracellular pH

15:50–16:05 (15 min) Coffee break

Chairpersons: Seiji Akimoto (Japan), Jóse A. Navarro (Spain), Azat Abdullatypov (Russia)

16:05–16:25 **Rajagopal Subramanyam** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India).* Role of stt7 kinase in acclimatization and organization of photosynthetic apparatus to salt in *Chlamydomonas reinhardtii*

16:25–16:45 **Arjun Tiwari** *(Department of Biochemistry, Molecular Plant Biology, University of Turku, Turku, Finland).* Photosystem I in photochemical and non-photochemical quenching of excitation energy

16:45–17:05 **Marina Kozuleva** *(Institute of Basic Biological Problems, RAS, Pushchino, Russia).* A new insight into mechanisms of oxygen photoreduction in the photosynthetic chain

17:05 Special Events

Committee: James Barber (UK), Ada Yonath (Israel), T. Nejat Veziroglu (USA), Tatsuya Tomo (Japan), Govindjee (USA), Anatoly Tsygankov (Russia), Suleyman Allakhverdiev (Russia)

Young Talents (8+8 awards/prizes)

The awards will be presented to young researchers who have done outstanding research in the field of photosynthesis research for sustainability and biohydrogen. All young researchers, including Ph.D. students and Post-Docs, may compete for awards.

Winners will be selected by the committee (see above), according to recommendation of chairpersons of poster sections.

19:00 Banquet

JUNE 24 (FRIDAY – 6TH DAY)

Chairpersons: Alena Volgusheva (Russia), Mahir Mamedov (Russia), Norio Murata (Japan)

9:00–9:25 **Lyudmila Vasilieva** *(Institute of Basic Biological Problems, RAS, Pushchino, Russia).* BChl ligation in bacterial photosynthetic reaction center: possible options

9:25–9:50 **Alexander Krasnovsky Jr.** *(A.N. Bach Institute of Biochemistry (Biotechnology Center, RAS) and M.V. Lomonosov Moscow University, Moscow, Russia).* Phosphorescence studies of the plant photosynthetic apparatus

9:50–10:15 **Victor Nadtochenko** *(Institute of Chemical Physics, RAS, Moscow, Russia).* Ultrafast charge separation events in Photosystem I *Synechocystis* sp. PCC 6803 under excitation of the Q_Y band red side: The mechanism of long-wavelength limit of photochemical energy conversion in Photosystem I

10:15–10:35 (20 min) Coffee break

Chairpersons: Lyudmila Vasilieva (Russia),
Rajagopal Subramanyam (India), Alexander Krasnovsky Jr (Russia).

10:35–11:00 **José A. Navarro** *(Instituto de Bioquímica Vegetal y Fotosíntesis, cicCartuja, Universidad de Sevilla-CSIC, Sevilla, Spain).* The photosynthetic cytochrome C550 from the diatom *Phaeodactylum tricornutum*

11:00–11:25 **Dmitry A. Cherepanov** *(Institute of Physical Chemistry and Electrochemistry, RAS, Moscow, Russia).* Dielectric behavior of the photosynthetic bacterial reaction center evaluated by the langevin and kirkwood-fröhlich models using molecular dynamics simulations

11:25–11:50 **Konstantin Neverov** *(A.N. Bach Institute of Biochemistry, Biotechnology Center, RAS, Moscow, Russia).* Chlorophyll triplet state in isolated PS II reaction centers and core complexes: Low temperature phosphorescence study

11:50–13:20 (90 min) Lunch

Chairpersons: Dmitry A. Cherepanov (Russia),
Alexander Krasnovsky Jr. (Russia), Vladimir Sukhov (Russia)

13:20–13:45 **Mahir Mamedov** *(Belozersky Institute of Physical-Chemical Biology, Moscow State University, Moscow, Russia).* Trehalose effects on real-time kinetics of electrogenic reactions due to catalytic cycle of water oxidizing complex of Photosystem II

13:45–14:10 **Eugene Lysenko** *(Institute of Plant Physiology RAS, Moscow, Russia).* Thylakoids win the competition for cadmium within plant chloroplasts

14:10–14:35 **Jianguo Liu** *(Institute of Oceanology CAS, Qingdao, China).* The multiple changes of photosynthetic behaviors, the role of photorespiration during the astaxanthin accumulation in *Haematococcus pluvialis* grown outdoors in tubular photobioreactors

14:35–15:00 **Alexei Solovchenko** *(Department of Bioengineering, Faculty of Biology, Moscow State University, Moscow, Russia).* Red or dead: Photosynthetic acclimation in a carotenogenic microalga *Haematococcus pluvialis* under stress

15:00–15:25 **Roman Pishchalnikov** *(Prokhorov General Physics Institute, RAS, Moscow, Russia).* Red exciton states: Localization in the photosystem I trimer complexes of *Arthrospira platensis* and their role in energy transfer

15:25–15:45 Coffee break

Chairpersons: Mahir Mamedov (Russia), Anjana Jajoo (India), Eugene Maksimov (Russia)

15:45–16:10 **Dmitry Zlenko** *(Moscow State University, Moscow, Russia).* Molecular model of PBS core and PBS association with photosystems

16:10–16:35 **Eugene Maksimov** *(Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, Russia).* Construction of a photoactive fluorescence sensor from the Orange Carotenoid Protein

16:35–17:00 **Vladimir Sukhov** *(N. I. Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia).* Proton signal as probable mechanism of photosynthetic response induced by variation potential in higher plants

17:00–17:25 **Nataly Belyaeva** *(Moscow State University, Moscow, Russia).* Thylakoid model parameters fitted to photosynthetic induction data

17:25 Closing Ceremony

James Barber (UK), Norio Murata (Japan), William Cramer (USA), Kimiyuki Satoh (Japan), Győző Garab (Hungary), Leslie Dutton (USA), Govindjee (USA), Suleyman Allakhverdiev (Russia), Tatsuya Tomo (Japan), Anatoly Tsugankov (Russia)

JUNE 25 (SATURDAY – 7TH DAY)

10:00–13:00

Excursions to Institutes of Pushchino Research Center.

Departure

Poster Session Schedule

JUNE 19

Section 1.1: Primary Processes of Photosynthesis

S1.1 Light-harvesting complexes from purple sulfur bacteria with modified *in vitro* carotenoid composition
Alexander Ashikhmin, Zoya Makhneva, Maksim Bolshakov, and Andrey Moskalenko

S1.2 Effect of light absorbed by carotenoids on growth of purple sulfur bacterium *Alc. vinosum*
Maksim Bolshakov, Zoya Makhneva, Alexander Ashikhmin, and Andrey Moskalenko

S1.3 Femtosecond processes of charge separation in two mutant reaction centers of *Rhodobacter sphaeroides* with increased midpoint potential at cryogenic temperature
Anton Khristin, Maria Leonova, Vladimir Shuvalov, and Anton Khmelnitskiy

S1.4 Comparison of the recombination rate of charges and lifetime of tryptophan fluorescence in RCs of *Rb. sphaeroides* in the temperature range from −180 to 25°C
Petr Knox, E. P. Lukashev, B. N. Korvatovskii, V. V. Gorokhov, N. P. Grishanova, N. Kh. Seifullina, and Vladimir Z. Paschenko

S1.5 Comparison of the recombination rate of charges and lifetime of tryptophan fluorescence in RCs of *Rb. sphaeroides* in the temperature range from −180 to 25°C
Petr Knox, E. P. Lukashev, B. N. Korvatovskii, V. V. Gorokhov, N. P. Grishanova, N. Kh. Seifullina, and Vladimir Z. Paschenko

S1.6 Two-photon spectroscopy of the LH1 complex and its subunit B820
Andrei Razjivin, Alexander Solov'ev, Victor Kompanets, Sergey Chekalin, and Andrey Moskalenko

S1.7 Can LH1 complexes of purple bacteria be assembled with partial filling of carotenoid pockets?
Alexander Solov'ev, Alexander Ashikhmin, and Andrey Moskalenko

S1.8 Coherent intradimer events in reaction centers of *Rhodobacter sphaeroides*
Andrey Yakovlev and Vladimir Shuvalov

S1.9 Study of the spectral and electron-transfer properties of *Rhodobater sphaeroides* R-26 reaction centers under vacuum conditions
Aleksey Zabelin, Anton Khristin, Taygib Iliyasov, Valentina Shkuropatova, and Anatoly Shkuropatov

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

S2.1 Thermostability of Photosystem I trimers and monomers from the cyanobacterium *Thermosynechococcus elongates*
Vladimir Shubin, Irina Terekhova, Yulia V. Bolychevtseva, Marta J. Kopczak, Eithar El-Mohsnawy, Matthias Rögner, Werner Maentele, and Enela Džafić

S2.2 Spectroscopic properties of the peripheral antennae from photosystem II
Alexander S. Belov and Daniil V. Khokhlov

S2.3 Evolution of metastable states in the process of plastocyanin and cytochrome *f* complex formation
Vladimir Fedorov, Sergei S. Khruschev, and Ilya Kovalenko

S2.4 The properties of two sources of carbonic anhydrase activity in photosystem II of higher plants
Lyudmila Ignatova, Elena Zhurikova, and Boris Ivanov

S2.5 Structural and functional features of photosynthetic apparatus of *Pinus sylvestris* in Baikal Siberia
Maria Ivanova and Galina Suvorova

S2.6 Simulation of electron transfer by plastocyanin in chloroplast thylakoid lumen by brownian dynamics
Ilya Kovalenko, Olga Knyazeva, Galina Riznichenko, and Andrey B. Rubin

S2.7 Molecular imprinted polymers in studying chlorophyll-protein interactions and development of sensors to determine herbicides
Mikhail Khristin

S2.8 Participation of carbonic anhydrase alpha-4 in the development of non-photochemical chlorophyll fluorescence quenching in *Arabidopsis thaliana*
Elena Zhurikova, Lyudmila Ignatova, Vilen Mudrik, and Boris Ivanov

Section 1.3: Photosystem II and Water Oxidation Mechanism

S3.1 Automatization of analysis of movement trajectories of small molecules in photosystem II
Bulat Fatkhullin and Azat Gabdulkhakov

S3.2 Involvement of molecular oxygen in the donor-side photoinhibition of Mn-depleted photosystem II membranes
Andrey Khorobrykh and Vyacheslav Klimov

S3.3 Spectral characteristics of photosystem II complexes isolated from chlorophyll *f* containing cyanobacterium
Toshiyuki Shinoda, Min Chen, Suleyman I. Allakhverdiev, and Tatsuya Tomo

S3.4 EPR and Fluorescence investigations of Fe cation interaction with Mn-binding sites in Mn-depleted PS II membranes
Boris Semin, Lira Davletshina, Jyotishman Dasgupta, G. Charles Dismukes, and Michael Seibert

S3.5 Trehalose induced stimulation of oxygen photoconsumption and electron transfer in manganese-depleted photosystem II preparations
Denis Yanykin, Andrey Khorobrykh, Mahir Mamedov, and Vyacheslav Klimov

Section 1.4: Energy Transfer and Trapping in Photosystems

S4.1 Energy migration in model quantum dot – aluminum phthalocyanine system
Daniil Gvozdev, Evgeniy Maksimov, M. G. Strakhovskaya, and Vladimir Z. Paschenko

S4.2 Characterization of the putative ferredoxin binding sites on photosystem I
Pini Marcu and Iftach Yacoby

S4.3 Excitation energy transfer in phycobiliproteins of *Acaryochloris marina* investigated by site-selective spectroscopy
Jörg Pieper, M. Rätsep, G. Gryliuk, S. Hildebrandt, K.-D. Irrgang, and H.-J. Eckert

S4.4 Accurate simulation of hole burning and fluorescence line-narrowing spectra
Jörg Pieper M. Rätsep, M. Pajusalu, P. Artene, and Arvi Freiberg

S4.5 Excitation energy transfer processes among photosystethic complexes in cyanobacterial cells
Yoshifumi Ueno, Shimpei Aikawa, Akihiko Kondo, and Seiji Akimoto

Section 1.5: Photosystem I and Bacterial Photosynthesis

S5.1 2,6-Dichlorophenolindophenol exhibits side effect on the acceptor side of photosystem I
Marina Kozuleva, Anastasia Petrova, Mahir Mamedov, Alexey Semenov, and Boris Ivanov

S5.2 Kinetic modeling of electron transfer in Photosystem I with variable internal and external acceptors
Georgy Milanovsky, Anastasia Petrova, Dmitry A. Cherepanov, and Alexey Semenov

S5.3 Studies on the monomeric form of Photosystem I
Sigal Y. Netzer-El and Nathan Nelson

S5.4 Interaction of the Photosystem I complexes containing different quinones in the A_1-site with exogenous electron acceptors
Anastasia Petrova, Georgy Milanovsky, B. K. Boskhomdzhieva, Mahir Mamedov, and Alexey Semenov

JUNE 20

Section 1.8: Regulation of Photosynthesis and Environmental Stress

S8.1 The function of FoF1-ATPase has an influence on the phycobilisome
Mina Agatsuma, Junji Uchiyama, Kento Funamizu, Haruna Ishikawa, Ayumi Matsuhashi, Yu Kanesaki, Hirofumi Yoshikawa, and Hisataka Ohta

S8.2 Response of photosynthetic apparatus, methobolic and antioxidant defense enzymes to phytoplasma infection in pepper (*Capsicum annuum* L.) leaves
Irada Huseynova, Gulnara Balakishiyeva, Durna Aliyeva, Ulduza Qurbanova, Jamila Bayramova, Ilgar Maharramov, and Jalal Aliyev

S8.3 Gas exchange parameters of wheat genotypes under soil water deficit
Tofig Allahverdiyev

S8.4 Luminescence and antioxidant responses of chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) to chilling stress during early vegetative stages
Alexey Baikov, Murat Gins, Mikhail Solntsev, and Alexander Tikhonov

S8.5 Effect of coherent radiation on some morphometric parameters and photosynthetic activity in regenerated plantlets of *Lavandula hybrid* Rev. cultivars
Valentina Brailko, Olga Mitrofanova, and Irina Mitrofanova

S8.6 High temperature effects and the photoprotective responses in chlorophyll *b* deficient wheat mutants
Marián Brestič, Marek Živčák, Katarína Olšovská, Kristýna Kunderlíková, and Suleyman I. Allakhverdiev

S8.7 Impact of annual changes of temperature and light (PAR) on induction of Chl *a* fluorescence *in situ* in *Stellaria media* (L.) and *Plantago maior* (L.)
Bogdan Nikolic, Dejan Dodig, Nina Djapic, Hadi Waisi, Violeta Petrovic, Nenad Milovanovic, and Sanja Djurovic

S8.8 Reorganization of pigment-protein complexes in *Ajuga reptans* leaves at overwintering
Olga Dymova, Mikhail Khristin, Ilya Zakhozhiy, and Tamara Golovko

S8.9 Roles of anionic lipids clarified with an SQDG-deficient mutant of *Thermosynechococcus elongatus* BP-1
Kaichiro Endo, Koichi Kobayashi, and Hajime Wada

S8.10 "Bicarbonate protective effect" on ATP synthesis
and possible role of carbonic anhydrase
Tatyana Fedorchuk, Vera Opanasenko, and Boris Ivanov

S8.11 Light-controlled variability of the size of an antenna unit
building block: experimental and theoretical studies
Andrey Yakovlev, Alexandra Taisova, Vladimir Shuvalov, Alexander Arutyunian, and Zoya Fetisova

S8.12 Improving resistance to acute UV stress in *Synechocystis* sp. PCC 6803
using rescue media derived from *Deinococcus radiodurans*
Thomas Friedrich

S8.13 Fluorescence procedures to assess the photosynthetic
resilience in scots pines after a surface fire
Irina Gette, Nina Pakharkova, and Ivan Kosov

S8.14 Assessment of the physiology state of photosynthetic
machinery and plant vitality by JIP-test and PAM-fluorometry
Vasilij N. Goltsev and Momchil Paunov

S8.15 Identification of nutrients deficiencies in growth medium of
Phaseolus vulgaris by chlorophyll fluorescence methods: application
of artificial neural networks as a tool for rapid recognition
Vasilij N. Goltsev, Vladimir Aleksandrov, Momchil Paunov, Violeta B. Velikova, Tsonko D. Tsonev, and Hazem M. Kalaji

S8.16 The impact of the environmental factors on
the photosynthetic activity of common pine (*Pinus sylvestris* L.)
in spring and in autumn in the region of Eastern Siberia
Natalya Korotaeva, Maria Ivanova, Galina Suvorova,
and Gennadii Borovskii

S8.17 Early detection of sulphur deficiency in radish plants
Izabela A. Samborska, Leszek Sieczko, Vasilij N. Goltsev, and Hazem M. Kalaji

S8.18 Potassium-deficiency induced changes in
electron transport chain of radish
Magdalena D. Cetner, Vasilij N. Goltsev, Katarzyna Kowalczyk,
and Hazem M. Kalaji

S8.19 Machine learning methods in detection of
nutrient deficiency of in winter rape
Hazem M. Kalaji, Izabela A. Samborska, Magdalena D. Cetner, Urszula Piszcz, Krzysztof Gediga, Krzysztof Bielecki, Kamila Karmowska, and Wojciech Bąba

S8.20 Spectral multi-exponential approximation of
the chlorophyll *a* fluorescence transient allows early detection
of stress caused by nitrogen or sulfur deprivation
Sergei S. Khruschev, Tatiana Plyusnina, Ivan V. Konyukhov, Elena N. Voronova, Taras K. Antal, Alena Volgusheva, Galina Riznichenko, and Andrey B. Rubin

S8.21 The effect of phytochrome A and B deficiency on the photosynthetic processes in *Arabidopsis thaliana*
Aleksandra Khudyakova, Aleksander Shmarev, Galina Shirshikova, and Vladimir Kreslavski

S8.22 Signaling forms of the orange carotenoid protein
Konstantin Klementiev, Evgeniy Maksimov, M. Moldenhauer, E. A. Shirshin, E. A. Parshina, N. N. Sluchanko, Georgy Tsoraev, Franz-Josef Schmitt, Vladimir Z. Paschenko, Thomas Friedrich, and Andrey B. Rubin

S8.23 Photosystem II of contrasting silver fir provenances in response to heat stress
Alena Konôpková, Daniel Kurjak, Jaroslav Kmeť, and Dušan Gömöry

S8.24 Investigation of deleterious effects of chromium phytotoxicity on photosynthesis in wheat plant
Sonal Mathur and Anjana Jajoo

S8.25 Sll1276, Slr2019 paralog, is essential for acid stress tolerance in *Synechocystis* sp. PCC 6803
Ayumi Matsuhashi, Yutaro Ito, Kengo Matsushima, Mina Agatsuma, Junji Uchiyama, and Hisataka Ohta

S8.26 Differences of induction curves chlorophyll fluorescence of the applle fruits and of the leaves under the natural development
Marina Pikulenko, Alexander Bulychev, Anna Komarova, and Tamara Kumakhova

S8.27 Physiological state of selected beech population during peak of growing season
Eva Pšidová, Jana Majerová, Srdjan Stojnić, Ľubica Ditmarová, Marek Ježík, Katarína Střelcová, and Dušan Gömöry

S8.28 Influence of α-carbonic anhydrase 4 gene knockout on photosystem II light-harvesting antenna in *Arabidopsis thaliana*
Natalia Rudenko, Tatyana Fedorchuk, Elena Zhurikova, Lyudmila Ignatova, and Boris Ivanov

S8.29 New antimony(III) complexes as potent inhibitors of photosystem II, carbonic anhydrase, and glutathione reductase
Margarita Rodionova, M. S. Karacan, T. Tunc, K. B. Venedik, S. Mamas, Aleksandr Shitov, N. Karacan, Sergey Zharmukhamedov, Vyacheslav Klimov, and Suleyman I. Allakhverdiev

S8.30 Regulation of photosynthesis by oxylipins generated in allene oxide synthase and hydroperoxide lyase pathways
Tatyana Savchenko, Andrey Khorobrykh, Denis Yanykin, Vyacheslav Klimov, and Katayoon Dehesh

S8.31 Variation potential influences the resistance of photosynthetic machinery to the thermal stress in pea
Lyubov Surova, Vladimir Vodeneev, and Vladimir Sukhov

S8.32 Rearrangements of photosynthetic antenna units in response to light conditions are regulated by the extent of ROS production
Daria Vetoshkina, Marina Kozuleva, Boris Ivanov, and Maria Borisova-Mubarakshina

S8.33 Influence of narrow-band red and blue light on barley chloroplast ultrastructure
Daria Gorshkova, Tatiana Vlasova, Elizaveta Bassarskaya, Galina Kochetova, Tatiana Zhigalova, and Olga Avercheva

S8.34 Phenotyping of photosynthetic traits in lettuce: the limits and possibilities of chlorophyll fluorescence imaging in drought stress studies
Marek Živčák, Marián Brestič, Katarína Olšovská, and Klaudia Brücková

S8.35 Exploring the power of parallel measurements of electron transport, CO_2 and H_2O in plant leaves
Richard L. Garcia

JUNE 22

Section 1.7: Artificial and Applied aspects of Photosynthesis

S7.1 Energy efficiency of C4 plants by the example of *Zea mays* L. and *Miscanthus sinensis* Anderss. on gray forest soils of Moscow region, Russia
Gennadiy Bulatkin, Gennadiy Mitenko, and Ivan Guriev

S7.2 Microalgae and cyanobacteria: induction of lipids and screening promising strains
Nadezhda I. Chernova and Sophia V. Kiseleva

S7.3 Optimization of a photosystem 1 and 2 based photovoltaic cell
Volker Hartmann, Tobias Vöpel, Fangyuan Zhao, Felipe Conzuelo, Simon Ebbinghaus, Marc M. Nowaczyk, Nicolas Plumeré, Wolfgang Schuhmann, and Matthias Rögner

S7.4 Effects of grapevine leafroll assosiated virus 3 on the photosynthesis and antioxidant compounds in field grown grapevine (*Vitis vinifera* L.) plants
Irada Huseynova, Durna Aliyeva, Nargiz Sultanova, Nargiz Bayramova, Tofig Allahverdiyev, and Jalal Aliyev

S7.5 Measuring system for investigation of photosynthetic apparatus components-based bio-solar cells
Roman Voloshin, D. A. Gabrielyan, V. S. Bedbenov, Vladimir Kreslavski, Sergey Zharmukhamedov, and Suleyman I. Allakhverdiev

Section 1.9: Systems Biology of Photosynthesis:

S9.1 Peculiarities of acetate assimilation in purple non-sulfur bacterium *Rhodobacter capsulatus* B10
Ekaterina P. Petushkova and Anatoly Tsygankov

S9.2 Integrated model of primary photosynthetic and metabolic processes in algae cells
Tatiana Plyusnina, Galina Riznichenko, Andrey B. Rubin

Section 1.11: Emerging Techniques for Studying Photosynthesis

S11.1 Applying small angle scattering methods to investigate cyanobacterial thylakoid membranes
Dainius Jakubauskas, Poul Erik Jensen, Kell Mortensen, and Jacob Kirkensgaard

Section 2.1: Energy for the Future – Hydrogen economy

S1.1 Biohydrogen purification using metalhydride technologies
Dmitry Blinov, Vasily Borzenko, and Dmitry Dunikov

S1.2 Self-ignition of pressurized hydrogen diluted by methane
Sergey Golovastov, Vladimir Bocharnikov, and Anastasiia Samoilova

S1.3 Scale effect in LaNi5-based alloys used in bio-hydrogen purification and storage
Ivan Romanov and Anna Pykhtina

S1.4 Detonation mitigation in hydrogen-fueled spark ignition engine by adding low-energetic components
Victor Zaitchenko, Mikhail Ivanov, and Anna Smygalina

Section 2.2: Elevating Climate Change

S2.1 Torrefaction technology
Julia Kuzmina, George Sytchev, and Victor Zaitchenko

S2.2 Unconventional and renewable energy resources for sustainable arctic development
Maria Morgunova, and Dmitriy Solovjov

Section 2.3: Biological hydrogen production

S3.1 Ferredoxin–hydrogenase fusion protein successfully diverts the photosynthetic electron flux towards hydrogen production *in vivo*
Haviva Eilenberg and Iftach Yacoby

S3.2 Photosynthesis and hydrogen photoproduction by the cyanobacterium *Anabaena* sp. 7120 and its mutants with modified nitrogenase under different light and temperature
Anastasia Gavrisheva, Hajime Masukawa, Masaharu Kitashima, Hidehiro Sakurai, Kazuhito Inoue, and Anatoly Tsygankov

S3.3 Advantages of mixed carbon fermentation in biological hydrogen production by *Rhodobacter sphaeroides*
Lilit Hakobyan, Lilit Gabrielyan, and Armen Trchounian

S3.4 Hydrogen photoproduction by Hup− mutant of *Rubruvivax gelatinosus* RL2 under microaerobic conditions
Tatyana Laurinavichene, Kenji V. P. Nagashima, Takeshi Sato, Kazuhito Inoue, and Anatoly Tsygankov

S3.5 Two-stage thermal conversion of biomass into hydrogen-containing gas mixture
Vladimir Lavrenov and Victor Zaitchenko

S3.6 Novel approaches to simultaneously combat the oxygen sensitivity of hydrogenase and its poor electron acceptance
Milrad Yuval and Yacoby Iftach

S3.7 The *in vitro* enhancment of [FeFe] hydrogenase activity by [Fe] superoxide dismutase
Oren Ben-Zvi and Iftach Yacoby

Section 2.4: Hydrogenases

S4.1 Peptides for immobilizing HydSL hydrogenase from *Thiocapsa roseopersicina*
Azat V. Abdullatypov, Sergey S Kiselev, Nikolay A. Zorin, and Anatoly Tsygankov

S4.2 Long-term storage of *Thiocapsa roseopersicina* BBS
Liliya Koshkarova, Andrey Shestakov, and Alexander Netrusov

S4.3 Inactivation of termostable HydSL hydrogenase from *Thiocapsa roseopersicina* by cyanide
Nikolay A. Zorin, Aleksey Zabelin, Anatoly Shkuropatov, and Anatoly Tsygankov

Part 1.
Photosynthesis Research for Sustainability

Section 1.1: Primary Processes of Photosynthesis

Lecture 1

Problems of biophysics and mechanisms of primary photosynthetic reactions

Andrey B. Rubin

Department of biophysics, Biological faculty, Lomonosov Moscow State University, Moscow

The problems of electron transfer in photosynthetic reaction centers are discussed with the respect to the electron-conformational interaction as the basis of functional activity of bio-macromolecules. The special emphasis is on the role of hydrogen bonds as possible regulators of electron transfer. Mathematical modeling of electron transfer kinetics provides data to discuss the applicability of classical kinetic approaches to understand dynamic regulation in biological systems at the molecular level. As examples photo-induced transitions in the OCP, cytochrome-plastocyanin interactions, hydrogen evolution mechanisms in algae will be presented.

Lecture 2

Half century of scientific wandering – Freedom, Serendipity and Joy

Nathan Nelson

Department of Biochemistry and Molecular Biology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Even in my BSc studies in TAU, more than half a century ago, I was fascinated by the structure and function of membrane proteins. Working for my PhD under the supervision of Joseph Neumann, I was captured by the marvel of photosynthetic research. My post-doctoral mentor Efraim Racker prompted me to quench my curiosity in the whole bio-energetic field, which I actually researched ever since. Firstly in the structure and function of photosynthetic membrane complexes (where I unraveled features of three out of the four); in the lab of Jeff Schatz we demonstrated the need of ATP for protein import into mitochondria; then in RIMB, to where I was brought by Ronald Kaback, I had 10 exciting years of molecular biology studies. I experienced the golden era of discoveries in the fields of Vacuolar-ATPase, metal ion transporters; we pioneered also in neurotransmitter transporters research as well as molecular biology of photosynthesis. I thought my research climaxed in this golden era, but then I came back to TAU in 1995 and there Adam Ben Shem became my student and with him I returned to structural biology, which now proved to me the jewel in the crown. My current research focuses on harnessing oxygenic photosynthesis for sustainable energy production.

Lecture 3

Structural constraints for excitation energy migration and trapping in photosynthetic bacteria

Manoop Chenchiliyan[1], Kõu Timpmann[1], and Arvi Freiberg[1,2]*

1 – Institute of Physics, University of Tartu, Tartu, Estonia
2 – Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia
* E-mail: arvi.freiberg@ut.ee

In photosynthesis solar photons are converted into molecular excitations in the so-called antenna pigment-protein complexes and the absorbed energy is eventually transported to the reaction center pigment-protein complex, where a conversion of the excitation energy into chemical energy occurs. The light harvesting efficiency is considered to be significantly enhanced by exciton interactions present between the pigments of the antenna complexes, which broadens their energy spectrum and also, by virtue of forming a suitable energetic ladder of exciton states, allows ultrafast energy diffusion and transfer in the photosynthetic membrane. Recent advances in atomic force microscopy combined with innovative synthetic biochemistry have provided evidence for nanoscale structural adaptation of photosynthetic membranes in response to changing habitats. Examples include mutable stoichiometry of the antenna and reaction center complexes with the light intensity experienced during the membrane development as well as varying architecture of the protein complexes such as dimeric and monomeric core complexes.

Here, we report on the following fundamental question: how the nanoscale structural remodeling of the photosynthetic membrane influence the rate of the delivery of photo-excitations into the reaction center traps and the efficiency of charge separation at the reaction centers. The answer was sought by analyzing steady state and picosecond time-resolved fluorescence spectra of photosynthetic membranes extracted from differently treated purple bacteria *Rhodobacter sphaeroides*. The data [1–3] imply a robust photosynthetic apparatus that functions surprisingly effectively under a wide variety of conditions.

1. K. Timpmann, M. Chenchiliyan, E. Jalviste, J.A. Timney, C.N. Hunter, A. Freiberg. Efficiency of light harvesting in a photosynthetic bacterium adapted to different levels of light. Biochim. Biophys. Acta, 1837, 1835 (2014)
2. M. Chenchiliyan, K. Timpmann, E. Jalviste, P.G. Adams, C.N. Hunter, A. Freiberg. Dimerization of core complexes as an efficient strategy for energy trapping in Rhodobacter sphaeroides. Biochim. Biophys. Acta, 1857, 634 (2016)
3. A. Freiberg, M. Chenchiliyan, M. Rätsep, K. Timpmann. Spectral and kinetic effects accompanying the assembly of core complexes of purple photosynthetic bacteria. Biochim. Biophys. Acta (2016), submitted

Lecture 4

Towards efficient photosynthesis: overexpression of C4 enzymes in C3 plants: a case study

Deepika Kondoi[1], Baishnab C. Tripathy[1], and Govindjee[2]*

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Ort et al. (Proc Natl Acad Sci USA 112 (28): 8529–8536, 2015) have beautifully presented various possible scenarios for redesigning photosynthesis to meet global food and bioenergy demand. Here, we shall discuss only one aspect. C4 plants, such as maize, where the primary carboxylation step, during carbon fixation, is the addition of HCO_3^- to a C3 intermediate, to make a C4 compound, are, under many, but not all, conditions, much more efficient in overall photosynthesis than are C3 plants, such as rice, where addition of CO_2 is on to a 5-C intermediate making 2 molecules of a C3 compound, and, hence the name C3 and C4 (see G. T. Edwards and D. Walker, 1983, Blackwell Science; A. S. Raghavendra and R. F. Sage (eds), Advances in Photosynthesis., Vol. 32, 2011, Springer; also see: Kubásek, et al., Physiol Plant 149: 528–539, 2013). Further, C4 plants, as compared to C3 plants, have higher solar radiation utilization efficiency, higher water use efficiency, and are tolerant to both water stress and salt stress. Thus, to improve sustainability, attempts are being made all over the World to introduce C4-like pathway into C3 plants (for a review, see Miyao et al., J Exp Bot 62:3021–3029, 2011). There are promising results, but there are problems. Although transgenic rice plants, which expressed C4 specific carboxylase at a high level, had a decreased sensitivity to inhibition by O_2, yet there was no improvement in the photosynthetic efficiency of the overexpressors (see e.g., Fukuyama et al., Photosynth Res 77 (2–3): 227–239, 2003). Here, we will review the available data on attempts made, thus far, for introducing C4 genes and pathways (into C3 plants), as well as problems and issues related to it. Our presentation will focus on the results of Kandoi et al. (Photosynth Res, 27 pages; DOI 10.1007/s11120-016-0224-3, 2016) on molecular characterization, pigment and protein content, chlorophyll *a* fluorescence, photosynthesis (electron transport, CO_2 fixation), and non photochemical quenching of chlorophyll *a* excited state, in the Phospho-Enol Pyruvate Carboxylase (PEPC) overexpressing transgenic plants of *Arabidopsis thaliana*; our results clearly show improved photosynthetic efficiency and tolerance to salt stress in the transgenic plants. In the transgenics, we had increased amino acid and protein synthesis, increased chlorophyll, increased photosynthesis (electron transport and CO_2 fixation), increased respiration, and increased starch content and dry weight, but lower non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence. Significantly, these transgenics were tolerant to high salt. We thank Lars Olof Björn for reading our abstract before its submission.

Lecture 5

Differences in light-harvesting and energy-transfer processes under different growth conditions

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Photosynthetic organisms regulate their primary process of photosynthesis to respond to changes in environmental conditions, such as light quality, light quantity, nutrient, and so on. Pigment compositions alter in response to light conditions. In the cyanobacterium *Anacystis nidulans* (*Synechococcus* sp.), the content ratio of phycocyanin to chlorophyll *a* decreased under strong orange light, and increased under strong red light [1]. Nutrient conditions also affect pigment contents; nitrogen- or phosphorus-deficiency induced degradation of phycobilisome in cyanobacterial cells [2, 3]. The nutrient stresses induced changes in the energy transfers within phycobilisome, from phycobilisome to photosystems, within photosystem II, and from photosystem II to photosystem I [3]. We will discuss differences in light-harvesting and energy-transfer processes under different environmental conditions, by means of time-resolved fluorescence spectroscopy.

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Lecture 6

Phosphorescence studies of the pigment apparatus of plants

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The studies of author's group on phosphorescence of triplet chlorophylls and chlorophyll-sensitized phosphorescence of singlet oxygen are briefly outlined. Phosphorescence of chlorophylls was detected at 77 K. It provides direct information on the energy and lifetimes of the pigment triplet states and allows for estimation of the rate constants of radiative deactivation of the triplet states and on the quantum yields of their population. Comparison with low-temperature fluorescence shows the energies of singlet-triplet splitting and relative yields of these light emissions. All parameters are very sensitive to environment, solvation and aggregation of pigment molecules. Similar phosphorescence is detected in green plants and algae. The strongest chlorophyll phosphorescence appeared in etiolated leaves at early stage of greening, in isolated complexes of PS II reaction centers and in the herbicide-treated leaves with blocked carotenoid biosynthesis. In normal mature leaves phosphorescence is 10^2–10^3 times weaker. It is emitted by the triplets of the short-wavelength forms of Chl *a*, which are coupled with the accessory antenna pigments. The intensity of phosphorescence at 77 K was shown to correlate with the rates of chloroplast damage at room temperature. The photosensitized phosphorescence of singlet oxygen is observed at room temperature in air-saturated pigment solutions in organic solvents, aqueous detergent and liposome dispersions and also in certain plant materials. It provides information on the quantum yields of singlet oxygen production and quenching of singlet oxygen by components of the photosynthetic apparatus. Combination of the obtained data allows for formulation of reasonable hypothesis on the mechanisms and efficiency of singlet oxygen production in the photosynthetic apparatus and on the work of protection systems in plants. Detailed information is given in recent papers cited below and in references therein.

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Lecture 7

Ultrafast charge separation events in Photosystem I from *Synechocystis* sp. PCC 6803 under excitation into the QY band red edge: the mechanism of long-wavelength limit of photochemical energy conversion in Photosystem I

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The energy and electron transfer in photosystem I (PS I) from cyanobacteria *Synechocystis* sp. PCC 6803 was studied by femtosecond pump-probe absorption spectroscopy. The excitation was performed using ~25-fs laser pulses centered at 720 nm, 740 nm, and 760 nm. The transient spectra induced by the femtosecond pump pulse were detected in the time delay window from 20 ps to 500 ps.

The main goal of the study was to clarify two important issues: 1) whether the formation of the primary and the secondary ion-radical pairs $P700^+A_0^-A_1$ and $P700^+A_0A_1^-$ in PS I can be formed under single-photon absorption at the far red edge of Q_Y band?; 2) what is a lifetime of the primary ion-radical $P700^+A_0^-A_1$ formation?

The effect of the pump power on the transient spectra was studied in detail. Transient spectra depend on the pump power. Detailed analysis of transient spectra shape as a function of the pump power energy shows that under red light excitation two channels of PS I absorption can be realized: linear single photon absorption and nonlinear multiphoton absorption. The linear single photon absorption leads to the appearance of specific spectral features in the transient spectra. These spectral features correspond to the transient spectra of $P700^+A_0^-A_1$ and $P700^+A_0A_1^-$ pairs. The analysis of transient spectra corresponding to single photon absorption at short time delays reveals that formation of $P700^+A_0^-A_1$ occurs within 100 fs. The formation of the secondary radical pair $P700^+A_0A_1^-$ due to electron transfer between A_0 and A_1 has characteristic time close to 20 ps. The obtained femtosecond laser spectroscopy data evidences that the ultrafast formation of primary $P700^+A_0^-A_1$ ion-radical pair in PS I from *Synechocystis* sp. PCC 6803 occurs with characteristic time less than 100 fs. This process can be due to single photon absorption by reaction center of PS I even when the reaction center of PS I is excited by low-energy photons into the far-red edge of Q_Y absorption band.

The work was supported by the Russian Science Foundation (grant 14-14-00789)

Lecture 8

Chlorophyll triplet state in isolated PS II reaction centers and core complexes: low-temperature phosphorescence studies

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Low-temperature (77 K) phosphorescence measurements were performed to study the triplet state of chlorophyll *a* (^{3}Chl) in photosystem II (PS II) reaction centers (D1D2cytb559 particles, RCs) and PS II core complexes isolated from spinach. Applying this technique, ^{3}Chl phosphorescence was detected in the RCs as well as in core complexes with doubly reduced primary quinone acceptor Q_A. The spectral parameters of Chl phosphorescence were comparable for both types of PS II preparations. The phosphorescence emission band was characterized by the main maximum at 952–955 nm (1.30 eV) and lifetime of 1.5–1.6 ms [1, 2]. The excitation spectra of the phosphorescence show maxima corresponding to the absorption bands of Chl, pheophytin *a* and β-carotene. The relative quantum yield of the Chl phosphorescence in RCs was equal to that of monomeric Chl in aqueous detergent dispersions, while in the case of core complexes with doubly reduced Q_A the yield decreased for one order of magnitude. The Chl phosphorescence was very sensitive to the redox state of PS II preparations and significantly went down (i) in the frozen RC samples containing either silicomolybdate (causing photoaccumulation of P_{680}^{+}Pheo state) or sodium dithionite (causing photoaccumulation of P_{680}Pheo^{-} state) [1] and (ii) in core complexes with oxidized Q_A [2]. Based on our data, we may conclude that the Chl triplet state responsible for the phosphorescence is generated due to charge recombination in the reaction center radical pair $P680^{+}$ $Pheo_{D1}^{-}$. Presumable localization of this Chl triplet state on one of RC cofactors will be discussed.

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Lecture 9

Trehalose effects on real-time kinetics of electrogenic reactions due to catalytic cycle of water oxidizing complex of Photosystem II

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The use of osmolytes in the stabilization of biomolecules is an old trick of Nature. Among these, in recent years trehalose, which is naturally produced by several species of eubacteria, archaea, some fungi, certain invertebrates, and plants, has received considerable attention.

Pigment-protein complex of photosystem II (PS II) mediates photoinduced oxidation of water to molecular oxygen at the lumenal side and reduction of plastoquinone to plastohydroquinone on the stromal side of the thylakoid membrane of oxygenic photosynthetic organisms. Asymmetric orientation of PS II core particles in liposomes (donor side location at the exterior of the proteoliposomes) allows to study the effect of added disaccharide trehalose on the electrogenic charge transfer at the water oxidizing side by direct electrometrical technique. Addition of trehalose to assay medium resulted in acceleration of the kinetics of electrogenic proton transport during S2→S3, and S4→S0 transitions, while it had no effect on the kinetics of S1→S2 transition (transfer of an electron from Mn to $Y_Z^{\bullet}$) of the water oxidizing complex induced by the 2nd, 3rd and 1st laser flashes, respectively in dark-adapted PS II samples. The data obtained suggest that trehalose keeps the water-oxidizing complex in a more optimal conformation for effective functioning.

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Lecture 10

Thylakoid model parameters fitted to photosynthetic induction data

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The light-dependent induction of the photosynthetic apparatus involves a series of electron and proton transfers. Electron fluxes through PS II and PS I can be estimated *in vivo* using Chl fluorescence (FL) and absorption spectroscopy measurements [1, 2]. The fluorescence induction (FI) and $P700^+$ oxidoreduction changes (ΔA_{810}) were monitored (at PFD 200 μmol photons m^{-2} s^{-1}) on dark-adapted pea leaves during the 20 s time range. The elaborated Thylakoid Model (TM) was improved compared to [3], which allowed fitting to both FI and ΔA_{810} data. The two-wave kinetics of O(JI)PSMT and OABCD were reproduced in their parallel and antiparallel phases due to the TM parameter quantification.

The PS II model developed earlier [4–6] was incorporated into the TM reaction scheme. Estimation of the antenna and reaction center dissipative losses allowed quantitative simulations of the variable FL levels at both the fast OJIP and slow SMT kinetic stages. Under assumption that 20% of F_0 is attributed to PS I, the TM was fitted to FI and ΔA_{810} data, while $\Delta\Psi(t)$ and pHL/S(t) dynamics was found physiologically relevant [1, 2, 5]. The increase of the buffer capacity in the model from 43 to 81 mM elevated the peak $\Delta\Psi(t)$ from 8 to 15 mV at reasonable ranges of H^+, K^+, Cl^- fluxes.

The previous TM simulations that assumed the unvaried activation state of FNR and stromal enzyme were refined. We introduced the time-dependent rate constant to model the $NADP^+$ reduction by ferredoxin supposing the FNR activation effect in the time domain of 20–40 s after the start of a low intensity illumination. Transitions between the linear and cyclic modes, concurrent with ATPase operation can be clarified over a wide time-scale due to quantitative analysis of the light induction stages preceding the stationary state.

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Poster 1

Light-harvesting complexes from purple sulfur bacteria with modified *in vitro* carotenoid composition

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Carotenoid biosynthesis in the cells of purple sulfur bacteria *Allochromatium minutissimum* and *Ectothiorhodospira haloalkaliphila* was inhibited using diphenylamine as previously reported [1]. Some carotenoids (ζ-carotene, rhodopin, spheroidene, spheroidenone, etc.) with different number of conjugated double bonds from 7 to 13 and various side-chain substituents were incorporated *in vitro* into the membranes (pigment-protein complexes) from these cells. The efficiency of carotenoid incorporation was from 40 to 100%. The distribution of carotenoids between the light-harvesting complexes LH1-RC and LH2 was studied. The analysis of fluorescence excitation spectra of complexes with incorporated carotenoids showed that carotenoids restore the ability to transfer energy to bacteriochlorophyll. Thermal stability and photostability of the LH2 complexes obtained by irradiation of blue-green light were studied. It was shown that some carotenoids initiated bacteriochlorophyll photooxidation with different efficiencies.

This work was supported by Russian Foundation for Basic Research (grant 15-04-02660a).

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Poster 2

Effect of light absorbed by carotenoids on growth of purple sulfur bacterium *Alc. vinosum*

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The effect of the intensity of light with different spectral composition on the growth of *Alc. vinosum* cells was estimated. Different types of LH2 complexes (B800-850, B800-840, and B800-820) were assembled in these cells in response to changes in light intensity. *Alc. vinosum* cells were grown on red (≥640 nm) and blue-green (430–585 nm) light with intensity of 0.4, 4 and 6 W/m^2. It was shown that blue-green light (0.4 W/m^2) inhibits cell growth in contrast to red light. These results are consistent with the known data that cells of some species of bacteria are not capable to growth at the illumination in carotenoid absorption region [1]. We assumed that this effect may be related to the ability of carotenoids to selectively sensitize the BChl850 photooxidation [2]. In other cases (red (0.4 W/m^2) or red and blue-green light (4 and 6 W/m^2), it was noted that cells grew with different rates. In these conditions B800-820 and B800-840 complexes was assembled, but complex B800-850 was not found. The analysis of carotenoid composition of these complexes was performed. Carotenoid composition of these complexes was similar. We studied the selectively sensitized photooxidation of longwave forms of BChl at the exposure these complexes to blue-green light. The hypothesis that the light absorbed by carotenoids inhibits growth of *Alc. vinosum* cells was not confirmed.

This work was supported by Russian Foundation for Basic Research (grant 15-04-02660a).

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Poster 3

Femtosecond processes of charge separation in two mutant reaction centers of *Rhodobacter sphaeroides* with increased midpoint potential at cryogenic temperature

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Despite the advances made in the study of photosynthetic reaction centers of purple bacteria, the mechanism of their functioning at the molecular level is not fully elucidated. This is especially true of studies of very early stages of light energy conversion in reaction centers (RCs) occurring in the femtosecond time range. A key element of the photosynthetic reaction center is a primary electron donor, which is a dimer of bacteriochlorophyll (BChl) molecules oriented to face each other, so-called special pair P.

Studies of 2 mutant reaction centers with similar increase in midpoint potential of the primary electron donor P were performed by using femtosecond absorption spectroscopy. In M197FH (Phe→His) and M160LH (Leu→His)+L131LH (Leu→His) mutant RCs redox potential of the primary donor increases by ~125 mV and by ~130 mV respectively. RCs were excited with 40 fs pulses at 890 nm at 90 K. Despite such a drastic increase in redox potential of P formation of a radical anion band of monomeric BChl B_A at 1025 nm was observed in transient absorbance difference spectra of both mutants. Having similar redox potential of P charge transfer dynamics was very different in two mutants. In M197FH RCs $P+B_A^-$ and $P+H_A^-$ states were formed in 3.3 ps and 260 ps respectively. In M160LH+L131LH RCs this values were 110 ps and 600 ps. Moreover amplitude of H_A^- band at 955 nm in M197FH mutant RCs was very low compared to amplitude of B_A^-. This discrepancy in electron transfer rates in two mutants and especially unexpectedly fast electron transfer from P* to B_A in M197FH mutant may be due to possible effect of M197FH amino acid replacement on B_A redox potential which may lead to stabilization of electron on B_A^-.

This work was supported by the RFBR project 16-34-00829.

Poster 4

Comparison of the recombination rate of charges and lifetime of tryptophan fluorescence in RCs of *Rb. sphaeroides* in the temperature range from −180 to 25°C

Petr Knox, E. P. Lukashev, B. N. Korvatovskii, V. V. Gorokhov, N. P. Grishanova, N. Kh. Seifullina, and Vladimir Z. Paschenko*

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Ion-radical pair $P870^{\bullet+}Q_A^{\bullet-}$ in the reaction centers (RC) structure is the source of the electric field influence on the charged groups of the protein, changing their spatial orientation. Indirectly this influence should be manifested in the fluorescence characteristics of tryptophan residues, which are internal indicator of the dynamic state of the RC protein.

In this investigation the temperature dependence of the recombination rate of charges separated between the photoactive bacteriochlorophyll P and primary quinone acceptor Q_A in the photosynthetic RC from purple bacteria *Rhodobacter sphaeroides* were studied. Recombination kinetics were measured in the individual absorption bands of the donor (600 nm) and an electron acceptor (335 and 450 nm) for the RC in the water-glycerol and trehalose environment. Before measurements the preparations were frozen to −180°C in the dark and under activating light, then in the heating process the T-dependence of the recombination rate was measured.

In similar conditions the fluorescence lifetime of tryptophanils (λ_{reg}=315 and 345 nm) were registered. It was established that the recombination rate constant in the RC preparations dissolved in water-glycerol medium and frozen in the dark when measured at 600 and 450 nm is equal. On the other hand in the RC with the isotopic substitution of H_2O for D_2O the recombination kinetics in the band of 450 nm was slower than that in the 600 nm band. In preparations frozen in the light of this difference we did not observe. A correlation was found between the temperature dependence of the recombination rate and lifetime of the tryptophanil fluorescence in RC protein complexes in different solvents. Additionally differences in the average lifetime (τ_m) of tryptophanils fluorescence in RC preparations frozen in the dark or under activating light were measured. These results are explained due to RC transitions between different conformational states as well as by processes of proton relaxation in the structure of the hydrogen bonds in the environment of RC cofactors.

Poster 5

Comparison of the recombination rate of charges and lifetime of tryptophan fluorescence in RCs of *Rb. sphaeroides* in the temperature range from −180 to 25°C

Petr Knox, E. P. Lukashev, B. N. Korvatovskii, V. V. Gorokhov, N. P. Grishanova, N. Kh. Seifullina, and Vladimir Z. Paschenko*

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Poster 6

Two-photon spectroscopy of the LH1 complex and its subunit B820

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Comparative experimental study of the exciton structure of the LH1 complex and its subunit B820 was carried out. We used chromatophores from *Rhodospirillum rubrum* cells (carless mutant G9). These chromatophores contain native LH1 complex in the form of an ensemble with the reaction center (LH1-RC). These chromatophores also were used to isolate the B820 subunit. The measurements were performed by two-photon pump-probe spectroscopy. Samples in the rotating 2 mm cell were excited by 70 fs pulses with a wavelength of 1290 nm and a frequency of 1 kHz. Photoinduced absorption changes were recorded in the spectral range between 780–1020 nm and the time delay of the probe pulse relative to the pump pulse between (−1.5)–11 ps. All measurements were performed at room temperature. Two-photon excitation caused bleaching of exciton bands of the circular aggregate of LH1 complex (k=0, k=±1) as it was shown earlier [1]. In the case of B820 subunit two-photon excitation within 1200–1400 range did not cause absorption changes in the spectral range of 780–1020 nm.

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Poster 7

Can LH1 complexes of purple bacteria be assembled with partial filling of carotenoid pockets?

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B820 subunits from purple sulfur bacterium *Ectothiorhodospira haloalkaliphila* ATCC 51935^T strain were obtained by treatment of carotenoid free LH1-RC complexes of this bacterium with β-octylglucopyranoside (β-OG). The same complexes with 100% carotenoid content were not able to dissociate to B820 subunits, but disintegrate to monomeric bacteriochlorophyll (BChl) regardless of their carotenoid composition. The degree of dissociation of the LH1-RC complexes with an intermediate content of carotenoids with the B820 formation was in inverse negative relationship to the amount of carotenoids in the samples. B820 subunits did not contain carotenoids. B820 subunits were easily aggregated to form a complex with an absorption peak at 880 nm with a decrease of β-OG concentration. Analysis of the spectra of LH1-RC complexes isolated from cells with different levels of carotenogenesis inhibition, led to the conclusion of the heterogeneity of the samples with a predominance in them a) the fraction with 100% of carotenoids and b) the fraction carotenoid free complexes. The obtained results are in good agreement with the data on heterogeneity of LH2 complexes, isolated from cells with different levels of inhibition carotenogenesis. These data were obtained by heat treatment of LH2 complexes [1, 2]. In turn, the coincidence of the results may point to the fundamental principles of the assembly of light-harvesting complexes in the cells of photosynthetic bacteria.

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Poster 8

Coherent intradimer events in reaction centers of *Rhodobacter sphaeroides*

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By applying the Redfield theory and linear susceptibility theory to a system of one ground and two different excited states, we modeled the coherent dynamics of the primary electron donor, bacteriochlorophyll dimer P, in *Rhodobacter sphaeroides* reaction centers. We calculated time-resolved spectral profiles of the stimulated emission from the excited state of P for the case of the femtosecond broad-band optical excitation of P. The model showed the possibility of the extremely fast transfer of both the coherence and the population from the locally excited state (P_1^*) to the spectrally different, optically dark excited state (P_2^*). This transfer is clearly seen in the kinetics of the stimulated emission at 870 and 960 nm, where mostly P_1^* and P_2^* states are presented, respectively. Our calculations are in accordance with experimental results. The assumption about the existence of the second excited state P_2^* helps to explain the complicated temporal behavior of the ΔA spectrum measured by pump-probe spectroscopy. In spite of a pronounced electronic coupling between the P_1^* and P_2^* states assumed in our model, the form of the coherent oscillations is mainly defined by pure vibrational coherence in the excited states. A possible nature of the P_2^* state is discussed.

Poster 9

Study of the spectral and electron-transfer properties of *Rhodobater sphaeroides* R-26 reaction centers under vacuum conditions

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The photosynthetic reaction center (RC) is an integral membrane pigment-protein complex that transforms energy of light quanta into electrochemical energy of charge-separated states via a series of ultrafast electron-transfer reactions. Study of RCs in vacuum is of great interest in connection with (i) elucidation of the role of tightly bound water molecules in microenvironment of pigment cofactors and (ii) with attempts to use RCs as a photoactive material in solid state bioelectronic devices. During devise fabrication it is necessary to preserve structural integrity of RCs placed in harsh vacuum conditions. We have studied the ability of detergents LDAO, Triton X100 and n-dodecyl-β-D-maltoside (DM) commonly used for solubilization of photosynthetic membrane proteins to stabilize *Rhodobacter* (*Rb.*) *sphaeroides* R-26 RCs upon dehydration in vacuum (10^{-2}–10^{-5} Torr). Structural and functional characteristics of the vacuum-dried RC films were determined by visible-NIR, mid-IR light-induced FTIR and femtosecond transient absorption spectroscopies. It was found that mild nonionic detergent DM provided the most friendly medium to maintain the original spectral and vibrational properties of RC in films exposed to vacuum drying. Time-resolved results showed that the kinetics of primary electron transfer in the *Rb. sphaeroides* R-26 RC films vacuum-dried in the presence of high DM concentrations were virtually identical to those in the DM-solubilized RCs in a buffered aqueous solution.

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

Lecture 1

On the mechanism of state transitions: redox- and structure- dependent interaction *in vitro* between Stt7 kinase and the cytochrome b_6f complex

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A trans-membrane signaling mechanism involving a serine-threonine kinase (Stt7) contributes to regulation of the distribution of light energy between the two photosystems of oxygenic photosynthesis (e. g., Lemeille, S. et al., (2009) Plos Biol 7, 664–675). Plastoquinol oxidation mediated by the cytochrome b_6f complex on the electrochemically positive (p) side of the thylakoid membrane is known to activate the kinase domain of Stt7 on the negative (n) side of the membrane, which leads to phosphorylation and redistribution ("state transition") of the major light-harvesting chlorophyll proteins between the photosystems. However, a molecular description of the Stt7 kinase and its interaction with the cytochrome b_6f complex is unknown or unclear. In the present study, the Stt7 kinase has been cloned, expressed, and purified in an active state. The purified kinase is shown (a) to be active *in vitro* in the presence of reductant, and (b) purified as a tetramer with a molecular weight of 332 kDa, consisting of an 83.41 kDa monomer, as determined by analytical ultracentrifugation and electrospray-ionization mass spectrometry. Far-UV circular dichroism spectra show (i) Stt7 to be mostly α-helical, and (ii) describe a structure-based interaction between the kinase and b_6f complex through increased thermal stability of the α-helical structure of Stt7 in the presence of the b_6f complex. These studies document a significant interaction between Stt7 and the b_6f complex, and a requirement of reducing conditions for activity of purified kinase. It is hypothesized that kinase activation occurs through reduction of the Stt7 p-side disulfide by superoxide previously shown (Baniulis et al. (2013) Biochemistry, 52: 8975–8983) to be generated at relatively high levels by plastoquinol redox reactions in the b_6f complex.

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Lecture 2

Bicarbonate-reversible inhibition of the iron-quinone acceptor complex of photosystem II lacking low-molecular-weight proteins or with targeted mutations to the D1 protein

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The chlorophyll-binding CP47 pre-assembly complex of Photosystem II (PSII) is associated with up to six low-molecular-weight proteins: PsbH, PsbL, PsbM, PsbT, PsbX and PsbY. We have used gene knockouts in *Synechocystis* sp. PCC 6803 to create a series of mutants to investigate the function of these proteins. In this presentation we will highlight novel findings surrounding the role of PsbT in stabilizing the bicarbonate ligand to the non-heme iron of PSII and we will present new data on the function of PsbH, PsbX and PsbY. These observations include the existence of a stable population of the reduced primary plastoquinone electron acceptor, Q_A^-, in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea in cells lacking PsbH indicating that the removal of this protein can substantially modify back reactions with the S_2 oxidation state of the oxygen-evolving complex. Our data also suggest a role for these proteins in cyclic electron transfer around PSII which may be important in protecting against photodamage to nascent PSII complexes during assembly. We have also targeted residues on the D1 protein that stabilize bicarbonate and that suggest this cofactor may play a role in the susceptibility of PSII to photodamage as well as contributing to the function of the iron-plastoquinone acceptor complex.

LECTURE 3

SMALL RESIDUES CONTROL PROTEIN-GATED ELECTRON TRANSFER IN PHOTOSYNTHETIC REACTION CENTERS

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The inter-quinone electron-transfer (ET) from the oxidizing to the reducing side of isolated reaction centers (RCs) of photosystem II (PSII-RC) and non-oxygenic bacteria is protein-gated. The gating frequency, k, between ET-active and inactive protein conformations, is given by $\ln(k/T)=f(1/T)$ down to cryogenic temperatures. However, the mechanism remains unresolved and could not be explored in whole cells following experimental limitations.

Data presented in this lecture shows that the temperature cooling effect can be mimicked by increasing the volume of a single residue (V_{res}) at the PSII-RC subunits crossing in whole cells of cyanobacteria. More specifically, V_{res} controls the intersubunits interactions and thereby determine the frequency of attaining an ET-active conformation. Our findings rationalize the conservation of small-residue motif at the subunit crossing of Type II RC in both oxygenic and non-oxygenic organisms. The experimental approach and resolved mechanism allow to assess the significance of ET rates for the whole cell physiology and offers new means for exploring protein-gated reactions *in vivo*.

Lecture 4

Roles of non-bilayer lipids and non-lamellar lipid phases in the assembly and structural dynamics of thylakoid membranes

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In chloroplast thylakoid membranes, similar to all energy-converting membranes, non-bilayer lipids account for about half of the lipid content. It is generally believed that these lipids are constituents of the membrane bilayer, a lipid phase that is essential for building up and utilizing $\Delta\mu_H^+$, the transmembrane electrochemical potential gradient, and non-bilayer phases are formed only transiently. Non-bilayer lipids play key role in the operation of violaxanthin de-epoxidase. It has been hypothesized that non-bilayer lipids, because of their segregation capability, regulate the protein-to-lipid ratio of the membranes, and might provide additional structural flexibility to the thylakoid membrane (Garab et al. 2000 Trends Plant Sci). Via ^{31}P-NMR and steady state and time-resolved fluorescence measurements using merocyanine 540 on spinach thylakoid membranes as well as experiments on *dgd1* mutant Arabidopsis we have shown that non-bilayer lipid phase(s) and the bilayer phase of thylakoid membranes coexist and are in close association with each other (see Garab et al. 2016 in: Nakamura and Li-Beisson (eds.) Lipids in Plant and Algae Development, and references therein). Here, by applying co-solute treatments on thylakoid membranes, we substantiate the conclusions of the coexisting phases and support our membrane model which is suggesting a dynamic equilibrium between the bilayer and non-bilayer lipid phases.

Lecture 5

Evolution and function of the OEC family proteins in chloroplasts

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The oxygen-evolving complex (OEC) proteins are membrane-extrinsic subunits of photosystem (PS) II. In addition, multiple isoforms and homologs for OEC proteins have been found in the chloroplast thylakoid lumen and shown to have various roles in photosynthetic electron transfer [1]. This suggests that diversification of OEC family proteins would be important for chloroplasts, enabling them to regulate efficient photosynthesis in changing environments. The PsbP protein, a specific OEC protein in green plants, is known to concentrate Ca^{2+} and Cl^{-} in the OEC and also required for PS II core complex assembly. The interaction of PsbP within the PS II complex has been investigated using a chemical zero-length cross-linker and it was found that PsbP directly interacts with the membrane-intrinsic protein, PsbE, and also with PsbR, a protein we suggest is mostly membrane-extrinsic; in addition, PsbP interacts with the CP26 and CP43 light-harvesting proteins of the PSII-LHCII supercomplex [2, 3]. Furthermore, PsbP modulates the redox properties of Cytochrome b559 involved in the side electron transfer pathway within PS II [4]. Molecular evolution and specific role of PsbP in regulating the function of the PS II complex will be discussed.

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Lecture 6

Chlorophyll degradation by Mg-dechelatase

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Conversion of chlorophyll *a* to pheophytin *a* is the first step of chlorophyll degradation. Pheophytin is synthesized by extracting Mg from chlorophyll. Although most of the enzymes in the chlorophyll biosynthesis and degradation pathway have been reported, the enzyme responsible for this reaction, Mg-dechelatase, has not been identified yet. Mendel's green cotyledon gene, *STAY-GREEN* (*SGR*), is the candidate of Mg-dechelatase, because chlorophyll degradation is suppressed in plants defect in *SGR* gene. The *Arabidopsis* genome has three *SGR* genes, *SGR1*, *SGR2* and *STAY-GREEN LIKE* (*SGRL*). To examine the enzymatic activity of SGR in Mg removal from chlorophyll, we prepared *Arabidopsis* recombinant SGR1 and SGRL. After incubation of chlorophyll *a* with recombinant SGR1, pheophytin *a* was detected, suggesting that SGR is Mg-dechelatase. Recombinant *Arabidopsis* SGR1 did not extract Mg from chlorophyllide *a*. In contrast, recombinant *Arabidopsis* SGRL extracted Mg from both chlorophyll *a* and chlorophyllide *a*. Neither SGR1 nor SGRL could extract Mg from chlorophyll *b*. Enzymatic experiments using light-harvesting complexes isolated by sucrose density gradient centrifugation showed that SGR extracted Mg from chlorophyll in the chlorophyll protein complexes. Furthermore, when *Arabidopsis* SGR1 was expressed in the *Arabidopsis* leaves, most of the chlorophyll and chlorophyll-binding proteins disappeared. These observations suggest that SGR is not only involved in chlorophyll degradation but also contributes to photosystem degradation.

LECTURE 7

THE PHOTOSYNTHETIC CYTOCHROME c_{550} FROM THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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Cytochrome c_{550} (Cc_{550}) is a c-type heme protein with a bis-histidinyl axial coordination adscribed as an extrinsic component in the luminal side of photosystem II (PS II), although its role within this complex is not yet well established. Cc_{550} is present in cyanobacteria as well as in eukaryotic algae from the red photosynthetic lineage, which includes diatoms. However, the protein is absent in the green lineage, which comprises green algae and plants. We have here characterized the Cc_{550} from the diatom *Phaeodactylum tricornutum*. Cc_{550} is mostly obtained from the soluble cell extract in relatively large amounts. In addition, the protein appeared to be truncated in the last hydrophobic residues of the C-terminal, as deduced by MS analysis and the comparison with the gene sequence. Interestingly, in cyanobacteria it has been described that the C-terminus of Cc_{550} forms a hydrophobic finger involved in the interaction with PS II. Cc_{550} was absent in solubilized PS II complex samples, that are not able to (re)incorporate the cytochrome after further incubation with an excess of the purified protein, thus indicating a low affinity of Cc_{550} for the PS II complex. Under iron-limiting conditions the amount of Cc_{550} decreases up to about 50% as compared to iron-replete cells, pointing to an iron-regulated production. Oxidized Cc_{550} has been further characterized using continuous wave (CW) EPR and pulse techniques, including ESEEM and HYSCORE, the results indicate a distortion of the heme-axial ligands and loss of the co-planarity of the imidazole ring, as well as relevant changes in the structure of the single occupied molecular orbital (SOMO) of the heme centre.

Lecture 8

Molecular model of PBS core and PBS association with photosystems

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The phycobilisome (PBS) is a major light-harvesting complex in cyanobacteria and red algae. The PBS consists of the central core and a number of peripheral phycobiliprotein rods. Various phycobiliproteins are known to pack in the crystal lattices in a fashion reminiscent their existence *in vivo*. Therefore, to obtain the detailed structure of the hemidiscoidal PBS core typically composed of three rods composed of allophycocyanin (APC) and minor polypeptide components, we analyzed all nine available from Protein Data Bank 3D structures of APCs from eight different cyanobacterial species and one red alga. We found several variants of crystal packing that potentially correspond to PBS core organization. After careful analysis of all APC crystal structures, combination of face-to-face APC trimer crystal packing presented in the *Porphyra yezoensis* (1KN1) with back-to-back APC hexamer packing from *Spirulina platensis* (1ALL) and *Thermosynechococcus vulcanus* (3DBJ) suggests two variants of the tricylindrical PBS core. Minimization of the distance between the APC phycobilin chromophores allows to reject the trimers lateral packing presented in 1KN1 lattice (48.5 Å) in favor of 1ALL/3DBJ variant (34.6 Å).

It is well known that, in cyanobacterial thylakoid membranes, photosystem II (PSII) is organized as a dimer and photosystem I (PSI) exists in form of monomers and trimers. It was eventually found out that PBS transfers the energy to both photosystems. To choose one of the PBS core structures based on two different trimer triples presented in 1ALL and 3DBJ crystal lattices we considered a spatial model of the super-complex of the PBS core and PSII dimer with minimized distance between the terminal PBS emitters and neighboring antenna chlorophylls. In the selected model, the distance between two types of pigments does not exceed 37 Å corresponding to the Förster mechanism of energy transfer. We also proposed a model of the PBS core and PSI monomer interaction showing a possibility of such a super-complex formation and direct energy transfer from the PBS to PSI similar to the energy transfer from PBS to PSII. The PSI trimer interaction with the PBS core of the determined structure is hardly possible due to the mismatch in the symmetry of the single groove between the PBS core lower cylinders and three ferredoxin binding protrusions on the PSI trimer surface.

Poster 1

Thermostability of Photosystem I trimers and monomers from the cyanobacterium *Thermosynechococcus elongates*

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The thermostability of photosystem I (PS I) trimers and monomers from the thermophilic cyanobacterium *Thermosynechococcus elongatus* was studied to probe heating induced perturbations on the level of secondary structure of the complexes. Changes have been monitored by Fourier transform infrared (FTIR) spectra which have been recorded in the region 1750–1500 cm^{-2} during slow heating (1°C per minute) of samples from 20°C to 100°C. These spectra show distinct changes in the Amide I region of PS I complexes which had been incubated for 24 h in D_2O phosphate buffer (pD 7.4), as a function of the rising temperature. The Amide I band of α-helices peaked at 1653 cm^{-1} for PS I monomers and 1656 cm^{-1} for PS I trimers, respectively, indicating a slightly different spatial arrangement of α-helices in those complexes. In PS I monomers, absorbance at the Amid I maximum drops in two temperature intervals, i.e. 60–75 and 80–90°C. In contrast, absorbance of PS I trimers (at Amid I maximum) drops only in the temperature interval 80–90°C. Also, the thermal profile of the spectral shift of α-helices bands in the region 1653–1642 cm^{-1} confirms two temperature intervals (60–75 and 80–90°C) for PS I monomers and only one interval (80–90°C) for trimers. Apparently, the observed absorbance changes at the Amide I maximum during heating of PS I monomers and trimers are caused by deformation and unfolding of α-helices. The absence of absorbance changes in the interval of 20–65°C in PS I trimers is probably caused by a greater stability of protein secondary structure as compared to that in monomers. During heating above 80°C a large part of α-helices both in trimers and monomers converts to unordered structure. Spectral changes of PS I trimers and monomers heated up to 100°C are irreversible due to denaturation of proteins and non-specific aggregation of complexes leading to new absorption bands at 1618–1620 cm^{-1}. We propose that monomers shield the denaturation sensitive sides at the monomer/monomer interface within a trimer, making the oligomer structure more stable against thermal stress.

Poster 2

Spectroscopic properties of the peripheral antennae from photosystem II

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Peripheral antennae LHCII, CP24, CP26 and CP29 play an important role in energy absorption and transfer in PS II light-harvesting supercomplex. Contributions of single chromophores to absorption and circular dichroism (CD) spectra reveal exciton structure and energy transfer mechanisms in the supercomplex. CD spectra are particularly useful in this case because they allow to distinguish individual excitonic states. In this work, we modeled the line-broadened spectra for the four antennae using molecular dynamics and excitonic Hamiltonian approach.

The structures of LHCII and CP29 complexes were determined from X-ray data by various authors, but no structural information is available for CP24 and CP26. For the latter two, we deduced the structure using amino acid sequences encoded by lhcb5 and lhcb6 genes which were aligned with a LHCII subpart as a template. Only the 500–700 nm region (the chlorophyll Q_y band) was studied, so excitation of carotenoids was neglected. Spectra were modeled using excitonic Hamiltonian approach including atom-atom Coulomb couplings. Excitation energies of chlorophylls in each binding site type were calculated using CASSCF[4,4] at cc-pVDZ level (three states averaging) with perturbation theory correction (XMCQDPT2). Pigment-pigment and pigment-protein interactions were calculated using ESP and TrESP [1] charges obtained by fitting matrix elements of electrostatic potential with the CASSCF wavefunctions. To account for spectral broadening, molecular dynamics was simulated with 2.5 ns duration giving 2500 nuclear geometries for spectra calculations. Averaging of linear spectra gave the resulting broadened spectra.

The obtained spectra fit well the experimental data. This work shows that the described approach can be used for non-empirical modeling of line broadening in the absorption and CD spectra. Based on the well-established TrESP method, this approach allows non-empirical modeling with the accuracy about 3–5 nm in the Q_y absorption region.

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Poster 3

Evolution of metastable states in the process of plastocyanin and cytochrome *f* complex formation

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Formation of plastocyanin-cytochrome *f* complex and electron transfer in the complex is an essential part of photosynthetic electron transport chain. We simulated protein-protein complex formation by Brownian and molecular dynamics methods and identified intermediate (metastable) states of this process using hierarchical cluster analysis. We obtained 2200 energetically favorable plastocyanin-cytochrome *f* encounter complexes with the energy of electrostatic attraction more than 8 kT by rigid body Brownian dynamics. These complexes were analyzed by hierarchical cluster analysis method and divided into two clearly separated groups containing 58% and 42% of structures.

We obtained two full-atom explicit solvent molecular dynamics trajectories of about 1 μs each, starting from the central structures of the clusters. One of them (from the bigger cluster) resulted in a stable complex with distance between cofactors on proteins of about 1.4 nm, similar to the complex obtained by NMR (PDB ID 2PCF). Another one resulted in a quite stable but different from experimental structure with nearly opposite orientation relatively to the NMR structure. Such unproductive metastable states can not easily be destroyed by the action of random Brownian forces. In the situation of low ionic strength the stability of such unproductive states increases due to lower screening of electric charges on protein surfaces. The existence of such states can be the main cause of decrease in the rate of protein-protein complex formation observed in experiments at low ionic strength values.

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Poster 4

The properties of two sources of carbonic anhydrase activity in photosystem II of higher plants

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Carbonic anhydrase (CA) is an enzyme performing interconversion of inorganic carbon forms. Several isoforms of the enzyme, soluble and membrane-bound ones may be detected in one and the same cell compartment. Chloroplasts of *Arabidopsis thaliana* contain αCA1, αCA4, βCA1, βCA5. Moreover, several sources of CA activity were found in the thylakoid membrane however nature of this CA activity was not established yet.

Thylakoid membrane preparations enriched with PS II (PS II-membranes) were isolated from pea, spinach and arabidopsis leaves. These PS II-membranes possessed CA activity increasing upon incubation with Triton X-100 and reaching a maximum values at Triton X-100/Chl ratio of 1. CA activity could be observed in the gel stained with bromothymol blue after the native electrophoresis of dodecylmaltoside-treated PS II-membranes. Gel color change occured in two places: in the area of high-molecular mass proteins of PS II (HMCA) and in the bottom part of the gel where the low molecular mass proteins (LMCA) usally remain. CA activities of eluates of corresponding bands were measured in presence of specific sulfamide inhibitor of CAs acetasolamide (AA) and ethoxyzolamide (EZ). CA activity of HMCA was not sensitive to EZ while AA inhibited it at nanomolar concentration. On the contrary, CA activity of LMCA was inhibited by EZ at nanomolar concentration and AA stimulated it at these concentration. These facts indicate the different origen of the CA activity sources. We believe that the HMCA may be formed from several proteins as CAs of γ-family. LMCA is the real CA belonging to the family of α-CA.

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Poster 5

Structural and functional features of photosynthetic apparatus of *Pinus sylvestris* in Baikal Siberia

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The aim of this work is to study the adaptive structural and functional rearrangements in the photosynthetic apparatus of pine (*Pinus sylvestris* L.) in the year cycle. It was established that mesophyll chloroplasts of the *P. sylvestris* needles mainly refer to the "light" type by inner structure under the conditions of Baikal Siberia. By vegetation conditions, the studied periods (2007 and 2008) were optimal for the photosynthetic productivity of pine. Owing to a higher illuminance in 2007, a "lighter" type of the organization of chloroplast membranes corresponded to higher values of the photosynthetic productivity. On the contrary, under lower illuminance in 2008, a less "light" type corresponded to lower values of the photosynthetic productivity. In the dynamics of the content of triene and tetraene fractions of unsaturated fatty acids (UFA) in lipids of needles in *P. sylvestris*, the peaks were observed in April, July, and October owing to the specific combinations of external and internal factors. In April, the increase in the UFA content in lipids of needles corresponded to the beginning of the photosynthetic activity, the low air and soil temperatures, and the first peak of the chlorophyll content in the light-harvesting complexes of photosynthetic units (LHC PSU). The increase in the UFA content in lipids of needles in July coincided with the peak of the contents of chlorophylls (*a*+*b*) in needles. It was assumed that UFA participated in the functional activity increase of chloroplasts in July (by the incorporation of chlorophylls into thylakoid membranes and the formation of additional LHC) related to the increased outflow of assimilates to the storage tissues. The peak of the UFA content in lipids of needles in September corresponds to the decrease in the total content of chlorophylls in needles and the increase in the chlorophyll *b* content in LHC as a response to the decrease in the illuminance and the air temperature in autumn. The dynamics of the UFA content coincides with the dynamics of the free and bound water fraction in the spring and summer periods. It was concluded that, the interdependent dynamics of the structural components (chloroplast membranes, contents of chlorophylls, and fatty acids), water status, and photosynthetic productivity of the assimilation apparatus is a manifestation of the complex adaptive mechanisms underlying the stability and high biological productivity of coniferous stands in the extreme conditions of Baikal Siberia.

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Poster 6

Simulation of electron transfer by plastocyanin in chloroplast thylakoid lumen by Brownian dynamics

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We introduce a Brownian dynamics (BD) computer model of limited diffusion of protein plastocyanin in chloroplast thylakoid lumen. The simulation area consists of two thylakoid membranes with the luminal space between them. On the lateral plane the simulation area is presented as a rectangle divided by the granal and stromal parts.

In the algorithm each protein molecule is represented as a 3D rigid body, its individual geometric surface is designed using the structure from the Protein Data Bank. The computer model includes 3D diffusion of plastocyanin in the narrow thylakoid lumen, electrostatic interaction and reactions of plastocyanin with photosystem I and cytochrome b_6f complexes fixed in the photosynthetic membranes: protein-protein transient complex formation, electron transfer inside the transient complexes, and complex dissociation. The model takes into account the geometry of the luminal space packed with many protein molecules and considers electrostatic interactions of plastocyanin with its reaction partners in the thylakoid membrane. The model simulates protein-protein complex formation, electron transfer and complex dissociation reactions proceeding after a short saturating light flash.

Results of the modeling showed fairly good correlation with experimental kinetics. The spatial organization and dimensions of the thylakoid membrane were shown to have a strong effect on the actual kinetics of P700 reduction and especially on cytochrome *f* oxidation. Computer simulation demonstrates that Brownian diffusion and electrostatic interactions in the complex interior of the photosynthetic membrane provide physical conditions for the directed electron flow along the electron transport chain.

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Poster 7

Molecular imprinted polymers in studying chlorophyll-protein interactions and development of sensors to determine herbicides

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Molecular imprinted polymers (MIP) technologies became widely used in Biotechnology, modeling of high efficient, selective sensors to determine concentration of medicines, herbicides, production of chromatographic materials. In the present work we used first MIP technologies to study chlorophyll-chlorophyll and chlorophyll-protein interactions. It has been shown possible determination of atrazine with the help of polymers imprinted with D1 protein which was isolated from *Chlamydomonas reinhardtii*. The polymer materials were prepared based on polyacrylamide gel, methacrylate, which contained purified preparations of chlorophyll-a, bacteriochlorophyll, isolated chlorophyll- protein complexes. After removal of these components from the gels with detergents and pre-electrophoresis the native electrophoresis of chlorophyll preparations (LHII, PS I, PS II, D1-D2-cyt.b559 complexes) was performed. It has been shown that in gels imprinted with chlorophyll and bacteriochlorophyll, the preparation of these pigments has been divided into two zones as compared with division in non imprinted gels. It is supposed that this effect is due to interaction of chlorophyll molecules from less mobile area with imprints in gel. These interactions are inhibited by addition of 15 mM histidine into the preparation before electrophoresis. PS I, PS II and LHII complexes are also differed by electrophoretic mobility in gels imprinted with chlorophyll. The gels imprinted with chlorophyll were obtained to make HPLC of the preparations of chlorophyll-protein complexes. Gels imprinted with chlorophyll have been shown to sorbate selectively the preparations of chlorophyll *a*, bacteriochlorophyll and apoproteins of chlorophyll-protein complexes. Possible selective binding of atrazine has been shown in chromatographic columns filled with polymer material, imprinted with D1 protein preparation. Thus, MIP technologies enable us to study chlorophyll-protein interactions, to elucidate and separate chlorophyll molecules placed superficially from the complexes, as well as to develop high selective sensors of herbicides for real world problems.

Poster 8

Participation of carbonic anhydrase alpha-4 in the development of non-photochemical chlorophyll fluorescence quenching in *Arabidopsis thaliana*

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Carbonic anhydrase (CA) is an universal enzyme that catalyzes the reversible hydration of CO_2 in the cell of all living organisms. In higher plant *Arabidopsis thaliana* 19 genes that encode CA belonging to α-, β- and γ- families, were found. In the chloroplasts of higher plants the presence of α-CA1 and β-CA1 in the stroma, α-CA4 in the thylakoid membrane and β-CA5 with an unknown location within the organelle have been established. Furthermore, the CA activity had been detected in membranes enriched with PS2 (two sources of activity) or PS1, as well as in the thylakoid lumen. The roles of chloroplast CAs during photosynthesis had not yet been established. The work aim was to identify the possible role of the thylakoid α-CA4 in photosynthesis.

We compared a number of photosynthetic characteristics of *Arabidopsis thaliana* mutant plants with knocked out *At4g20990* gene encoding α-CA4 with those of wild-type (WT) plants. Weight of mutant leaves was greater by 10–20% due to a higher starch content; electron microscopy showed that the chloroplasts of mutant contain the much larger starch grains as compared with WT plants. The fluorescent characteristics of mutants that were measured in the conditions close to growth conditions, 100 μmol quantum/m^2s and 450 ppm CO_2, hardly differed from those of WT plants. The measurements of chlorophyll *a* fluorescence in actinic light of 500 μmol quanta/m^2s at 800 and 1500 ppm CO_2 revealed that in the mutants the effective quantum yield of PS2 under steady illumination (Y) was higher by 10%, while the CO_2 assimilation rate was lower than in WT plants by 20%. In the conditions of both high illumination and a high CO_2 concentration, a hydrogen peroxide content in the mutant's leaves was higher by 20–30%; and this was possibly explained by increased rate of oxygen reduction leading to a higher Y. The distinctive feature of mutant plants was a reduced energy-dependent component of non-photochemical quenching of chlorophyll fluorescence by 30–40%. The data implied that α-CA4 is located near PS2 and probably participates in the development of excitation energy dissipation, providing with protons the components engaging in this process.

This work is supported by Russian Foundation for Basic Research (grant number 15-04-03883).

Section 1.3: Photosystem II and Water Oxidation Mechanism

Lecture 1

The Mn_4Ca-catalyst of the photosynthetic oxygen evolving centre: structure, function and evolution

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Photosystem II is the chlorophyll containing enzyme in which the very first chemical energy storing reaction of photosynthesis occurs. It does so by splitting water into molecular oxygen and hydrogen equivalents at a catalytic centre composed of four Mn ions and one Ca^{+}_{2}. All the oxygen in the atmosphere is derived from this reaction and without it the biosphere, as we know it, would not exist. Indeed its appearance about 3 billion years ago gave rise to the "big bang of evolution". Thus understanding the structure and functioning of this metal cluster is a major topic in science and I will discuss it in terms of research over of the last twelve years dating back to when it was first proposed to be a Mn_3CaO_4 cubane with a fourth Mn attached to cubane by one of its oxo bridging bonds. In so doing a number of novel properties emerge for this metallo-proteins with implication for its evolutionary origin.

Lecture 2

Energetics of proton release on the first oxidation step in the water oxidizing enzyme

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In Photosystem II (PS II), the Mn_4CaO_5 cluster catalyzes the water splitting reaction. The recent high-resolution crystal structures of PS II show the presence of a hydrogen-bond water molecule directly linked to O4. Using a quantum mechanical/molecular mechanical (QM/MM) approach, here we show the detailed properties of the H-bonds associated with the Mn_4CaO_5 cluster. When O4 was taken as a μ-hydroxo bridge acting as an H-bond donor to water539 (W539), the S_0 redox state best described the unusually short O4–O_{W539} distance (2.5 Å) seen in the crystal structure. We found that in S_1, O4 easily released the proton into a chain of eight strongly H-bonded water molecules. The corresponding H-bond network is absent for O5 in S_1. The present study suggests that the O4-water chain could facilitate the initial deprotonation event in PS II . This unexpected insight is likely to be of real relevance to mechanistic models for water oxidation.

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Lecture 3

Combined SAXS-SANS structure study of isolated PS II Core Complex

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Photosynthesis is a fundamental natural process that enables the conversion of solar energy into storable chemical energy under the release of molecular oxygen derived from water. The essential processes of photosynthetic water splitting take place in a membrane-bound protein assembly denoted as Photosystem II (PS II). So far structure investigations of the PS II pigment-protein complexes by X-ray diffraction have been limited to low temperatures. However, the knowledge of the protein structures at close to native conditions is the missing link to understand the functioning of PS II at physiological conditions.

Here we present, combined SAXS-SANS structure study of the PS II core complex and the detergent structure at physiological temperature. The PS II core complex was isolated and solubilized in a solution of the dodecyl-β-D-maltoside (βDM) detergent and of the octaethylene glycol monodecyl ($C_{12}E_8$) detergent. In order to investigate independently the protein and detergent structure, SANS contrast variation experiment was applied. Thus, the topological shape of PS II was determined according to the *ab initio* shape restoration methods from the SANS data at the contrast match point of the detergents. The full PS II-detergent complex was studied from the SAXS curves and also from the SANS curves, measured at the contrast match point for the protein and in 75% D_2O contrast.

Our data analysis shows that there is a monolayer of the detergent surrounding the protein as a kind of protection belt. This work proves that natively structure PS II can be produced for functional characterization at physiological conditions.

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Lecture 4

Role of D1-Pro173 of photosystem II in water oxidation

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The main cofactors in photosystem II (PSII) are borne by the D1 and D2 subunits. In the cyanobacterium *Thermosynechococcus elongatus*, there are three psbA genes encoding D1. Among the 344 residues constituting D1, there are 21 substitutions between PsbA1 and PsbA3, 31 between PsbA1 and PsbA2, and 27 between PsbA2 and PsbA3. Here, we present the study of PsbA2-PSII. Using several spectroscopies, we show that: (i) $Tyr_Z^{\bullet}$ in the $(S_3Tyr_Z^{\bullet})'$ is modified; (ii) $Tyr_Z^{\bullet}$ in the $(S_2Tyr_Z^{\bullet})'$ state induced by near-infrared illumination at 4.2K of the S_3Tyr_Z state is significantly modified; and (iii) the slow phases of $P680^{\bullet+}$ reduction by Tyr_Z are slowed down from the hundreds of μs time range to the ms time range, whereas both the $S_1Tyr_Z^{\bullet}$ to S_2Tyr_Z and the $S_3Tyr_Z^{\bullet}$ to $S_0Tyr_Z + O_2$ transition kinetics remained similar to those in PsbA(1/3)-PSII. These results show that the geometry of the Tyr_Z phenol and its environment, likely the Tyr-O···H···Ne-His bonding, are modified in PsbA2-PSII when compared with PsbA(1/3)-PSII. They also point to the dynamics of the proton-coupled electron transfer processes associated with the oxidation of Tyr_Z being affected. From sequence comparison, we propose that the P173M substitution in PsbA2-PSII is responsible for these changes.

Lecture 5

Energetics of the proton transfer from tyrosine D in photosystem II: comparison with tyrosine Z

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A water-oxidization enzyme, photosystem II (PS II), has two redox active tyrosines TyrZ (D1-Tyr161) and TyrD (D2-Tyr160). When TyrZ is oxidized by the central chlorophyll dimer P680, it releases the proton of the phenolic group to the nitrogen of the imidazole group of the adjacent D1-His190 and neutral tyrosine radical (TyrZ-O$^{\bullet}$) forms. After that, the TyrZ-O$^{\bullet}$ is again reduced from the catalytic site Mn_4CaO_5. On the reduction, the proton at D1-His190 returns back to TyrZ (the so-called proton rocking mechanism) [1]. In contrast to TyrZ, when TyrD is oxidized, the released proton from TyrD-O$^{\bullet}$ is transferred not to the adjacent histidine but to the protein bulk surface [2, 3]. Therefore once TyrD-O$^{\bullet}$ formed, it is observed to be very stable for many hours under physiological condition. In this study, we investigated the detailed energetics of the proton transfer (PT) from TyrD by using quantum mechanical/molecular mechanical calculations [4].

The PT pathway for TyrD is an H-bond network that involves D2-Arg180 and a series of water molecules. The potential-energy profile along the PT pathway indicates that the overall PT from TyrD is energetically downhill. D2-Arg180 plays a key role in the PT pathway, providing a driving force for PT, maintaining the H-bond network structure, stabilizing P680$^{\bullet+}$, and thus deprotonating TyrD-OH to TyrD-O$^{\bullet}$. A hydrophobic environment near TyrD enhances the electrostatic interactions between TyrD and redox active groups, e.g., P680 and the catalytic Mn_4CaO_5 cluster, which is also linked with the protonation state of TyrD, i.e., release of the proton from TyrD. Thus, the PT pathway from TyrD may ultimately contribute to the conversion of S_0 to S_1 in the dark in order to stabilize the Mn_4CaO_5 cluster when the photocycle is interrupted in S_0

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Poster 1

Automatization of analysis of movement trajectories of small molecules in photosystem II

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The lipid-pigment-protein complex from thylakoids of cyanobacteria and other photosynthesizing organisms, photosystem II (PS II), uses the energy of light and creates molecular oxygen from water. Oxygen-evolving complex is located deep within the luminal part of PS II. Water molecules and oxygen need to pass through protein's environment to enter and exit the active site of the oxygen-evolving complex. Previous studies on the detection of oxygen and water channels in PS II were based on the analysis of internal cavities of static molecular structures, or on experiments using introduction of noble gases atoms in crystals of PS II under pressure. It allowed to detect some possible exit paths for molecular oxygen.

Present study describes the creation of computer program for automatization of analysis of small molecules movements in molecular dynamics model of PS II. Such necessity arose as a consequence of the large number of water molecules that have been used in molecular dynamics model. Manual processing of such a high number of molecules is difficult.

The program, written in MatLab, uses several criteria for detection of molecules passing through proposed channels, already known from X-ray diffraction data, as well as through new previously unknown channels. As a result, we identified low intensity of molecular movement at proposed hydrogen channels and high intensity of movement in oxygen and water channels. We also found previously unknown possible water channel with dominant traffic of water molecules in photosystem II.

The study was supported by the Program "Molecular and Cellular Biology" of the Presidium of the Russian Academy of Sciences and the Russian Foundation for Basic Research (project no. 15-04-03041a).

Poster 2

Involvement of molecular oxygen in the donor-side photoinhibition of Mn-depleted photosystem II membranes

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It has been shown [1–3] that removal of Mn from PS II led to the appearance of O_2 photoconsumption on the donor side of PS II. The light-induced O_2 consumption is accompanied with hydroperoxide formation, and it was suggested that the events are related to the oxidative photoinhibition of PS II. In this work, we experimentally verified the suggestion. The degree of photoinhibition was determined by the loss of the capability of exogenous electron donors (Mn^{2+} or sodium ascorbate) to the reactivation of electron transport (measured by the light-induced changes of chlorophyll fluorescence yield (ΔF)) in Mn-depleted PS II membranes. The transition from anaerobic conditions to aerobic ones significantly activated photoinhibition of Mn-depleted PS II membranes both in the absence and in the presence of exogenous electron acceptor, ferricyanide. The photoinhibition of Mn-depleted PS II membranes was suppressed upon theaddition of exogenous electron donors (Mn^{2+}, diphenylcarbazide, ferrocyanide). The addition of superoxide dismutase did not affect the photoinhibition of Mn-depleted PS II membranes. It is concluded that the interaction of molecular oxygen (rather than superoxide anion radical formed on the acceptor side of PS II) with the oxidized components of the donor side of PS II reflects the involvement of O_2 in the donor-side photoinhibition of Mn-depleted photosystem II membranes rather than superoxide anion radical formed on the acceptor side of PS II.

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Poster 3

Spectral characteristics of photosystem II complexes isolated from chlorophyll *f* containing cyanobacterium

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Chlorophylls (Chls) play important roles in light harvesting, energy transfer, charge separation and electron transfer during photosynthetic reaction. Recently, more red-shifted Chl *f* was found in cyanobacterium *Halomicronema hongdechloris*. The absorption maximum of Chl *f* in organic solvents occurs at a wavelength approximately 40 nm longer than that of Chl *a*. The structure of Chl *f* was determined to be [2-formyl]-Chl *a* by mass spectroscopy and NMR analysis. The Chl content of *H. hongdechloris* varied under different light conditions. When under far-red light (>700 nm), the Chl *f* content increased to ca. 10% of total Chls. When under white fluorescent light, the Chl *f* content decreased negligibly. The photochemical and photophysical functions of Chl *f* are not known in photosystem II complexes. Therefore, we isolated photosystem II complexes from cells grown under far-red light or white light. We discuss characteristics of photosystem II complexes isolated from chlorophyll *f* containing cyanobacterium *H. hongdechloris*.

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Poster 4

EPR and Fluorescence investigations of Fe cation interaction with Mn-binding sites in Mn-depleted PS II membranes

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Photosystem II (PS II) powers 100% of redox energy production and some proton energy production in all known oxygenic phototrophs. The PS II reaction center (RC) is highly conserved and uses a compositionally and structurally identical cluster (Mn_4CaO_5) to catalyze the oxidation of water to dioxygen. Despite many redox active metals that are available in nature for catalyzing this reaction, only manganese is utilized *in vivo*. In order to start to understand natural selection of Mn^{2+} over Fe^{2+} during biogenesis of the cluster, we have studied the interaction of Fe^{2+} with Mn-depleted spinach PS II membranes (PS II[−Mn]) using chlorophyll fluorescence and EPR spectroscopies. It is known that light-induced Fe^{2+} oxidation at the high-affinity, Mn-binding site (HA_Z) blocks subsequent electron donation by exogenous Mn^{2+} at this site in PS II(−Mn), due to the formation of a cluster or clusters containing up to 4.5±0.9 cations of Fe per RC. The light-induced oxidation of Fe(II) by PS II(−Mn) samples is accompanied by formation of three light-induced signals at g=4.2, 5.5 and 9.3, but not the recovery of water-oxidation activity. The light-induced EPR signals at g=4.2 and 9.3 are associated with photooxidation of bound Fe^{2+} to form a monomeric, high-spin Fe^{3+} possessing rhombic ligand field symmetry (zero-field splitting, D>E/3) at HA_Z. These signal do not form upon adding Fe^{3+} to PS II(−Mn), neither in dark nor light, nor in holo-enzyme. The previously unreported site at g=5.5 is tentatively attributed to a spin-coupled Fe_2(II, III) cluster in intermediate spin state on the basis of comparison to model dinuclear complexes of this type. Higher nuclearity mixed valence iron clusters may also produce this signal.

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Poster 5

Trehalose induced stimulation of oxygen photoconsumption and electron transfer in manganese-depleted photosystem II preparations

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The effect of trehalose on the oxygen photoconsumption (OPC) in Mn-depleted photosystem 2 (PS2) preparations (apo-WOC-PS2) was investigated. A more than two-fold increase of the OPC on both donor and acceptor sides of PS2 is revealed upon the addition of 1 M trehalose. It was shown that the addition of trehalose induces: (i) increase in the rate of superoxide-anion radical photoproduction on the electron-acceptor side of PS2; (ii) a significant increase in the ability of exogenous Mn^{2+} to electron donation to the reaction center of PS2; (iii) slowing down the photoaccumulation of the primary quinone electron acceptor of PS2 (Q_A^-) under aerobic conditions; (iv) acceleration of the reoxidation of Q_A^- by Q_B (and by Q_B^-) as well as the replacement of Q_B^{2-} by a fully oxidized plastoquinone; (v) restoration of the electron transfer between the quinone electron carriers in the so-called "closed reaction centers of PS2". It is suggested that the trehalose induces structural changes leading to both a decrease in the proportion of the "closed PS2 reaction centers" and an increase in the electron transfer rate in PS2.

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Section 1.4: Energy Transfer and Trapping in Photosystems

Lecture 1

Excitonic coupling and protein dynamics in the water-soluble chlorophyll protein (WSCP)

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The recombinant class-IIa water-soluble chlorophyll (Chl)-binding protein (WSCP) from cauliflower is a suitable model system for analyzing pigment-pigment and pigment-protein interactions [1], because it can be reconstituted with either two or four Chl molecules only.

We have investigated the spectroscopic properties of excitonically coupled Chl dimers in WSCP using a combination of hole-burning, difference fluorescence line-narrowing, circular dichroism, neutron scattering and molecular dynamics simulations.

Persistent spectral hole-burning at 4.5 K [2] resolved a doublet of broad satellite holes which is assigned to the excitonically coupled Chl dimer. Assuming identical site energies, the dipole-dipole interaction energy J is determined to be 85 and 100 cm^{-1} for Chl *b*- and Chl *a*-WSCP, respectively. The Gaussian low-energy absorption band at 4.5 K has a width of ~120 cm^{-1}. Electron-phonon coupling of the lower exciton level [3] is shown to have moderate coupling strength with Huang-Rhys factors S in the order of 0.81–0.85. The one-phonon profile is highly structured with a peak phonon frequency (ω_m) of ~24 cm^{-1} and further discernible peaks at 48 and 85 cm^{-1}, respectively.

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Poster 1

Energy migration in model quantum dot – aluminum phthalocyanine system

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Ferster resonance energy transfer (FRET) from an excited donor molecule to acceptor plays a major role in photosynthesis, delivering an excitation energy from chlorophyll molecules to the photosynthetic reaction center. It is known that the efficiency of energy transfer by this mechanism is affected by many different factors such as the distance between the energy donor and acceptor, their spectral properties and conformational state. In turn, environmental conditions indirectly influence on processes of energy migration through the above mentioned parameters. The study of the energy transfer efficiency in a pair of donor-acceptor and its regulation mechanisms will allow to know more about the features of the photosynthetic apparatus functioning. In this study, we used a model system consist of polycationic aluminum phthalocyanine (Pc) related porphyrins as an acceptor of energy, and semiconductor crystal (quantum dots, QDs) as a light-harvesting complex – the donor of energy. The donor-acceptor complex of Pc and QD formed by self-assembly, based on electrostatic interactions of Pc and the negatively charged surface of the QD. It was found that the interaction of QD with the phthalocyanine alters Pc's spectral characteristics, particularly increases fluorescence intensity and the lifetime of the singlet excited state of Pc molecule. In addition, absorption and fluorescence spectra of Pc are shifted to longer wavelengths. We have shown that the value of the identified changes in Pc absorption and fluorescence parameters depends on the size of the QD. In this work we discuss the relationship between the spectral characteristics of the phthalocyanine and conformational state of Pc molecule. Furthermore, we have demonstrated an increase in efficiency of energy transfer in QD-Pc complex with increasing of the solution ionic strength (in the range of 0.05–0.2 M) due to a significant increase in the Pc absorption cross-section after adding sodium chloride to the solution.

Poster 2

Characterization of the putative ferredoxin binding sites on photosystem I

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Despite the impressive progress made in recent years in understanding the early steps in charge separation within the photosynthetic reaction centers, our knowledge of how ferredoxin (Fd) interacts with the acceptor side of Photosystem I (PS I) is much less well developed. This lack of knowledge is particularly striking when one considers that Fd represent the sole link between the light reactions and critical downstream metabolic enzymes such as ferredoxin:$NADP^+$ oxidoreductase (FNR) and hydrogenase (HydA). Our recent *in vitro* work (Yacoby et al. 2011) demonstrated a preference for electron divergence towards NADPH production by FNR at the expense of hydrogen production by hydrogenase. We showed that a novel ferredoxin-hydrogenase fusion protein bypasses this diversion. However the mechanism is unclear. Our results as well as others (Setif 1994 1995) suggest that PS I contains multiple Fd binding sites. To assess the notion of more than a single binding site we characterized the binding constants of Fd to PS I by isothermal titration calorimetry (ITC). We studied *Chlamydomonas reinhardtii* purified components such as PS I, PS I mutant (psaC), Fd, Fd mutant and several Fd-fusions. Our results show two distinct binding events when Fd is titrated against PS I. The first corresponds to a Kd of 5–20 nM and the second ranges between 200–400 nM. While a binding event as the latter was suggested by the literature, the other event, of 5–20 nM Kd, was never described before. In summary, our initial kinetic data that suggested occurrence of multiple binding sites for Fd at the stromal face of PS I is now supported by two defined dissociation constants for Fd at PS I.

Poster 3

Excitation energy transfer in phycobiliproteins of *Acaryochloris marina* investigated by site-selective spectroscopy

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In adaption to its specific environmental conditions, the cyanobacterium *Acaryochloris marina* developed two different types of light-harvesting complexes: chlorophyll-*d*-containing membrane-intrinsic complexes and phycocyanobilin (PCB)-containing phycobiliprotein (PBP) complexes (see [1] and references therein). We have used small angle neutron scattering to verify the rod-shaped structure of PBPs, which are believed to contain three homo-hexamers of phycocyanin (PC), one hetero-hexamer of phycocyanin and allophycocyanin (APC). Spectral hole burning and difference fluorescence line-narrowing [2] were employed to study excitation energy transfer and electron-phonon coupling in PBPs. The data reveal a rich spectral substructure with a total of four low-energy electronic states whose absorption bands peak at 633 nm, 644 nm, 654 nm, and at about 673 nm. The electronic states at ~633 and 644 nm can be tentatively attributed to PC and APC, respectively. The remaining low-energy electronic states including the terminal emitter at 673 nm may be associated with different isoforms of PC or of the linker protein. Furthermore, the hole-burning data reveal a large number of ground state vibrational frequencies, which are characteristic for the chromophore PCB.

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Poster 4

Accurate simulation of hole burning and fluorescence line-narrowing spectra

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Spectral hole-burning (SHB) and (delta-) fluorescence line-narrowing (FLN) are routinely used as powerful experimental tools for investigations of electron-phonon coupling in photosynthetic pigment-protein complexes and in amorphous systems in general (see e.g. [1]). Nevertheless, Huang-Rhys factors S and one-phonon profiles obtained by SHB and/or (delta-) FLN over the past few years have differed significantly for some photosynthetic pigment-protein complexes. This indicates that there are systematic properties of both types of line-narrowed spectroscopies influencing the results. For this reason, we have studied the electron-phonon coupling of the antenna complex CP29 by both SHB and FLN spectroscopies simultaneously. The properties of both types of line-narrowed spectroscopy are discussed in detail based on extensive theoretical simulations. We show that delta-FLN spectra provide direct access to the homogeneously broadened spectrum in the low-fluence limit, so that Huang-Rhys factors S and one-phonon profile can be directly obtained from the measured spectra. In contrast, SHB utilizing the pseudo-phonon sideband may underestimate S in case of narrow inhomogeneous distribution functions IDF [2] or strongly overestimate S in case of overly high burn fluences [3].

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3.Pieper, J. et al., submitted

Poster 5

Excitation energy transfer processes among photosystethic complexes in cyanobacterial cells

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Efficient photosynthesis in oxygenic photosynthetic organisms relies on a balanced excitation between photosystem I (PS I) and photosystem II (PS II). Cyanobacteria contain specific antenna pigment-protein complexes called phycobilisomes (PBSs), which transfer light energy to PS II or PS I. Excitation energy transfer (EET) processes within or among PBS, PS I, and PS II have been controversial for many years. However, these EET processes, especially PBS→PS I EET pathways remain unclear. In the present study, we examined the EET processes in the cyanobacterium *Synechocystis* sp. PCC 6803 cells by means of picosecond time-resolved fluorescence spectroscopy. Time-resolved fluorescence was measured with a time-correlated single photon counting systems at 77 K, and the excitation wavelength was set to 408 nm or 633 nm, which mainly excites PS or PBS, respectively. Delayed fluorescence spectra on PS- or PBS-selective excitation indicated that PS II→PS I EET (spillover) is present in spite of the existence of PBS. Additionally, the fluorescence decay kinetics of PS I confirmed that direct PBS→PS I EET also occurs.

Section 1.5: Photosystem I and Bacterial Photosynthesis

Lecture 1

Charge separation in *Heliobacterium modesticaldum*: An exemplar of an early homodimeric Type I photosynthetic reaction center

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The homodimeric Type I reaction center in Heliobacteria is arguably the simplest known pigment-protein complex capable of carrying out (bacterio)chlorophyll-based conversion of light into chemical energy. Despite its structural simplicity, the thermodynamics of the electron transfer cofactors on the acceptor side have not been fully investigated. In this work, we measured the midpoint potential of the terminal $[4Fe\text{-}4S]^{2+/1+}$ cluster (F_X) in reaction centers from *Heliobacterium modesticaldum*. The F_X cluster was titrated chemically and monitored by (i) the decrease of stable P_{800} photobleaching by optical spectroscopy, (ii) the loss of the light induced g≈2 radical from $P_{800}^{+\bullet}$ following a single turnover flash, (iii) the increase in the low-field resonance at 140 mT attributed to the S=3/2 ground spin state of F_X^-, and (iv) the loss of the spin-correlated $P_{800}^+F_X^-$ radical pair following a single turnover flash.

These four techniques led to similar estimations of the midpoint potential for F_X of −502±3 mV (n=0.99), −496±2 mV (n=0.99), −517±10 mV (n=0.65), and −501±4 mV (n=0.84), respectively, with a consensus value of −504±10 mV (converging to n=1). Under conditions in which F_X is reduced, the long-lived (~15 ms) $P_{800}^+F_X^-$ state is replaced by a rapidly recombining (~15 ns) $P_{800}^+A_0^-$ state, as shown by ultra-fast optical experiments. There was no evidence for the presence of a $P_{800}^+A_1^-$ spin-correlated radical pair by EPR under these conditions. The midpoint potentials of the two $[4Fe\text{-}4S]^{2+/1+}$ clusters in the low

molecular mass ferredoxins were found to be −480±11 mV/−524±13 mV for PshBI, −453±6 mV/−527±6 mV for PshBII and −452±5 mV/−533±8 mV for HM1_2505 by EPR spectroscopy. F_X is therefore suitably poised to reduce one $[4Fe\text{-}4S]^{2+/1+}$ cluster in these mobile electron carriers. Using the measured midpoint potential of F_X and a quasi-equilibrium model of charge recombination, the midpoint potential of A_0 was estimated to be −854 mV at room temperature. The midpoint potentials of A_0 and F_X are therefore 150 to 200 mV more oxidizing than their respective counterparts in Photosystem I of cyanobacteria and plants. Transient EPR (TREPR) and optically detected magnetic resonance (ODMR) spectra of $^3P_{800}$ show that although the spin polarized triplet can be detected by ODMR, it is not observed by TREPR. We demonstrate that is a result of the fact that the zero-field splitting tensor is maximally rhombic, which results in complete cancellation of the absorptive and emissive polarization in randomly oriented samples. However, in partially oriented samples, the cancellation is not complete making it possible to detect the TREPR spectrum of $^3P_{800}$.

Lecture 2

Charge recombination in Photosystem I under conditions of restricted protein mobility

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The role of protein conformational dynamics in long-range electron transfer was studied in cyanobacterial Photosystem I (PS I) complexes after its incorporation into room temperature trehalose glasses at different hydration levels. In dry glasses, PS I was stable for months at room temperature. Flash-induced charge recombination kinetics were measured by time-resolved optical spectroscopy at 820 nm. In hydrated conditions, the kinetics mostly reflected the reduction of the photooxidized primary donor $P700^+$ by the reduced terminal iron-sulfur centers $[F_A/F_B]^-$; following dehydration, the decay accelerated and became more distributed. Continuous distributions of lifetimes, τ, were obtained by analyzing the kinetics with a constrained regularization method (CONTIN). Upon dehydration, the contributions of the fastest component (τ~150 μs) and of additional distributed phases with intermediate lifetimes (attributed to recombination from the phylloquinone A_1^- and F_X^- cluster, correspondingly) increased at the expense of the two slowest components (τ~300 ms and ~60 ms), assigned to $P700^+[F_A/F_B]^-$ recombination.

The charge recombination kinetics in PS I in a water-glycerol system was also studied in a wide temperature range. It was shown that the contribution from the slower kinetic components ascribed to $P700^+[F_A/F_B]^-$ recombination kinetics diminished as the temperature was lowered simultaneously with an increase of the faster components attributed to $P700^+$ reduction from the preceding acceptors A_1^- and F_X^-. In general, the effect of temperature was similar, but not identical, to the effect of dehydration in the trehalose glass. The dehydration of PS I in trehalose matrix did not affect the fast recombination between A_1^- and $P700^+$, unlike a temperature decrease. The recombination kinetics in dry trehalose glass generally mimicked the kinetics of PS I in water-glycerol at the temperature of the protein-glass transition (~170–180 K). This study demonstrates the essential role of protein-solvent dynamics in long-distance electron transfer in PS I.

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Lecture 3

Characterization of a non-detergent isolated form of a cyanobacterial trimeric photosystem I using styrene-maleic acid co-polymers

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Cyanobacterial Photosystem I is the largest membrane protein complex to have had its structure solved by X-ray diffraction. This complex has 51 transmembrane domains, 33 different subunits, >330 non-covalently bound cofactors and a molecular weight of ~1.4 mDa. It has been isolated and characterized primarily in a detergent micelle using the non-ionic detergent dodecylmaltoside (DDM). We have now succeeded in isolating this complex without the use of detergents using the self-association of styrene-maleic acid co-polymers. PSI isolated by this method is in the form of a Styrene-Maleic Acid Lipid Particle (SMALP). A host of different biochemical, biophysical and functional assays, has been applied to characterize this non-detergent form of PSI. The PSI SMALP has a slightly different sedimentation coefficient and has both quantitative and qualitative differences in the lipid composition. We have shown that it is still functional yet has selectively lost one transmembrane subunit, PsaF. This loss of a single subunit reflects either a more labile interaction of PsaF and the core complex or a selective exclusion during co-polymer assembly. The loss of PsaF altered the interaction with both native and non-native cytochromes verifying its role as a docking site for native electron transfer. The increase in the size of the boundary lipid content reflects a more native form of PSI and suggests this non-detergent isolation may be a new approach for the structural characterization of PSI structure, function and assembly.

Lecture 4

Comparative study on reaction kinetics of $NADP^+/H$ reduction/oxidation catalyzed by ferredoxin-NAD(P)H oxidoreductases from photosynthetic and non-photosynthetic bacteria

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Ferredoxin-$NAD(P)^+$ oxidoreductase (FNR) is a ubiquitous enzyme catalyzing redox reactions between soluble small iron-sulfur protein ferredoxin and $NADP^+/H$. FNRs from photosynthetic green sulfur bacterium *Chlorobaculum tepidum* (CtFNR), purple non-sulfur bacterium *Rhodopseudomonas palustris* (RpFNR) and heterotrophic gram-positive bacterium *Bacillus subtilis* (BsFNR) are homo-dimeric flavoprotein with significant structural homology to NADPH-thioredoxin reductase and distinct from the other monomeric FNRs from plastids, cyanobacteria and vertebrate. Although the redox reaction catalyzed by FNR is theoretically reversible, the direction of the reaction is generally optimized to either $NADP^+$ reduction in many photosynthetic organisms, or NAD(P)H oxidation in non-photosynthetic organisms and tissues. Thus comparative studies on CtFNR, RpFNR and BsFNR will provide valuable information on the structure-function relationships of FNRs and related enzymes. In this presentation, the differences in the catalytic mechanism will be discussed based on the pre-steady-state kinetics of the reactions between FNRs and $NADP^+$/NADPH. Obtained results indicated that CtFNR and RpFNR catalyze $NADP^+$/NADPH reduction/oxidation reactions reversibly, whereas BsFNR-catalyzing reaction is rather optimized for NADPH oxidation direction, which are in good agreement with physiological requirements. Although both CtFNR and RpFNR catalyze the reaction reversibly, $NADP^+$ reduction rate of CtFNR was much slower. Interestingly, kinetics analysis of CtFNR-catalyzing reaction suggested that transient $NADP^+$-CtFNR complex in $NADP^+$ reduction reaction had a different conformation from that in NADPH oxidation reaction. UV-vis spectra of reduced FNRs suggested the difference in conformation of the isoalloxazine ring portion.

Lecture 5

BChl ligation in bacterial photosynthetic reaction center: possible options

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In photosynthetic reaction center (RC) of purple bacteria interactions of bacteriochlorophyll (BChl) molecules with their protein environment affect important parameters of the primary electron transfer such as value of E_m P/P^+, levels of the free energy of P* and $P^+B_A^-$ states, inter-dimer BChl interactions, quantum yield of the photochemical charge separation and stability of RC complex. Fundamentally important BChl-protein interaction is 5th-coordination of the central Mg^{2+} atom. In bacterial photosynthetic RC Mg^{2+} atoms of BChl are always penta-coordinated from α-side of the macrocycle through histidine ligands that are always situated perpendicular to the BChl planes. Using site-directed mutagenesis in this work it was demonstrated that these rules are not strict. For example, His ligands of the special pair BChls could be dismissed from positions L173 (BChl P_A) and M202 (BChl P_B) to positions L177 and M206, correspondently. Such ligand displacement in RCs H(M202)L+I(M206)H and H(L173)L+I(L177)H results in considerable blue-shifts of the Q_Y P bands. By means of protein environment modifications in the vicinity of the monomer BChl B_B it is possible to exchange this BChl for bacteriopheophytin in RC H(M182)L, to change Mg^{2+} α-coordination for β-coordination in RC I(L177)H+H(M182)L or to get hexa-coordinated BChl B_B in RC I(L177)H. It is also possible to exchange His-ligand of monomer BChl B_A for Cys or Met with no effect on RC's photochemistry and stability. Exchange of the His-ligand for Leu and Tyr severely affects stability of the complex and results in loss of the BChl BA from the RC H(L153)Y.

Lecture 6

Dielectric behavior of the photosynthetic bacterial reaction center evaluated by the Langevin and Kirkwood-Fröhlich models using molecular dynamics simulations

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Molecular dynamics simulations allow direct investigation of the molecular mechanics of charge separation reactions in photosynthetic bacterial reaction center (BRC). A molecular model of BRC was developed with the aim to examine the impact of microscopic movements of protein, lipid bilayer and surrounding water on different charge transfer reactions in BRC. The primary charge separation is difficult for theoretical explication: the reaction kinetics, which does not change over a wide range of temperature and free energy, reveals oscillatory features. Here the reaction mechanism was analyzed within a phenomenological Langevin approach. The spectral function of polarization around the special pair P_L/P_M and the dielectric response upon the formation of P_L^+/P_M^- dipole were calculated. The system response was approximated by Langevin oscillators, the respective frequencies, friction and energy coupling coefficients were determined. The protein dynamics around P_L and P_M was shown to be highly asymmetric. The polarization around P_L was described largely by a single Debye mode with relaxation time of 80 fs and the amplitude of 130 mV; the protein response around P_M could be largely described by two oscillatory modes with frequencies of 90 and 150 cm^{-1} and the total amplitude of 50 mV. The revealed polarization dynamics was in agreement with the observed oscillatory behavior and could rationalize the other properties of primary charge separation. The spatially heterogeneous distribution of the dielectric permittivity in BRC was calculated using the Kirkwood-Fröhlich's approach. Based on this distribution we developed a Poisson-Boltzmann model of BRC, which included several dielectric strata; its numeric solution was in quantitative agreement with diverse experimental data obtained in our laboratory by the direct electrometric measurements.

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Lecture 7

Red exciton states: localization in the Photosystem I trimer complexes of *Arthrospira platensis* and their role in energy transfer

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In order to determine the red chlorophylls location in the pigment-protein complex of the photosystem I (PS I) trimer, a simultaneous fit of the pump-probe kinetics and the linear spectra has been done. To create an exciton model of the energy transport in the PS I trimer, we used the available crystal structure and slightly modified it during the fit. The extended dipole-dipole approximation has been applied, to get the proper coupling energies between chlorophylls at distances less than 10 Å. Since more than 500 Chls have been taken into account for evaluation of the exciton dynamics, we optimized our computational routines and run them on a computer cluster using an optimization procedure known as the Differential evolution.

To reproduce the pump-probe experimental data, which reveal the exciton transfer from antenna to the P700 reaction center with the rate of 40 ps at room temperature, it was assumed that the red exciton states directly involved in the energy transport process. It was shown that the excited chlorophyll molecules, which correspond to the red exciton states, are localized on the peripheral part of the PS I monomer, particularly in the places of contacts of monomers in the trimer. The role of the red exciton states in PSI energy transport is discussed in details.

Poster 1

2,6-Dichlorophenolindophenol exhibits side effect on the acceptor side of photosystem I

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In photosynthetic electron transport chain, Photosystem I (PS I) performs oxidation of plastocyanin or cytochrome c_6 and reduction of ferredoxin or flavodoxin. Besides that, PS I also reduces O_2 leading to superoxide anion radical production. Investigation of activity of isolated PS I complexes requires an efficient electron supply to $P700^+$, which is provided by artificial compounds such as *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and 2,6-dichlorophenolindophenol (DCPIP) capable of immediate reduction of $P700^+$. Still far, there are few data describing TMPD and DCPIP as electron donors to PS I under continuous illumination. The aim of the presented work is to characterize TMPD and DCPIP (both in the presence of ascorbate) as electron donors to PS I isolated from *Synechocystis* sp. PCC 6803.

A single PS I turnover resulted in superoxide radical generation leading to O_2 uptake detectable by Clark type O_2 electrode. Methyl viologen (MV), an efficient autooxidable electron acceptor from PS I, was used to evaluate efficiency of electron donors since the reduction of $P700^+$ becomes a rate-limiting step in the MV presence. The rate of O_2 uptake was proportional to TMPD or DCPIP concentration in the presence of MV, with rates being higher in the presence of DCPIP than that of TMPD. That argues in favor of DCPIP being a better electron donor to $P700^+$ in PS I. In the absence of MV, the rate of O_2 uptake did not depended on TMPD concentration, while the increase in DCPIP concentration promoted the increase in the O_2 uptake rate. The latter effect was not observed in F_A/F_B-depleted F_X-core complexes. The apparent K_m (MV) in native PS I was higher when DCPIP was used as electron donor to $P700^+$ than that of TMPD (4.92±0.13 μM vs. 1.39±0.13 μM). The above results show that DCPIP can accept electrons from F_A/F_B and then reduce O_2. Therefore, despite TMPD being less efficient donor to $P700^+$ than DCPIP, it seems to be more appropriate donor, revealing no side reactions.

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Poster 2

Kinetic modeling of electron transfer in Photosystem I with variable internal and external acceptors

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Photosynthetic reaction center complexes contain a number of the redox-cofactors participating in various forward and backward electron transfer reactions, mechanisms of which is still a matter of debate. This complicates the analysis of experimentally obtained time-resolved kinetics of charge recombination. In general, the fitting of such complex kinetics is an ill-posed problem, i.e. a single dataset can be fitted by a large number of possible solutions, with arbitrary deviations from each other. To obtain reasonable guess on the processes underlying the observed kinetics, Tikhonov-Philips regularization algorithm, implemented in CONTIN program, was used to obtain the deconvolution of charge recombination kinetics in photosystem I (PS I) from cyanobacteria *Synechocystis* sp. PCC 6803 with minimal amount of *a priori* assumptions. The obtained kinetic parameters were used to construct the kinetic model of PS I electron transfer chain with reasonable values of intrinsic electron transfer reactions rates. Changes of electron transfer rates caused by substitutions of secondary electron acceptor phylloquinone in the A_1-site by plastoquinone and 2,3-dichloro-naphtoquinone, addition of external electron acceptor and incorporation in trehalose matrix were studied. In particular, obtained reaction rates of PS I with substituted quinones allow to estimate the redox-potentials of plastoquinone and 2,3-dichloro-napthoquinone bound in the A_{1A} and A_{1B}-sites.

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Poster 3

Studies on the monomeric form of Photosystem I

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Oxygenic photosynthesis provides the organic material, oxygen and fuel, which sustain life on earth, as we know it. Light energy is converted by two photosystems – photosystem I (PS I) and photosystem II (PS II). In nature PS I takes both monomeric and trimeric forms. In cyanobacteria both forms of PS I are present, while the trimeric one being the major one. The fact that the trimer to monomer ratio changes in respect to environmental changes suggests that PS I monomer may take a functional role by itself. To test this hypotheses we manipulated PS I genes in the Cyanobacterium *Synechocystis* sp. PCC 6803. We mutated the C-terminus of PsaL to interfere with the trimerization domain of PS I, and by that rendered a mutant generating mostly the monomeric form. After obtaining the desirable mutant strain we extracted the PS I and tested for crystallization conditions to solve its structure. So far we obtained crystals diffracting to 8 Å.

Poster 4

Interaction of the Photosystem I complexes containing different quinones in the A_1-site with exogenous electron acceptors

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The time-resolved near-IR absorption spectrometry was used to study the charge recombination kinetics in Photosystem I (PS I) from the wild type (WT) cyanobacteria *Synechocystis* sp. PCC 6803 containing native phylloquinone (PhQ), and from the deletion mutant strain *menB* containing plastoquinone (*menB*-PQ) or high-potential 2,3-dichloro-1,4-naphtoquinone (*menB*-Cl_2NQ) in the A_1-site in the presence of external acceptors methyl viologen (MV) or Cl_2NQ. As it was shown earlier, in the *menB*-PQ PS I forward electron transfer (ET) $A_1 \rightarrow F_X$ is ~1000 slower, than in the WT (Semenov et al., 2000, J Biol Chem 275: 23429), while substitution of PQ by Cl_2NQ resulted in complete prevention of the forward ET beyond A_1 (Mula et al., 2012, Photochem Photobiol Sci 11: 946). These data indicated that successive increase of the redox-potential (E_m) of the quinones in the A_1-site, leading to the decrease of the free energy (ΔG) difference, results in slowing and finally to termination of the $A_1 \rightarrow F_X$ ET.

In this work, we studied the effect of increasing concentrations of MV and Cl_2NQ on charge recombination kinetics in WT, *menB*-PQ and *menB*-Cl_2NQ PS I samples. It was shown that both MV and Cl_2NQ fully prevent the charge recombination in WT PS I at 10 and 1 μM, respectively. In *menB*-PQ PS I the saturating concentrations of MV and Cl_2NQ, were about an order of magnitude higher. In *menB*-Cl_2NQ PS I samples both external acceptors only partially prevented charge recombination even at the highest concentration used (~1 mM). These data suggest that the thermodynamic barrier of an ET from the high-potential Cl_2NQ to the terminal iron-sulfur clusters can be partially overcome by addition of high concentrations of exogenous acceptors. The kinetic modeling of the experimental data will be used for more precise evaluation of the E_m values of PhQ, PQ and Cl_2NQ in the A_{1A} and A_{1B}-binding sites.

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Section 1.6: Carbon Fixation and Photorespiration

Lecture 1

Biochemical and molecular basis of modulation by oxidative stress of photorespiratory components

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Photorespiration is now widely accepted as an adaptive response of plants to oxidative stress conditions, induced by both abiotic and biotic factors. The up-regulation of photorespiratory components during drought or salinity or excess light are reported in several C3 species. An inevitable consequence of the oxygenase activity of Rubisco under the present oxygen rich atmosphere produces P-glycolate, a toxic metabolite. The reactions of photorespiration metabolize such toxic P-glycolate into 3-phosphoglycerate, an important component of Calvin cycle, while at the same time limiting the loss of carbon. Photorespiratory reactions dissipate excess reducing equivalents and energy either by utilizing ATP/NAD(P)H/reduced ferredoxin or by sustained photosynthetic cyclic electron flow/an internal CO_2 pool. Thus, photorespiratory reactions contribute to the redox-homeostasis, optimize photosynthesis and protect against photoinhibition. Further, photorespiratory H_2O_2 could be a signal to modulate the expression of genes involved in the stress adaptations. Photorespiration is a typical phenomenon of inter-organellar interactions, involving chloroplasts, peroxisomes, mitochondria and cytoplasm. The crosstalk between the cellular redox status and photorespiration demonstrates an interesting network of flexible systems within plant cells, to prevent oxidative damage. We have been studying the consequences of the modulation of mitochondrial metabolism by suitable inhibitors and mutants. We have employed ROS inducers in different cellular compartments: acifluorfen methyl ester (AFM) (chloroplasts), paraquat (chloroplasts), menadione (MD) (mitochondria) and abscisic acid (ABA) (plasma membrane). The oxidative stress created by these ROS inducers caused an increase in ROS, up-regulation of catalase/anti-oxidative enzymes, increase in proline, and enhanced the operation of alternative pathway. Further the oxidative stress restricted not only photosynthesis but also photorespiratory glycolate metabolism. Besides the activity, protein and transcripts levels of three peroxisomal enzymes: catalase, glycolate oxidase and hydroxypyruvate reductase, were altered. The levels of key metabolites related to photorespiration revealed that the photorespiratory turnover was increased under redox modulation. Based on our results and literature, we propose that mitochondrial or chloroplast redox could act as a major signal to modulate the photorespiratory components in chloroplasts, mitochondria and peroxisomes.

Section 1.7: Artificial and Applied aspects of Photosynthesis

Lecture 1

Key issues in contemporary medicine, the microbiome and environmental sustainability

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Resistance to antibiotics and the spread of antibiotics metabolites are severe problems in contemporary medicine and ecology. Structures of complexes of eubacterial-ribosomes with antibiotics paralyzing them illuminated common pathways in inhibitory-actions, synergism, differentiation and resistance. Recent structures of ribosomes from a multi-resistant pathogens identified features that can account for species-specific diversity in infectious-diseases susceptibility. These may lead to design of environmental-friendly degradable antibiotics, which will also be species-specific antibiotics-drugs, thus the basis for a revolution in the antibiotics field, which its current preference for wide-spectrum drugs. Thus, reducing resistance while protecting the environment and preserving the microbiome.

Lecture 2

Photosystem I: From protein composition to photo-bio-nano-electronics, a personal prespective dedicated to Prof. Nathan Nelson

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Photosystem I (PS I), is a multi-subunit complex that functions as plastocyanin-ferredoxin oxido-reductase at the reducing-site of the photosynthetic electron transfer chain. At the late 1960's, PS I was identified by native gel electrophoresis as CP1: Chlorophyll Protein 1. In the mid 1970's, in Prof. Nathan Nelson laboratory, PS I complexes (called PSI-RC) were isolated by biochemical anion exchange and sucrose density gradient chromatographies. Three more decades of research that included purification of numerous cyanobacterial, algal and plants' PS I complexes passed till the atomic structures of cyanobacterial PS I and holo-PSI from plants containing also the PS I's Light Harvesting Complexes, were determined. The holo-PSI was solved in Prof. Nathan Nelson laboratory and published in NATURE in 2003 and 2007. The availability of high resolution structures facilitated the current 'engineering' state of PS I research in which PS I is bound to electrodes and used as a photo-absorbing charge separating unit to generate fuel (H_2) or electricity (photocurrent). The research focused on assembly of such photo-bio-electrochemical cells, based on native PS I, is attracting substantial interest as means to convert light energy into electrochemical/biofuel power, is still in its infancy. One of the prerequisites is that the PS I will be bound to the electrodes in a functional configuration that allows electron transfer communication between the primary photogenerated electron/hole species in PS I and the electrode surface. For that purpose, redox active polymers are used as charge carriers. Alternatively, metallic nanoparticles or conductive nanomaterials (e.g. grapheme/semiconductor) are used as electron transporting materials, and redox proteins (e.g. Cytochrome c) are used as electron transfer mediators/molecular relay units. These challanges will be discussed in my presentation that is dedicated to Prof. Nelson who already in 2009 stated: "Plant Photosystem I – The Most Efficient Nano-Photochemical Machine" (Journal of Nanoscience and Nanotechnology).

Lecture 3

From bacteriophytochromes to iRFP: Optimization of fluorescence by "local" and "remote" amino acid substitutions

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Bacteriophytochromes are promising tools for tissue microscopy and imaging due to their fluorescence in the near-infrared region. These applications require optimization of the originally low fluorescence quantum yields via genetic engineering. Factors that favour fluorescence over other non-radiative excited state decay channels are yet poorly understood. In this work we employed resonance Raman and fluorescence spectroscopy to analyse the consequences of multiple amino acid substitutions on fluorescence of the iRFP713 benchmark protein. Two groups of mutations distinguishing iRFP from its precursor, the PAS-GAF domain of the bacteriophytochrome P2 from *Rhodopseudomonas palustris*, have qualitatively different effects on the biliverdin cofactor, which exists in a fluorescent (state II) and a non-fluorescent conformer (state I). Substitution of three critical amino acids in the chromophore binding pocket increases the intrinsic fluorescence quantum yield of state II from 1.7 to 5.0% due to slight structural changes of the tetrapyrrole chromophore. Whereas these changes are accompanied by an enrichment of state II from ~40 to 50%, a major shift to ~90% is achieved by remote amino acid substitutions. Additionally, an increase of the intrinsic fluorescence quantum yield of this conformer to ~40% is achieved. The present results have important implications for future design strategies of biofluorophores.

Lecture 4

Fabrication of bio-conjugate photosystems using reconstituted photosystem I and II with molecular wires

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Photosynthesis in nature exhibits excellent photoelectrochemical properties. For example, the quantum efficiency of photoelectric conversion is about 100%. If it is possible to utilize this ultimate function, devices with maximal performance can be acquired. The high efficiency of photoelectric conversion in photosynthesis is achieved through the potential cascade called Z-scheme, in which the photo-excited electron transfers step by step to the redox species with optimal spatial arrangement. In our study, one of the redox species in the electron transfer pathway is replaced with the artificial molecular wire with a view to extract electrons from photosystems by the reconstitution process to form bio-conjugate photosystems. The bio-conjugate systems using photosystem I or II reconstituted with metal nanoparticles terminated molecular wire will be presented at the meeting.

Lecture 5

Formation of biohybrid device between photosysten I and carbon material

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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 I (PSI) for biohybrid device is one of the key research themes for sustainable energy. Carbon materials are also potential candidate as a next-generation device. Among them, single-walled carbon nanotubes (SWNTs) are new materials for their remarkable electronic properties, especially. In this study, we formed a complex between PSI and SWNTs which were reasonable to expect the high-efficient electron transfer device. The PSI attached to the SWNTs surface via binding peptides, which were introduced to the reducing site of PSI complex by genetic transformation since terminal electron acceptor of PSI is close to the PsaE subunit. Therefore, the SWNT is expected to accept electrons from the Fe-S cluster of PSI by photo-irradiation. We will discuss the optical response of the biohybrid device at the meeting.

Lecture 6

Light-driven whole-cell biocatalysis with recombinant cyanobacteria

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Biocatalysis has emerged as a key technology for the pharmaceutical and chemical industries. Oxidoreductases, highly selective enzymes that catalyze oxidative redox reactions under mild conditions, are already established for the environmental friendly production of pharmaceuticals and fine chemicals. However, one inherent limitation for the use of oxidoreductases is the stoichiometric requirement for organic co-substrates (e.g. glucose, isopropanol, etc.) as electron donors in heterotrophic production systems like *Escherichia coli*. These co-substrates are only partly oxidized in the process and most of the energy remains in side products that have to be removed costly from the reaction. The use of water as electron donor would avoid the demand for co-substrates and the generation of unwanted waste products.

Recombinant biocatalysts are usually produced with heterotrophic production systems like *E. coli*. In order to test the feasibility of photosynthetic biocatalyst production, two enantioselective model enzymes were expressed in *Synechocystis* sp. PCC 6803, cell extracts were used for biotransformations and synthesis of products was proven [1]. Subsequently, a stereoselective, NADPH-dependent enoate reductase was expressed in *Synechocystis* and whole-cells were applied for light-driven asymmetric C=C reductions. The efficiency of the reaction was comparable to typical whole-cell biotransformations in *E. coli*. Under optimized conditions, a solution of 100 mg prochiral 2-methylmaleimide was reduced to optically pure 2-methylsuccinimide (99% ee, 80% yield of isolated product). Moreover, volumetric productivities of up to 10 mm h^{-1} and product titers of up to 2 g L^{-1} were obtained [2].

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Lecture 7

The multiple changes of photosynthetic behaviors, the role of photorespiration during the astaxanthin accumulation in *Haematococcus pluvialis* grown outdoors in tubular photobioreactors

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Unicellular green alga *Haematococcus pluvialis* can accumulate amounts of astaxanthin. The multiple changes of photosynthetic behaviors during astaxanthin accumulation in outdoors cultured *H. pluvialis* were studied. In the initial 3 d of incubation, the capacities of light absorption and photosynthetic electron transport became more efficient with increased time. After 5 d of incubation, during amounts of astaxanthin accumulation, the pool size of the PS I end electron acceptors decreased significantly, leading to an over-reduction of the PS I acceptor side, indicated by a decrease in the maximal amplitude in the I-P phase of the chlorophyll *a* fluorescence (OJIP) transient. The L-bands and K-bands of the OJIP transient enhanced significantly, and the performance index (PIABS) as well as its individual partial components declined gradually with increased time after 5 d of incubation, indicating that the capacity of photosynthetic energy utilization decreased significantly during amounts of astaxanthin accumulation, leading to imbalance between photosynthetic light absorption and energy utilization. It was also indicated by the observation that the light absorption per active reaction center (ABS/RC) increased but the electron transport per active reaction center (ETo/RC), the efficiency of electron transport (ψ_o) and the quantum yield of electron transport (φ_{Eo}) decreased with increased time. The over-reduction of the PS I acceptor side and over-excitation of PS II reaction centers during amounts of astaxanthin accumulation would inevitably aggravate photoinhibition under high light, e.g. at midday. However, the sensitivity of photoinhibition in *H. pluvialis* decreased during the incubation, which might be because the accumulation astaxanthin can protect cells from photoinhibition. Interestingly, during the period of astaxanthin accumulation, the astaxanthin content was reduced significantly when photorespiration was inhibited by its specific inhibitor, carboxymethoxylamine. The inhibition of photorespiration did not change the dry weight, chlorophyll content and OJIP transients during the incubation; however, the inhibition of photorespiration significantly decreased the photochemistry of photosystem II and total photosynthetic O_2 evolution capacity. Moreover, the restriction in photorespiration was synchronized with a decrease of astaxanthin accumulation. These results suggest that the photorespiratory pathway in *H. pluvialis* can accelerate astaxanthin accumulation.

Lecture 8

Construction of a photoactive fluorescence sensor from the Orange Carotenoid Protein

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Orange carotenoid protein (OCP) undergoes significant rearrangements upon photoconversion and transition from the stable orange to the signaling red state. This includes a 12 Å translocation of the carotenoid cofactor and separation of N- and C-terminal domains. Here, we labeled OCP of *Synechocystis* with tetramethylrhodamine-maleimide (TMR) and obtained a photoactive OCP-TMR complex, whose fluorescence is highly sensitive to the protein state, reflecting changes in protein conformation and the distances from TMR to the carotenoid during the photocycle. Based on the observed Förster resonance energy transfer, we determined that the distance between TMR bound to a cysteine in the C-terminal domain and the carotenoid increased by 18 Å upon photoconversion with simultaneous translocation of the echinenone (ECN) into the N-terminal domain. Time-resolved fluorescence anisotropy revealed a significant decrease of the OCP rotation rate in the red state, indicating that the light-triggered conversion of the protein is accompanied by an increase of its hydrodynamic radius. Thus, our results support the idea of significant structural rearrangements of OCP, providing a completely novel approach to study its photocycle and non-photochemical quenching. Furthermore, we show that the obtained OCP-TMR complex is sensitive to light intensity, temperature and viscosity of the solvent, and, therefore, may serve as a promising markers for the development of novel biosensors.

Poster 1

Energy efficiency of C4 plants by the example of *Zea mays* L. and *Miscanthus sinensis* Anderss. on gray forest soils of Moscow region, Russia

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Solar radiation and technical energy are the main input energy flows into agroecosystems. Study of the efficiency of their usage is an important trend in current photobiology.

4-year field experiments have been made to estimate crops productivity, the value of input-output energy flows and efficiency of their usage by the crops at different level of fertilization.

Miscanthus is a class of perennial herbaceous plants, cereals family. Annually it can be cultivated for 15–20 years at one area. Two variants were provided: 1) the control (without any fertilizers) and 2) with applying fertilizers once for three years at $N_{120}P_{100}K_{100}$. The technique developed by IBBP RAS was taken as a base. The planting was made at 20x70 cm. Irrigation was made as often as required. Aboveground biomass was harvested in autumn before frosts. Three variants were planned in experiments with maize: control, $N_{90}P_{60}K_{40}$ and $N_{150}P_{190}K_{190}$, annually. Averagely for 4 years aboveground biomass of Chinese silver grass in the variant without any fertilizers was made up 7.5 t/ha dry matter and at applying fertilizer – 10.0 t/ha per year. Maize harvest was averagely 6.7 t/ha dry matter of aboveground biomass in the variant without any fertilizers, and – 9.0 and 11.0 t/ha, respectively, at applying fertilizers. Accounting the influence on soil fertility, energy efficiency (the ratio of the energy supplied in aboveground biomass to technical energy expended for cultivation and harvest) at Chinese silver grass was made up 13.9 in the control on average for 4 years, at fertilized variant – 12.1; at maize – only 6.7 – in the control and 5.9 – at fertilized variant or 2 times lower as compared to Chinese silver grass. PAR efficiency during cultures vegetation at Chinese silver grass in the control was made up 0.91, at fertilizers application – 1.34. At maize plants PAR efficiency equaled, on average, 1.38 – in the control and 1.91 and 2.34 at fertilized variants. However, due to longer vegetation period which starts early in spring from raise of average daily air temperature above +5°C, Chinese silver grass forms high aboveground biomass. Thus, Chinese silver grass uses natural and anthropogenic energy resources more efficiently as compared to maize at current technique of its cultivation. The lower energy efficiency of maize associates with the fact that higher technical energy expenses are required to restore soil fertility after its cultivation.

Poster 2

Microalgae and cyanobacteria: induction of lipids and screening promising strains

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Research interests of authors is related to the bioenergetics, screening of microalgae and cyanobacteria for biofuel production, the ways to increase effectiveness of cultivation microalgae as a source of biofuels. Significant trend in modern investigations of microalgae and cyanobacteria as advanced feedstock for biodiesel production is search for efficient stressors which could provide for lipid induction. Therefore, search for microalgae – potential lipids producers adapted to Russian natural conditions, including lipids producers tolerant to culturing in low temperatures – is rather an important task. To achieve this, samples have been withdrawn on expeditions to Russian regions in 2008–2014 to isolate lipid-containing microalgae from them. It has been shown that *A. platensis* could be not only the source of BAA, but have promise as producers of neutral lipids. Candidate strains have been investigated according to the following schedule:

1) Description and identification by genus or by species based on morphometric and cultural characteristics. Phylogenetic position identification of eight strains has been carried out by biomolecular methods;

2) Experimental selection of technological parameters to produce strain biomass under culturing standard optimal conditions and under stress conditions.

3) Experimental selection of technological parameters to produce strain biomass with lipid high content under stress conditions.

4) Biochemical analysis of biomass, produced at both culturing stages.

The effect of the following stressors has been under investigation: a) increased and decreased insolation (from (2÷4) μE/(m^2×s) to (450±25) μE/(m^2×s)); b) suboptimal temperatures (from (25±1)°C to (9±1)°C); c) lack of nitrogen and phosphorus in culture media; d) sparging by air, containing 2% of CO_2. Effectiveness of lipid-containing microalgae screening technique by fluorescent dye Nile red staining has been determined. It shows the spectrum of stressors providing lipids accumulation in two-stage microalgae culturing. Eight strains have demonstrated positive stress reaction while in two strains lipid content has decreased.

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Poster 3

Optimization of a photosystem 1 and 2 based photovoltaic cell

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Photosystem 2 (PS2) and Photosystem 1 (PS1) are the key components of natural photosynthesis. Light induced charge separations within the photosystems drive the electron transfer, and finally energy is stored in chemical compounds like NADPH. Moreover, PS2 catalyzes the unique light induced oxidation of water that allows access to an in principle unlimited source of electrons. Isolated photosystems can be immobilized by redox active polymers on electrode surfaces and generate electrical power during illumination [1, 2]. Both half-cells can be optimized separately.

A major problem of the PS2 containing anodic half-cell is the poor long term stability. To gain insight into the photo-degradation process, we combined electrochemical measurements with the simultaneous detection of reactive oxygen species (ROS) by fluorescence microscopy [3] and, based on the combination of PS1 with Pt-nanoparticles as catalysts for hydrogen production, we developed a cathodic half-cell that enables light driven generation of hydrogen. Local hydrogen production of the redox polymer embedded biohybrids was proven by scanning electrochemical microscopy (SECM) [4]. Coupling of both half-cells would in principle generate a biophotovoltaic fuel-cell that facilitates bias-free release of hydrogen from water and the simultaneous production of electrical power.

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Poster 4

Effects of grapevine leafroll assosiated virus 3 on the photosynthesis and antioxidant compounds in field grown grapevine (*Vitis vinifera* L.) plants

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Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops and is widely cultivated for the wine industry, as well as for the production of fresh and dried fruit in Azerbaijan. Grape leaf curl disease (GLD) affects the vines throughout the world and is considered to be the most economically destructive among all virus and virus-like diseases. Therefore, multiple phytopathological monitorings were carried out to identify GLD symptomatic vines in the major production areas, such as Ganja, Samukh, Absheron peninsula during the summer of 2015. By immunochromatographic test, the sandwich enzyme-linked immunosorbent assay (ELISA) and reverse-transcription polymerase chain reaction (RT-PCR) analysis, GLRaV-3 was detected in symptomatic vines. Changes in relative water content, leaf pigment content, soluble proteins, thylakoid membrane proteins and photochemical efficiency were studied in virus-infected leaves. The content of malondialdehyde, proline, ascorbate, hydrogen peroxide and intercellular phenol compounds were also investigated. It was found that GLRaV-3 caused an increase of malondialdehyde, hydrogen peroxide and proline contents in infected leaves compared with healthy samples. The level of total soluble proteins and ascorbate were also reduced in all virus-infected vine varieties. The content of intercellular phenol compounds was not significantly affected by GLRaV-3. Gradual reduction in green pigments like chlorophyll, carotenoids and anthocyanin was observed in all varieties. Similar results were observed for photosystem II efficiency by Chl fluorescence measurements. Some differences were recorded in thylakod proteins, leaf net photosynthetic rate, stomatal conductance and the transpiration rate in grapevine leaves.

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Poster 5

Measuring system for investigation of photosynthetic apparatus components-based bio-solar cells

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Development of the bio-solar cells on the basis of the components of photosynthetic apparatus (the thylakoid membranes, isolated complexes of photosystems 1 and 2) is an actively developing field of alternative energetic. The advantage of these solar cells is that they have a low cost and their production and use is environmentally-friendly.

The output current and efficiency of solar cells are dependent on environmental conditions: temperature, light intensity, spectral composition of light. This relationship is an important issue in the development and optimization of solar cells. Primarily it can be explained by high sensitivity of the components of photosynthetic apparatus to high and low temperature and high light intensity. Information about the dependence of cell efficiency on these parameters can be used for further optimization, the modification and improvement of solar cells.

In our laboratory, we developed and built the measuring system, which allows to read output current of solar cell at the defined environmental conditions: temperature, light intensity and quality. The system allows maintaining the solar cells at a constant temperature of 10 to 60°C. The maximum intensity of the light depends on the light source used. The user can set the desired source before measuring. The light intensity can be reduced by using neutral density filters. The spectrum of incident radiation is changed by color filters. With this system, one can conduct a comparative analysis of the sensitivity of various solar cells to low or high temperatures and high light. With the help of the measuring system one can determine the optimum operating conditions for a specific solar cell.

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Section 1.8: Regulation of Photosynthesis and Environmental Stress

Lecture 1

Calredoxin – a novel calcium-dependent sensor-responder connected to regulation of photosynthesis

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Calcium (Ca^{2+}) and redox signaling play important roles in acclimation processes from archaea to eukaryotic organisms. Herein we characterized a unique protein from *Chlamydomonas reinhardtii* that has the competence to integrate Ca^{2+} and redox-related signaling. This protein, originally identified in a proteomic study [1], was designated as calredoxin (CRX), as it combines four Ca^{2+}-binding EF-hands and a thioredoxin (TRX) domain. A protein crystal structure of CRX, at 1.6 Å resolution, revealed an unusual calmodulin-fold of the Ca^{2+}-binding EF-hands, which is functionally linked via an inter-domain communication path with the enzymatically active TRX domain [2]. CRX is chloroplast-localized and interacted with a chloroplast 2-Cys-peroxiredoxin (PRX1) [2]. Ca^{2+}-binding to CRX is critical for its TRX activity and for efficient binding and reduction of PRX1. Thereby CRX represents a new class of Ca^{2+}-dependent "sensor-responder" proteins. Genetically engineered *Chlamydomonas* strains with strongly diminished amounts of CRX, revealed altered photosynthetic electron transfer, a decreased expression of thioredoxin *f* upon high light treatment and impact in ROS defense underpinning a function of CRX in chloroplast redox and stress acclimation.

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2. Hochmal et al. Calredoxin represents a novel type of calcium-dependent sensor-responder connected to redox regulation in the chloroplast. Nature Communications, accepted (2016)

Lecture 2

ATP is the driving force of the repair of photosystem II during photoinhibition: Important role of cyclic electron transport

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The extent of photoinhibition of photosystem II (PS II) is a result of a balance between the rate of photodamage (light-induced inactivation) to PS II and the rate of repair of the photodamaged PS II. We have demonstrated that reactive oxygen species inhibit the repair of PS II by oxidizing and inactivating elongation factors of translation [1]. Therefore, the supply of electrons from PS I is essential for reduction and activation of the elongation factors, resulting in the stimulated synthesis of the D1 protein. Several other studies have suggested that ATP is essential for the D1 synthesis and the PS II repair. In this presentation, we will suggest that ATP is the driving force of the D1 synthesis and PS II repair. In the D1 synthesis, more than 2,000 molecules of ATP and other NTP are consumed. ATP is also necessary for the activation of FtsH, the protease for the degradation of D1 protein, and for the activation of some heat-shock proteins, which are involved in the PS II repair. The important contribution of ATP to the repair is evidenced by a number of results, in which the D1 synthesis and the repair of PS II are completely inhibited by mutational inactivation of ATP synthase, the presence of inhibitors of ATP synthase, and the presence of uncouplers of ATP synthesis. Therefore, ATP and activated elongation factors are two major contributors to the PSII repair. In addition, we will discuss an important role of cyclic electron transport in the synthesis of ATP, which drives the D1 synthesis and the PS II repair.

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Lecture 3

Elucidating the regulatory network of light harvesting in photoheterotrophic microalgae

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Light harvesting and energy dissipation processes are primary key events for the efficient formation of energy and reducing equivalents required for CO_2 fixation. In order to exploit this capability it is crucial to understand molecular details of the complex light conversion processes [1]. Adjustment of light harvesting is needed to efficiently drive photosynthesis, but is also important to prevent potential cell damage caused by excess of light energy. A precise blueprint of the regulation of light conversion in microalgae will eventually fuel into ongoing biotechnologically driven approaches aiming to enhance photon conversion efficiency rates. Novel insights into the function of a distinct LHCII subunit in energy quenching processes [2], and in the regulation of light-harvesting following altered carbon source availability [3] and redox-dependent fine tuning of LHC translation activity [4] have been recently obtained. In the microalga *Chlamidomonas reinhardtii* light-harvesting activity is controlled on different levels including translation regulation of LHC transcripts with the repressor NAB1 as a central control hub. Fine tuning of the antenna size includes (de)nitrosylation of NAB1 and regulation of the nuclear NAB1 promoter, which is activated when linear photosynthetic electron flow is restricted by CO_2-limitation in a photoheterotrophic context. Translation control as a newly identified long-term response to prolonged CO_2-limitation replaces LHCII state transitions as a fast response to PSII over-excitation. A regulation mechansism is proposed operating on distinct time-scales and in different cellular compartments to fine-tune light-harvesting in photoheterotrophic eukaryotes.

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Lecture 4

Variation of redox state of plastoquinone pool in cyanobcteria revealed by photochemical quenching and non-photochemical quenching of chlorophyll fluorescence

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As photosynthetic prokaryotes, cyanobacteria harbor photosynthesis and respiration in a single cell compartment with direct interaction between the two metabolic pathways. Plastoquinone (PQ) pool is shared by the respiratory and photosynthetic electron transfer, resulting in the reduction of PQ pool in the dark. This complicated the interpretation of chlorophyll fluorescence measurements in cyanobacteria and special attention should be paid for the assessment of photosynthesis by chlorophyll fluorescence. We further found that the redox state of PQ pool in the dark varies depending on the cyanobacterial species. In some species, PQ pool is oxidized in the dark as in land plants. The observed difference indicates that the degree of interaction between respiratory electron transfer and photosynthetic electron transfer differs among different cyanobacterial species. The variation could not be ascribed to the phylogenetic differences but possibly to the light environment of the original habitat. It must be noted that 'non-regulated' thermal dissipation increased under high-light conditions in all cyanobacterial species tested. Such 'non-regulated' thermal dissipation may serve as 'regulatory' mechanism for the acclimation to high-light conditions.

Lecture 5

Kinetic and computer multiparticle modeling of photosynthetic regulation

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Kinetic models describing H^+ fluxes across thylakoid membrane and electron transport processes in PSII were developed. The model parameters were identified using experimental fluorescence induction data, registered at the initial and late stages of nitrogen starvation. The simulation results demonstrate switching of the electron transport from the active state to the inactive one, controlled via local stromal H^+ concentration, which increases during the period of nutrient starvation.

Multiparticle Brownian simulation of Fd molecule interaction with FNR (which couples light reactions with the Calvin-Benson cycle) and Hydrogenase (which produces hydrogen) demonstrates an increase in the rate of Fd-Hd complex formation at higher pH (pH ~ 8).

Complicated geometric organization of the photosynthetic membrane was taken into account in a complex computer model which combines different simulation techniques. The processes inside photosynthetic multienzyme complexes were described by ODE for their state transitions, the interactions of mobile carriers with the complexes – by multiparticle Brownian dynamics, ΔpH evolution – by partial differential equations for H^+ concentration in lumenal and stromal spaces. The processes were simulated in heterogeneous system, where PSII complexes are located in granal, and PSI complexes – in stromal areas. Simulations showed that after the suppression of PSII activity as a result of starvation, the local H^+ concentration got higher in granal, and lower – in stromal areas. These results support the hypothesis that pH can act as a regulating factor switching the electron flow from the normal path to the Calvin-Benson cycle to the path of hydrogen evolution.

The work was supported by grants of the RFBR NN 14-04-00302, 14-04-00326

Lecture 6

A feed-forward loop consisting of the response regulator RpaB and the small RNA PsrR1 controls light acclimation of photosystem I gene expression in *Synechocystis* sp. PCC 6803

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Down-regulation of genes encoding subunits of photosystem I (PS I gene) is one of the key characteristics of the high-light (HL) acclimation in the cyanobacterium *Synechocystis* sp. PCC 6803. The transcriptional regulator RpaB and the small RNA PsrR1 (photosynthesis regulatory RNA1) have been suggested to be the two most critical factors for this response. In this study, we found that RpaB binds to the recognition sequence HLR1 highly conserved in the core promoter region of the *psrR1* gene orthologs. RNA gel blot analysis together with chromatin affinity purification (ChAP) analysis suggested that PS I genes are activated and the *psrR1* gene is repressed by the binding of RpaB under low-light (LL) conditions. DNA binding affinity of RpaB declines within 5 min after the shift from LL to HL conditions, leading to the prompt decrease in PS I promoter activity together with derepression of *psrR1* gene expression. Accumulating PsrR1 molecules then prevent translation from pre-existing PS I transcripts. Our findings suggest that RpaB and PsrR1 constitute a feed-forward loop for the regulation of PS I gene expression to achieve a rapid acclimation response to the damaging HL conditions.

LECTURE 7

DEG PROTEASES IN THE THYLAKOID LUMEN AND THEIR ROLE IN RESPONSE TO STRESS

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The photosynthetic machinery is constantly prone to oxidative damage that leads to photoinhibition. A key step in the repair cycle of damaged photosynthetic complexes is the proteolytic removal of oxidized subunits and their replacement by newly synthesized ones. Proteases of two families have been implicated in this process: the ATP-dependent FtsH metallo-proteases and the ATP-independent Deg serine proteases. Of the 16 *Arabidopsis Deg* genes, the products of *Deg2* and *Deg7* are located in the chloroplast stroma, whereas Deg1, Deg5 and Deg8 are found in the thylakoid lumen. Deg1 forms active homo-hexamers at acidic pH, degrading photosynthetic proteins, especially in relation to the PS II repair cycle. Deg5 and Deg8 form hetero-complexes, performing apparently similar functions, raising the question whether the two complexes are redundant. To answer this, we generated a full set of single, double and triple KO mutants and compared their phenotypes. We found that under optimal growth conditions Deg5-Deg8 mutants look like WT, but Deg1 mutants are smaller and show higher sensitivity to photoinhibition. Under harsher conditions, Deg5-Deg8 mutants are also affected, although less than Deg1 mutants. However, the functions of the two complexes are somewhat redundant, as overexpression of Deg5-Deg8 can partially compensate for the loss of Deg1. Comparative proteomics revealed moderate up-regulation of thylakoid proteins involved in folding, translocation, assembly and degradation, and down-regulation of components of all photosynthetic complexes. Testing the steady-state level of the thylakoid Deg proteases in WT plants demonstrated that Deg1 is approximately two-fold more abundant than the Deg5-Deg8 complex. Moreover, recombinant Deg1 had higher *in vitro* proteolytic activity compared with Deg5, Deg8 and the combination of the two. These results suggest that differences in abundance and proteolytic activity are the source of the differential importance of the two complexes *in vivo*. Deciphering the 3D structure of Deg1 revealed a novel activation mechanism. Upon protonation of His244 in the inactive monomer, the N-terminal α-helix is stabilized in a proper orientation for interaction with a neighboring protomer, allowing trimerization. Once trimers are formed, they dimerize to form proteolytically active hexamers. Thus, the same light conditions that run photosynthetic electron transport, but also cause oxidative damage, activate the Deg1 protease that is involved in the repair cycle of damaged proteins.

Lecture 8

Role of stt7 kinase in acclimatization and organization of photosynthetic apparatus to salt in *Chlamydomonas reinhardtii*

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Salt is a major threatening factor for growth of plants/algae, here, our study was mainly focused on the organization of photosynthetic apparatuses to the long term responses of NaCl in *Chlamydomonas reinhardtii*. We have ascertained cell wall as the reason for palmelloid formation in *C. reinhardtii* cells when grown under salt and it was determined using a cell wall mutant CC-503, stt7 and pgrl-1. Increase in F_o and drop in the F_v/F_m ratio, derived from Chlorophyll fluorescence transients reflect the redox state i.e., reduction of PQ pool. Also, low temperature emission spectra emanated that the PS I emission is increased in salt grown cells in wt but not in stt7 and pgrl-1 mutants indicates that, is stt7 dependent reduction of PQ pool. For the first time, we are showing the STT7 kinase dependent light harvesting complex II (LHC) phosphorylation in both short and long term treatments with various NaCl concentrations and furthermore, confirmed the STT7 kinase mediated LHCII phosphorylation using *stt7* mutant, which is defective in state transitions. Thus, we report here the short and long term NaCl induced state transitions, similar to the light induced state transitions. Surprisingly, as a part of acclimation responses in the long term treatments, increase in trimeric LHCII complexes and LHCII phosphorylation reflects the necessity of continuous PQ pool reduction. Blue native PAGE, protein profile analysis had shown that the increased LHCII trimers along with the damaged photosystem (PS) II reaction centers quench the excess unused energy and protect PS II from salt. Protein profile analyses indicate that PS II core proteins were more prone to damage by salt stress, however, most of the PS I core and LHCI proteins were increased since they may facilitate the protection during salt stress. Additionally, glycine betaine has retarded the salt effect since it has the highest cellular osmoprotective efficiency. Also, pgrl-1 mutant shows that the abundance of PsaH and G, of PS I is not changed indicates that cyclic electron transport is active in acclimitization. Inference drawn from our results is that stt7 plays a key role in implementing the state transitions as short term and photosystem stoichiometry adjustment as long term responses by sensing the redox state of PQ pool.

Lecture 9

Photosystem I in photochemical and non-photochemical quenching of excitation energy

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Photosystem I (PSI) uses light energy and electrons from water-splitting photosystem II (PSII) to produce NADPH. The Iron-sulphur (FeS) clusters of PSI are extremely sensitive to photodamage when receiving excess of electrons from PSII. We demonstrate a vital importance to maintain high $P700^+/P700$ ratio even upon increase in light intensity in order to protect the FeS-clusters of PSI from photooxidative damage [1]. For this purpose, the photosynthetic machinery in plants is endowed with a strong ΔpH-dependent control mechanism of linear electron transfer (LET) from Cyt b_6f to PSI [2]. Concomitantly, excess energy is dissipated by the PSBS- as well as the LHCII-PSII core phosphorylation-dependent mechanisms. However, in the absence of ΔpH, PSI takes a central role in excess energy dissipation and control of LET. The *proton gradient regulation 5* (*pgr5*) mutant lacks the formation of a strong trans-thylakoid ΔpH under high light, resulting in gradual damage of the FeS-clusters and subsequent slow-down of LET [3, 4]. Notably, the non-functional PSI centres turn into non-photochemical quenchers (PSI-NPQ) of excitation energy and dissipate thermally the surplus of excitation energy [1]. Moreover, photodamage of PSI increases the antenna cross-section of PSI, and conversely decreases that of PSII, via thylakoid protein phosphorylation dependent mechanisms [5], thus maximizing the amount of excess energy dissipation by PSI. It is conceivable that the novel transition mechanism of PSI from photochemical to non-photochemical quencher of excitation energy, as demonstrated here, is of remarkable physiological significance, for example, in survival of evergreen plants in nature.

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Lecture 10

A new insight into mechanisms of oxygen photoreduction in the photosynthetic chain

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Reduction of O_2 by components of the photosynthetic electron transport chain in the light represents one of the alternative pathways of excessively absorbed light energy dissipation. Electron flow to O_2 occurs simultaneously with $NADP^+$ reduction and results in production of reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2). Our works revealed that production of both $O_2^{\bullet-}$ and H_2O_2 occurs simultaneously outside thylakoid membrane and within it [1–3]. The contribution of ferredoxin to O_2 reduction has been established to be negligible when $NADP^+$ was present [4]. Phylloquinones occupying the A_1-sites of photosystem I have been recognized as an essential O_2 reducing components in high light [5], which could be responsible for $O_2^{\bullet-}$ appearance within the membrane [6]. Plastoquinol of the plastoquinone pool has been proposed as the main antioxidant reducing intramembranous $O_2^{\bullet-}$ to H_2O_2 within the membrane [7, 8]. The evolutionary adaptations of the photosynthetic apparatus providing a control of electron flow to O_2 [6] and possible signaling function of H_2O_2 produced with photosystem I and the plastoquinone pool involvement [9] are discussed. The work was supported by Russian Science Foundation #14-14-00535.

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Lecture 11

Thylakoids win the competition for cadmium within plant chloroplasts

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Cadmium is one of the most toxic heavy metals and widespread pollutant. Photosynthesis is important target for Cd action, and photosystem II (PSII) is highly sensitive to Cd *in vitro*. Plants apply a number of strategies to protect their chloroplasts from Cd penetration. However Cd *in vivo* can inhibit photosynthetic processes.

In a whole terrestrial plant the most regular Cd effect on photosynthesis is inhibition of Calvin-Benson cycle, the effect on processes in PSII is not so assured. In contrast to *in vitro* studies, the Cd effect *in vivo* can be achieved through direct or indirect action. For example, Cd inhibits formation of stomata and their opening which limits CO_2 uptake and consequently – Calvin-Benson cycle. We tried to find direct Cd effect on chloroplastic processes.

In our previous work [1] we found that in hydroponically grown barley plants chloroplasts accumulate relatively high portion of Cd. We revealed one process that correlated with Cd accumulation in chloroplasts – photoinhibition of PSII (NPQ-qI).

Further we suggested that a direct effect can correlate with Cd accumulation. We used gentle method of chloroplast envelope disintegration (hyperosmotic shock) and centrifugation to separate stroma and envelope from thylakoids. Less Cd portion remains in stroma (and envelope) but most – in thylakoids. This is in good agreement with previously obtained results [1]: the level of mRNAs in chloroplast stroma was not changed and the level of proteins in thylakoids decreased. Therefore we can expect that Cd in chloroplasts of terrestrial plants inhibits mainly processes in thylakoids (electron transport) and to a lesser extent in stroma (Calvin-Benson cycle, gene expression).

To verify the specificity of Cd distribution we studied accumulation of another two heavy metals – Cu and Fe – between stroma and thylakoids. Also we studied the effect of Cd, Cu or Fe stress on the accumulation of essential cations Ca, Mg, Mn, Zn in both stromal and thylakoidal fractions. The results obtained will be discussed in the report.

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Lecture 12

Red or dead: photosynthetic acclimation in a carotenogenic microalga *Haematococcus pluvialis* under stress

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We report on peculiar mechanisms of stress acclimation in the photosynthetic apparatus (PSA) of *Haematococcus pluvialis* (Chlorophyceae). Under favorable conditions, the microalga exists as green cells converted under stress to red haematocysts (HC) enriched with ketocarotenoid astaxanthin, an added-value nutraceutical much sought after on the world market. The extremely stress-tolerant HC were thought of as resting, metabolically quiescent cells. Recent evidence indicates that the HC formation involves deep changes in cell ultrastructure, metabolism and energy acquisition mode. We followed the engagement of photoprotective mechanisms (i) under favorable conditions, (ii) in the course of stress-induced haematocyst formation and (iii) during recovery from the stress. In the case of the stressed cells converting to HC (or recovering from the stress) their PSA was reduced and energy-dependent photoprotective mechanisms such as qT and NPQ, were down-regulated along with astaxanthin accumulation. On this background, a transient up-regulation of PSA was detected (after 20–50 h of the stress) tentatively related with the peak of metabolic activity found earlier in the forming haematocysts.

The stress response in this system encompasses secondary carotenogenesis together with a reversible transition from 'active' (energy-dependent) to 'passive' photoprotective mechanisms. The processes confer *H. pluvialis* its unique ability to endure harsh habitats from deserts and mountains to Arctic seas.

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Lecture 13

Proton signal as probable mechanism of photosynthetic response induced by variation potential in higher plants

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Local damages induce unique electrical signal in higher plants (variation potential, VP). VP propagates and inactivates photosynthetic processes in undamaged leaves; the inactivation changes resistance of photosynthetic machinery. It is known that decrease of activity of H^+-ATPase in plasma membrane is main mechanism of VP generation. We suppose that the activity decrease is a key mechanism of VP-induced inactivation of photosynthesis. Two groups of arguments support this hypothesis. Firstly, preliminary leaf treatment by a specific inhibitor of H^+-ATPase (orthovanadate, OV) decreases magnitude of VP-induced inactivation of photosynthesis; the treatment by specific activator of H^+-ATPase (fusicoccin) increases this magnitude. Secondly, application of OV induces inactivation of photosynthesis in protoplasts; the inactivation is similar with VP-induced photosynthetic response. Changes in apoplastic and cytoplasmic pH caused by decrease of H^+-ATPase activity are probable mechanism of VP-induced inactivation of photosynthesis. The supposition is supported by (i) high correlation between changes in photosynthetic parameters and pH, (ii) dependence of quantum yields of photosystem I and II of isolated chloroplasts on pH in incubation medium, (iii) inactivation of photosynthesis after artificial H^+ flux into cells of leaf, and (iv) results of simulation of VP influence on photosynthesis. Two potential mechanisms can connect photosynthetic processes with changes in apoplastic and cytoplasmic pH: decrease of CO_2 influx under VP-induced alkalization of apoplast and inactivation of dark and light reactions of photosynthesis under acidification of stroma of chloroplasts. Thus, H^+-ATPase plays a key role in VP-induced inactivation of photosynthesis; this result can be used for development of new methods of regulation of plant photosynthetic response to stressors. In particular, our results show that treatment of some phytohormones can change H^+-ATPase activity and increases VP-induced inactivation of photosynthesis. This work was supported by the Russian Science Foundation (Project No. 14-26-00098).

Poster 1

The function of FoF1-ATPase has an influence on the phycobilisome

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In previous study, we performed a series of evolution experiments to investigate the adaptive evolution of *Synechocystis* sp. PCC 6803 under acid stress conditions. We obtained acid-tolerant strains from long-term culture experiments. Based on whole-genome sequencing, we detected 11 mutations compared with the parent strain, including the FoF1-ATPase operon. In this study, we aim to clarify the detail contributions of FoF1-ATPase to acid stress tolerance.

To determine whether mutation of *sll1321* involved in acid stress tolerance, we constructed *sll1321* complement strain, which strain did not have native *sll1321* and have the mutation of *sll1321* was put behind the constitutive P_{cpc560} promoter and integrated into the *slr2030-slr2031* neutral site. The growth of the mutant strain was significantly inhibited compared with that of WT cells under non-stress condition. Since this promoter did not induce under acid condition, this strain did not exhibit acid stress tolerance. Now we construct the strain which have a native promoter.

Sll1321 mutation caused that transcript of *nblA* increased, CpcC2 slightly decreased under non stress condition. In addition, the mutation strain abolished light-induced proton extrusion activity. Therefore Sll1321 mutation might control photosynthesis by degradation of phycobilisome to prevent acidification in thylakoid lumen.

Poster 2

Response of photosynthetic apparatus, methobolic and antioxidant defense enzymes to phytoplasma infection in pepper (*Capsicum annuum* L.) leaves

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Phytoplasmas are cell wall-less, Gram-positive bacteria with low G+C content phylogenetically belonged to the class Mollicutes. These microorganisms are pathogens of important annual crops as well as perennial cultures, causing a wide variety of symptoms that ranges from mild yellowing to death of infected plants. All these pathological changes are closely related to the reduced rates of photosynthesis, also to the effects on metabolic and antioxidant enzyme activities. The aim of the present work was to study the changes in photosynthetic apparatus, methobolic and antioxidant enzyme activities in field grown pepper plants (*Capsicum annuum* L.) infected by phytoplasmas. Phytoplasmas were detected in the symptomatic pepper samples by nested PCR amplification of their 16S rDNA with universal primers for phytoplasmas. Phylogenetical analyses of the amplified 16S rDNA showed that the phytoplasmas infecting pepper plants corresponded to "*Candidatus* Phytoplasma solani". Photosynthetic characteristics, activities of some metabolic and antioxidant defense enzymes were evaluated in pepper-phytoplasma pathosystem. Phytoplasma infection considerably decreased leaf net photosynthetic rate, stomatal conductance and the transpiration rate in pepper leaves, and also strongly reduced pigments and soluble proteins. A significant reduction in the level of total chlorophyll, and carotenoids was observed in all infected pepper plants. The activities of metabolic enzymes, including NAD-malatdehidrogenase (NAD-MDH), aspartataminotransferase (AsAT) and alaninaminotransferase (AlAT) increased in the leaves of infected plants compared with healthy plants. Considerable differences for antioxidant enzymes and PS II efficiency were also induced by phytoplasma infection.

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Poster 3

Gas exchange parameters of wheat genotypes under soil water deficit

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We studied the effect of soil water deficit on gas exchange parameters of 8 durum wheat (Garagylchyg-2, Vugar, Shiraslan-23, Barakatli-95, Alinja-84, Tartar, Sharg, Gyrmyzy bugda) and 14 bread wheat (Nurlu-99, Gobustan, Akinchi-84, Giymatli-2\17, Gyrmyzy gul-1, Azamatli-95, Tale-38, Ruzi-84, Pirshahin-1, 12ndFAWWON №97, 4thFEFWSN №50, Gunashli, Dagdash, Saratovskaya-29) genotypes grown in irrigated and non-irrigated field conditions. Gas exchange was measured by using LI-COR 6400XT Portable Photosynthesis System from boot stage to grain formation. Drought caused a decrease of photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (E). The P_n greatest in flowering stage, however g_s and E greatest in boot stage. An increase in the intercellular CO_2 concentration (C_i) in the condition of water deficit was observed in grain formation stage especially in genotypes Garagylchyg-2, Barakatli-95, Gobustan, Akinchi-84, Ruzi-84, Pirshain-1, 12ndFAWWON №97 and Gunashli. A relatively high P_n was maintained to the stage of grain formation in flag leaf of genotypes Shiraslan-23, Sharg, Gyrmyzy gul-1, 4thFEFWSN №50, Dagdash in drought condition. Mesophyll conductance, calculated from P_n/C_i ratio and water use efficiency, calculated from P_n/E ratio were higher in genotypes Tartar, Sharg, Gyrmyzy bugda, Gyrmyzy gul-1, Tale-38, 4thFEFWSN №50, Dagdash in the flowering and grain formation stages in water stress condition. The C_i/C_a ratio increased in water stress condition. We also found that leaf temperature in the chamber was higher in irrigated plants than in plants exposed to water deficiency, which can be associated with concomitant enhanced photosynthesis non-photochemical quenching. The P_n was positively correlated with crop growth rate, water use efficiency, relative water content and Chl (*a*+*b*) content of flag leaf, stem and spike dry mass.

Poster 4

Luminescence and antioxidant responses of chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) to chilling stress during early vegetative stages

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Chinese cabbage hybrid F1 Nika seeds were germinated under controlled environment conditions with an irradiance of 225±25 μmol m^{-2} s^{-1}, a 20±2°C temperature regime, and a 12-h photoperiod. After 20 days, at the 4th true leaf stage, some plants were shifted to a −2°C temperature regime with the same photoperiod and irradiance for 3 days. All measurements were made on 3rd fully expanded leaves of either warm-grown control (20±2°C) or treated with low temperature (−2°C) plants. The effect of chilling stress has been examined by analyzing the Chl *a* fluorescence induction kinetics and thermoluminescence (TL) glow curves with well-resolved TL bands at about −2°C, 35°C, and 75°C, corresponding to Q-, B-, and high-temperature chemiluminescence bands. There was no significant effect on the maximum quantum yield (F_v/F_m), which was around 0.8. The performance index $PI_{ABS}=(RC/ABS)(F_v/F_0)(\psi_0/(1-\psi_0))$ corresponds to the efficiency by which a trapped photon could provide electron transfer beyond Q_A^-. Its value declines by 29–33%. We have analyzed the effects of chilling stress on individual components of PI_{ABS}. The reaction centers density (parameter *RC/ABS*) was affected but insignificantly. The ratio F_v/F_0 decreased by 12–14%, indicating that there may be some structural disorganization in the size of PS II antenna. The ratio $\psi_0/(1-\psi_0)$ decreased, as compared to control, more significantly (by 22–23%), indicating that the efficiency of the forward electron transfer was decreased. The increase (by 36–43%) in the amplitude of the Q-band of TL in treated plants suggests that accumulation of Q_A^- might enhance a probability of the non-radiation energy dissipation in PS II, which might serve as thephotoprotection mechanism under stress conditions. The TL band at 75°C, which intensity may serve as an indicator of oxidative stress, revealed asignificant increase in its amplitude by 64–87%. This observation correlates with the decrease in the total antioxidant content (TAC) in treated leaves by 32–42%. Parameters PI_{ABS}, TAC and the amplitude of the high-temperature TL peak can be used as convenient indicators to monitor chilling stress in Chinese cabbage plants.

Poster 5

Effect of coherent radiation on some morphometric parameters and photosynthetic activity in regenerated plantlets of *Lavandula hybrid* Rev. cultivars

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Lavandin (*Lavandula hybrida* Rev.) is a valuable medicinal and fragrant crop. Its crop yield and essential oil yield are in 2–4 times higher than those of productive lavender cultivars. The survey of lavender and Lavandin plants in the Crimea revealed that many of them were damaged with viral diseases. Methods of organ and tissue culture *in vitro* are widely used in breeding, propagation and preservation of valuable garden plant species and cultivars. Their use is particularly important for plants vegetative propagated only and need the cleaning up. In order to improve the ways of multiplication for the unique Lavandin genotypes ('Rabat' and 'Snezhnyi Bars') previously cleaned up by chemotherapy *in vitro*, we first used the laser light (Computerized laser irradiation system for plant objects LPI-2/C650/60), morphometric indexes were estimated and functional state of plants was analyzed in the parameters of slow chlorophyll fluorescence induction (with fluorometer LPT-3/CFL). Before the irradiation microshoots were cultured on MS medium for 8–12 months from the introduction of plants' meristem *in vitro*. Observations were made every 5 days for 30 days after the impact of the coherent radiation. Irradiation was carried out once. Various irradiation power densities were used: from 2 to 15 mW/m^2 and the time of exposure from 60 to 480 seconds. The best results of increase growth (at 30–65% compared to the control) and the formation of new clustering nodes were noticed at lower power densities (2–4 mW/m^2) and under shorter duration of exposure (60 seconds). Photoinhibition wasn't observed. The relative indexes of slow fluorescence $(F_m - F_{st})/F_m$ were 0.43–0.71 a.u., the viability index (F_m/F_{st}) – 1.12–2.03 a.u. The results can be used to accelerate breeding process and production of virus free planting material of Lavandin.

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Poster 6

High temperature effects and the photoprotective responses in chlorophyll *b* deficient wheat mutants

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Chlorophyll *b*-deficient mutants represent an interesting model for studies aimed at regulation of electron transport. The effects of high temperature on carbon assimilation, photosynthetic electron and proton transport as well as photoprotective responses were carried out in chlorophyll *b*-deficient mutant lines (ANK-32A and ANK-32B) and wild type (WT) of wheat (*Triticum aestivum* L.). Despite the low chlorophyll content and chlorophyll *a* to *b* ratio, the non-stressed mutant plants had the similar level of CO_2 assimilation and photosynthetic responses as WT. However, in ANK mutant plants exposed to prolonged high temperature episode (42°C for ~10 hours), we observed lower CO_2 assimilation compared to WT, especially when a high CO_2 supply was provided. In all heat-exposed plants we found approximately the same level of PS II photoinhibition, but the decrease in content of photooxidizable PS I was higher in ANK mutant plants compared to WT. The PS I damage can be well explained by the level of overreduction of PS I acceptor side observed in plants exposed to high temperature, which was, in turn, the result of the insufficient transthylakoid proton gradient associated with low non-photochemical quenching and lack of ability to downregulate the linear electron transport to keep the reduction state of PS I acceptor side low enough. Compared to WT, the ANK mutant lines had lower capacity to drive the cyclic electron transport around PS I in moderate and high light; it confirms the protective role of cyclic electron transport for the protection of PS I against photoinhibition. Our results, however, also suggest that the inactivation of PS I in heat stress conditions can be the protective mechanism against photooxidative damage of chloroplast and cell structures.

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Poster 7

Impact of annual changes of temperature and light (PAR) on induction of Chl *a* fluorescence *in situ* in *Stellaria media* (L.) and *Plantago maior* (L.)

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Chl *a* fluorescence, a mode for estimation of photosynthesis, especially is sensitive to changes of temperature and intensity of light (PAR). This enables to follow the influence of these ecophysiological parameters on photosynthesis of native plants. Investigated were the influence of annuall changes of temperature and PAR on parameters of Chl *a* fluorescence in *Stellaria media* (L.), plant species more represented in colder part of the year and *Plantago maior* (L.), plant species more represented in warmer part of the year. Lower values of PAR and temperature slowed electron transport in PS II, but lower temperatures reduce quantum efficacy of PS II and improved processes in system of antennae and size of plastoquinone pool of PS II in *Stellaria media* (L.). Lower temperatures reduced quantum efficacy and slowed electron transport in PS II in *Plantago maior* (L.). The fact that PAR (besides temperature) influences photosynthesis and that lower temperatures activated processes in system of antennae and on acceptor side of PS II, as a «counterbalance» of inhibition of quantum efficacy and electron transport in PS II, caused by low temperatures, indicated possible reasons of low temperature resistance to photosynthesis in *Stellaria media* (L.). On the contrary, in *Plantago maior* (L.) low temperatures caused inhibition of quantum efficacy electron transport in PS II, whatever caused low temperature photoinhibition and stepped quenching all physiological processes in that species. This might be a reason for different acclimation to low temperatures and different life strategies for these species which settled at very neighbouring sites.

Poster 8

Reorganization of pigment-protein complexes in *Ajuga reptans* leaves at overwintering

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In seasonal climate the photosynthetic apparatus (PSA) of evergreen and wintergreen species have structural and functional changes caused by acclimation to unfavorable conditions during the late fall–winter–early spring period. Protective mechanisms of PSA are most fully studied in conifers. The data concerning the herbs wintering under snow cover are practically absent. Seasonal changes in photosystem II (PS II) organization and the pigment content in wintergreen herbaceous *Ajuga reptans* were studied to show the adaptive reactions of PSA to overwintering in natural habitats (61°67' N, 50°77' E). The experiments were performed in 2014–2015. Maximum accumulation of chlorophyll (Chl) in mature summergreen leaves occurred in September. The highest values of PS II photochemical activity [variable fluorescence (F_v)/maximum fluorescence (F_m)], equaled to 0.80–0.82, were observed in July–September. The earliest changes occurred at the beginning of November, at freezing stress, with partial losses of Chl and degradation of PSII-LHCII complexes. In November there was a decrease in NPQ (nonphotochemical fluorescence quenching) and F_v/F_m value (0.62). The carotenoid composition varied seasonally with a double fold increase in β-carotene, which was a precursor of zeaxanthin (Z), during the late fall and early spring. The combined pool of violaxanthin (V), antheraxanthin (A) and Z increased during winter, and deepoxidation state (DEPS) of xanthophylls (Z+0.5A/V+A+Z) increased from 20 to 34% from November to April, reflecting a conversion of V to Z. The loss of Chl continued during winter, however by 50% maximum. The timing of fall and winter changes indicated that there was some inverse correlation between an increase in DEPS and a decrease in F_v/F_m. In May a recovery of photochemistry of PS II, pigment rearrangements and V deepoxidation occurred concomitantly. The recovery of photosynthesis in wintergreen leaves was completed in spring (April) under optimal conditions, with rapid increases in F_o (instantaneous fluorescence), as well as DEPS, followed by a recovery of F_v/F_m; all without any net increase in Chl. The late fall and early spring reorganization of PS II and the light-harvesting complexes allow *Ajuga* to maintain a large stock of Chl in the quenched, photoprotected state, enabling a rapid recovery of photosynthesis in spring.

Poster 9

Roles of anionic lipids clarified with an SQDG-deficient mutant of *Thermosynechococcus elongatus* BP-1

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Sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) were known as anionic lipids, which have negative charge at neutral pH. PG plays important roles in constitution of thylakoid membranes and maintenance of photosynthetic activity in both cyanobacteria and higher plants. SQDG-deficient mutants were isolated from cyanobacteria, *Chlamydomonas* and *Arabidopsis*, and used for functional analysis of SQDG, however, effects of SQDG deficiency on growth and photosynthesis were different among these organisms. Although SQDG and PG might complement a part of roles each other, it is unclear which part of PG function can be substituted with SQDG.

In this study, we made an SQDG-deficient mutant of *Thermosynechococcus elongatus* BP-1 by inactivation of the sqdB gene for UDP-sulfoquinovose synthase involved in the biosynthesis of SQDG. The mutant grew photoautotrophically, but its growth rate was slightly slower than that of wild type. The mutant cells lacked SQDG and had an increased content of PG, indicating that SQDG was replaced with PG in the mutant and the lack of SQDG was complemented by PG. We will discuss the role of SQDG and PG for photosynthesis and requirement of SQDG for growth and photosynthesis under phosphate-limited conditions.

Poster 10

"Bicarbonate protective effect" on ATP synthesis and possible role of carbonic anhydrase

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The light-dependent proton exchange inside of plant cell provides energy to form transmembrane proton gradient across the thylakoid membrane (ΔpH). It is converted into chemical energy of ATP, the universal energy source for many biochemical processes.

Thylakoid aging or addition of uncoupler (NH_4^+) in "non-lethal" concentrations lead to decrease of membrane phosphorylation due to membrane deterioration. Adding bicarbonate stimulated process of phosphorylation and in deteriorated thylakoids it was higher than in stable thylakoids. Basing on these data, we can assume that bicarbonate is a universal protector retaining the ability to generate ΔpH, and thus providing condition for ATP synthesis.

The participation of carbonic anhydrase (CA), an enzyme that catalyzes the reversible dehydration of bicarbonate in this process was suggested after disappearing of observed "bicarbonate protective effect" when thylakoids were incubated with CA inhibitors, acetazolamide or ethoxyzolamide, in the presence of exogenously added bicarbonate. According to Arabidopsis thaliana genome analysis, its chloroplasts contain at least six CA and their functions is still unclear. Probably, the function of at least one of them can be connected to alteration of the of proton donation rate to ATP synthase complexes.

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Poster 11

Light-controlled variability of the size of an antenna unit building block: experimental and theoretical studies

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Most photosynthetic organisms are able to adapt to low light intensities with a remarkable increase in the PSU size due to peripheral antennae thus, however, making the problem of structure optimization of variable antennae more acute because larger antennae place even more stringent requirements for optimization. Earlier, we have shown theoretically that the effect of antenna pigment aggregation, being in itself one of basic structural optimizing factors, is more pronounced with increasing the unit aggregate size thus ensuring the high functioning efficiency of the atenna regardless of the antenna size. Here we confirm that this design principle is actually realized in the chlorosomal oligomeric BChl *c* antenna of the green bacterium *Chloroflexus* (Cf.) *aurantiacus* which is a unique example of an ordered antenna structure that is shaped by functional criteria. Recently, we discovered that an increase in the BChl *c* antenna size observed upon lowering growth light intensities led to enhancement of the hyperchromism of the BChl *c* Q_y absorption band showing an increase in the size of a unit BChl *c* aggregate. This conclusion was confirmed by us independently with femtosecond difference absorption spectroscopy. It was shown that the amplitude of bleaching of the oligomeric BChl *c* Q_y band (as compared to that for monomeric BChl *a*) increased with increasing BChl *c* content in chlorosomes. In particular, this BChl *c* bleaching amplitude was about doubled as the PSU size was about trebled. Besides, the measured absorption and circular dichroism spectra of chlorosomes under investigation were in a good agreement with the calculated ones. Thus, all sets of findings for *Cf. aurantiacus* chlorosomes suggest that the light control over the size of a unit BChl *c* aggregate in the chlorosome does exist thus optimizing the antenna structure according to functional criteria.

Poster 12

Improving resistance to acute UV stress in *Synechocystis* sp. PCC 6803 using rescue media derived from *Deinococcus radiodurans*

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Deinococcus radiodurans is known for its ultra-high resistance to ionizing radiation (IR), since it can survive acute radiation doses of up to 15 kGy (lethal dose D_{10} is 13 kGy) [1] and has thus been nick-named as "Conan the Bacterium". A study by Daly et al. found that the major effect against cell damage is exerted by certain cytoplasmic compounds that increase the protection of cellular proteins against reactive oxygen species [2]. Ultrafiltrate prepared from *D. radiodurans* was found to be enriched in Mn^{2+}, phosphate, certain nucleosides, nucleobases, and peptides and conferred substantial IR resistance to normally IR-sensitive cells like *E. coli* and human Jurkat T cells when added to the growth medium. By testing candidate compounds individually and in combination, major radioprotective effects on *E. coli* were already observed upon addition of 3 mM uridine, 1 μM Mn^{2+} and 13 mM phosphate to normal TGY growth medium [2].

We asked, whether these components would have a protective effect on photosynthetic cyanobacteria (*Synechocystis* sp. PCC 6803) against acute UV radiation. It was first established that *Synechosystis* is ionizing radiation-sensitive (cells died at 500 Gy radiation from a ^{60}Co source). Subsequently, cell suspensions grown to a certain OD were subjected to intense illumination with light from a 100 W mercury arc lamp that was applied to the cell samples via a large-diameter light guide. Substantial cell death was observed upon 3 h-long illumination with about 5 kW/m^2 total intensity at the fibre output. Subsequently, addition or substitution of components in the normal BG11 medium were tested to establish a modified BG11 medium enriched in Mn^{2+}, phosphate, uridine and adenosine that still allowed for good cell proliferation. After adaptation of *Synechocystis* to the new growth medium (containing 10 mM Na-phosphate, 1 mM $MnCl_2$ and only 7 mM Na_2NO_3, all other components as in normal BG11 medium) for several weeks, substantially higher cell recovery or survival rates were obtained after illumination with up to 8 kW/m^2 over hours, but results varied between the batches of cells adapted to the new medium. Further improved illumination stress resistance was obtained upon addition of 0.5 mM uridine and 0.2 mM adenine to the adaptation medium. While this study is far from being quantitatively conclusive, the observations suggest that rescue media containing solute components from the cytoplasm of IR-resistant organisms may help to improve the performance of IR-sensitive cyanobacteria in sustainable biomass production by solar energy-harvesting bioreactors.

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Poster 13

Fluorescence procedures to assess the photosynthetic resilience in Scots pines after a surface fire

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Siberian forests play a major role in the global climate system, they are crucial for terrestrial biodiversity, and they are a supply of major natural resources. Forest fires happen frequently in Siberia. From 4.5 to 27 thousand forest fires are annually observed in this part of the world. Sixty percent of forest fires occur in pine forests. A close correlation trends to be observed between air temperature dynamics and total number of wildfires in Siberia. Climate change of recent decades has significantly increased the threat and distribution of forest fires.

Fire, as a stress factor, may induce metabolic changes in trees. Knowing the features and durations of the metabolic changes in plants is important for the use of prescribed fire. This paper aims to find out if Pine trees have resilience to repeated heat stress after low-intensity fires.

The study site with 20-year-old *Pinus sylvestris* L. stand was located in a forest-steppe zone of Krasnoyarsk region. Two sample plots were chosen. The first sample plot was the one with a surface fire of the year 2014. The second sample plot was the one with a surface fire of the year 2015. The forest stands that were not coved with fire were control plots. A generalized test was carried out on cut branches under laboratory conditions. We used the method of artificial stress influence. The branches were heated in thermostat at temperatures of 43–47°C. Then the indexes of delayed were measured. The parameters of delayed fluorescence were indicated on a fluorimeter "Foton–10" (Russia).

Based on the results of the index of chlorophyll fluorescence it was shown that all burned trees from a sample plot with a surface fire of 2014 were characterized by higher level of photosynthetic activity after repeated heat stress. This can lead to a conclusion that some physiological processes in plants are modified by ex-stress events. These changes can have a positive effect during repeated actions of stress factors. Thus, prescribed low-intensity fires can be planned for *Pinus sylvestris* L. stands to prevent higher tree mortality due to repeated heat stresses. This approach may increase the stability of valuable trees in the future, due to a rapidly changing climate and as a consequence of forest fires. Furthermore, fluorescence method can be used to diagnose pine needle thermic resilience and assess high temperature effects.

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Poster 14

Assessment of the physiology state of photosynthetic machinery and plant vitality by JIP-test and PAM-fluorometry

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Nowadays, the analysis of fluorescence emitted by Photosystem II (PS II) antenna complex chlorophyll molecules has become the most used experimental technique not only for monitoring of plant physiological state, their vitality and of the stress response at *in vivo* and in situ conditions, but also for photosynthesis research. There are two main experimental approaches of the fluorescence signal measuring: a) analysis of the dynamics of fluorescence directly excited by actinic light (OJIP-test) and; b) analysis of changes induced by saturating light pulses in yield of low intensive modulated light (PAM fluorometry). The mechanisms of the fluorescence quantum yield changes caused by different photosynthetic reactions and processes that allow us from the fluorescent signal to obtain the structural and functional information concerning the two photosystems and electron transport chain between them. On the basis of the energy fluxes theory, developed by Prof. R. Strasser, specific informative parameters could be calculated from the data received from fast phase of the chlorophyll fluorescence rise. The parameters characterize the primary photochemical reaction efficiency in the PS II reaction center and in different parts of the electron transport chain from water to PS I acceptors, relative size of the electron acceptor pool, the density of active reaction centers of PS II, the size of antenna complexes and probability of their connectivity. Slow dynamics (minutes) of processes taking place during the transition of the photosynthetic apparatus from the dark to the light adapted state, can be analyzed by testing of photosynthetic samples by series of short saturating pulses, using PAM fluorometry. This method allows us to estimate the contribution of different types of excitation quenching in chlorophylls and the actual photosynthetic electron transport rate as well as the primary photochemistry quantum yield.

Poster 15

Identification of nutrients deficiencies in growth medium of *Phaseolus vulgaris* by chlorophyll fluorescence methods: application of artificial neural networks as a tool for rapid recognition

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A deficiency of macro- and microelements has a huge impact on the life cycle of the plants. The determination of missing minerals requires analysis of the soil or plant tissue content, or a combined analysis. In this study the deficiency of macro- and microelements in nutrient solution was evaluated by the stress response of the plants estimated by leaves' photosynthetic activity. Influence of deficiency of some macro (Ca, K, Mg) and micronutrients (Fe, Cu, Zn) on bean plants grown in hydroponic Hoagland solution was investigated. Plants were grown in a nutrient solution with permanent deficiency of chosen macro or microelement. Control plants grew in complete Hoagland solution and they were compared with plants, which grew in solution with deficiency of minerals. Bean plants were decapitated after 7 days of growth. The photosynthetic activity of plants was measured after 10 days of growth and it was estimated by the application of analysis of the chlorophyll *a* fluorescence using JIP-test. The artificial neural networks and self-organized map combined with the Independent Component Analysis were applied to fluorescence data as a tool for rapid recognition of nutrient deficiency.

Poster 16

The impact of the environmental factors on the photosynthetic activity of common pine (*Pinus sylvestris* L.) in spring and in autumn in the region of Eastern Siberia

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Combination of high solar radiation, cold and drought is a specific peculiarity of the climate in Eastern Siberia, where during spring photosynthetic activity of common pine depends mostly on soil temperature. In autumn it depends on the factor which is beyond the optimal value more than others. In this study, we compare the relations between the intensity of photosynthesis of common pine growing in Eastern Siberia and the environmental conditions at the beginning and at the end of the growing season during the years, which differ in their weather conditions. Photosynthetic activity was monitored round the clock for three days of each week during spring and autumn. Daily photosynthetic activity maxima were selected according to the hourly parameters of the processes during each day. Standard linear regressions of the dependence of daily photosynthetic activity maxima from the level of environmental factors (soil and air temperature, irradiance and available soil water supply in the 0–50 cm soil layer), coefficient of determination (r^2) and Spearman's correlation (r_s) were used to assess the direction and level of the relation between photosynthesis level and environmental factors. The vegetation period of the first year of the investigation may be characterized as abnormally warm and productive and more favorable, than the second. The vegetation period of the second year characterized as warm, favorable from the viewpoint of humidity except spring. In spring close positive correlation of maximal daily photosynthesis rate was identified with only one environmental factor for each year. In autumn significant correlation was shown with two and four factors in different years. It may be presumed, that weaker connection of photosynthetic activity with changes in environmental factors in spring, as compared to autumn, is explained by the multidirectional influence of environmental conditions on photosynthesis in this period and by the necessity of earlier photosynthesis onset, despite unfavorable conditions.

Poster 17

Early detection of sulphur deficiency in radish plants

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Nutrients deficiency in plants are well understood and frequently diagnosed by visible symptoms in plants, however, this type of diagnostics is inadequate since that, at that stage, usually it is too late to act and restore the optimal demands. Some of nutrients deficiency are more difficult in recognition. Sulphur is macronutrient, which is an essential element for growth in plants. It plays significant role in conformation, structure and function of enzymes and proteins in vegetative plant tissue. Sulphur deficiency in plants is very difficult to observe. In radish (*Raphanus sativus* var. sativus) plants visible symptoms are very late and it is rather not distinguished by naked eye. The research related to nutrient deficiency on functioning and structure in plants are usually invasive and apply expensive methods e.g. analysis of chemical composition. We believe that, in sulphur deficiency study more advanced techniques are necessary. Therefore, we suggest to use measurements of chlorophyll *a* fluorescence, which can be used easily and *in vivo*. In this work, the reactions of the photosynthetic apparatus of radish plants is evaluated by the use of this technique and artificial neural networks (ANN). Our study suggests some parameters related to photosynthetic apparatus efficiency for early detection of sulphur deficiency in radish plant.

Poster 18

Potassium-deficiency induced changes in electron transport chain of radish

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Diagnosis of mineral deficiencies in plants is based either on visible symptoms or the chemical composition analysis. Visible symptoms might be nonspecific, while an analysis of chemical composition is a destructive, expensive and time-consuming technique. Non-invasive chlorophyll *a* fluorescence measurements are used in researches on plants' reaction to various kinds of environmental stresses, including nutrient deficiencies. The aim of the research was to study the efficiency of energy transfer in thylakoid membranes of radish plants grown under conditions of potassium deficiency.

Radish (*Raphanus sativus* var. sativus) plants were grown in controlled conditions (in a Phytotron), in hydroponics. The efficiency of light-dependent reactions was evaluated with chlorophyll *a* prompt fluorescence measurements, which provides an access to understanding the changes of photosynthetic apparatus efficiency, the architecture of light harvesting protein-pigment complexes (LHC), photosystem II (PS II) activity, redox state of photosystem I (PS I) primary acceptors, and the fate of absorbed light energy. Information on the efficiency of light-dependent reactions was enhanced by conducting: (i) measurements of plant gas exchange *in vivo* with an infrared gas analyzer (IRGA), (ii) determination of chlorophyll content in plants' assimilative tissues and (iii) flavonoid concentration *in vivo*, using a portable chlorophyll meter. The accuracy of research conclusions was verified by the chemical composition analysis of plants' tissues. Analysis of selected JIP test parameters as well as chlorophyll fluorescence induction curve comparison showed some disorders in the electron transport chain, which might be helpful in diagnosing of potassium deficiency at an early stage i.e. before the appearance of visible symptoms of nutrient deficiency stress.

Poster 19

Machine learning methods in detection of nutrient deficiency of in winter rape

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Usually, the nutrition state of the plants is diagnosed by various methods based on morphological or chemical changes. In this work we suggest the use of chlorophyll fluorescence (ChFl) measurements, which can be used easily and *in vivo* to underline plant nutritional status/demands in non-invasive way. Winter rape plant grown under controlled conditions in growth chamber in pots filled with 60 different soils types collected in Lower Silesia (Poland). Full analysis of plants and soils nutrients component (micro- and macro-elements content) were performed. Principal Component Analyses (PCA) and k-means classifications were applied to find the regions of high-density of points in the space defined by the PCA axes. In this way, the classification of (i) soil types, and (ii) groups of leaves on the basis of micro- and macro-elements content were obtained. The differences in the soil and plants were tested with one way-ANOVA with subsequent Tukey HSD test. Moreover, in order to find the relationships between 50 chosen ChFl parameters and micro- and macro-element contents in winter rape leaves, the Canonical Correspondence Analysis (CCA) were used. Lastly, the artificial neural network (ANN) models with one hidden layer and back propagation were used in order to predict the values of the Zn, Cu, Mn, and P in leaves. All the calculations were performed with R CRAN. The PCA with k-means clustering analysis revealed the main gradients in the soil chemical compositions and optimal four groups of samples, related to different soil types. The CCA analysis confirmed the relationships among higher leaf content: Zn concentration and ABS/CS_0, V_J, F_0 and F1; Ca and kN, ABS/RC, $\gamma(1-\gamma)$; Mg and TR_0/RC; Cu and TR/CSm, F_v; P and AM, kP, φRo; Fe and SM, RE_0/RC; Mn and TR/CS_0. The ANN models were suitable in prediction the values of Zn, Cu, P in leaves (R^2=0.79–0.96). Our research suggests that, machine learning methods are very efficient in detecting the higher Zn, Cu, Fe, Ca Mn, and P concentrations in winter rape plants.

Poster 20

Spectral multi-exponential approximation of the chlorophyll *a* fluorescence transient allows early detection of stress caused by nitrogen or sulfur deprivation

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The measurement of the kinetics of chlorophyll fluorescence transient of algae and plants is a widely used method for determining the state of photosynthetic apparatus. The time dependence of the fluorescence intensity is a complex multiphase curve, which depicts the various stages of the electron transfer in the electron transport chain of chloroplasts.

We proposed the method of spectral multi-exponential approximation (SMEA) which allows to formalize phase identification, to estimate quantitative characteristics of individual phases of the induction curve (amplitudes and characteristic times), and to reveal hidden phases of the curve. Occurrence of additional phases on the induction curve points to a change in the functioning of the photosynthetic apparatus in response to changing growth conditions.

The proposed method is implemented in pyPhotoSyn software, which allows carrying out a comprehensive analysis of large sets of experimental data. This allows monitoring of evolution of fluorescence transient curves in various growth conditions.

In this work we used SMEA to study the fluorescence transient data measured for microalgae (*Chlorella* sp., *Chlamidimonas reinhardtii*) cultivated under nitrogen or sulfur deprivation. In both cases of mineral starvation we observed the appearance of an additional phase in microseconds range. We suppose that occurrence of this phase points to the processes emerging in response to starvation. A high sensitivity of SMEA allows to suggest it for detection of early cell response to stress.

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Poster 21

The effect of phytochrome A and B deficiency on the photosynthetic processes in *Arabidopsis thaliana*

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One of the most key issues for the plant photobiology is the role of the phytochrome system in the regulation of photosynthesis under physiologically normal and stress conditions. Lack of at least some types of phytochrome can reduce activity of photosynthetic apparatus (PA), first of all photosystem 2 (PS II).

The role of phytochromes in the regulation photosynthetic processes was studied by using 25-days-old *Arabidopsis thaliana* mutant plants, deficient both in phytochrome B and A (PhyB and PhyA) – the double mutant (DM) and *hy3* mutant deficient in apoprotein of PhyB. Plants were grown in white light with an intensity of 110 μmol quanta m^{-2} s^{-1} and photoperiod 12 h. the effects of UV-B (30 min, λ_{max}=311 nm, I=1 W m^{-2}) and UV-A (2 h, λ_{max}=365 nm, I=12 W m^{-2}) irradiation on the Chl *a* fluorescence parameters of wild type plants (WT), DM and *hy3* were studied.

There was a small difference in PS II activity between WT and mutants without stress conditions. the value of PI_{ABS} which is an index of the performance of PS II was lower in DM by 29% compared to WT, which characterizes a decrease in the efficiency of PS II in DM plants. However, we did not indicate a difference between WT and mutants in value of maximum quantum yield of PS II (F_v/F_m). Under UV-B conditions the value of F_v/F_m was reduced by 26% in DM and by 21% in DT. UV-A irradiation also resulted in a greater decrease F_v/F_m values in DM than in DT, 10.0%±0.1 and 8.6±0.1%, respectively. Consequently, PS II resistance of *Arabidopsis* plants to UV-radiation is lower in mutant deficient in DM as compared to WT. the decrease of photosynthesis at light saturation after UV-A-irradiation was 51% in DM, whereas in *hy3* – 44% and in WT – 24%.

Thus, there is an interaction of PhyA and PhyB in resistance of PA to UV-radiation. the mutant deficient in PhyB is more resistant to UV than mutant deficient in PhyB and PhyA simultaneously, but less resistant than WT.

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Poster 22

Signaling forms of the orange carotenoid protein

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To protect themselves from the high intensity of solar radiation cyanobacteria use watersoluble photoactive protein, Orange Carotenoid Protein (OCP). It is responsible for a photoactive triggering of the non-photochemical quenching (NPQ). OCP converts into quenching red form under blue-green illumination. Functional analogue of the red active form can be obtained by chemical activation with high concentrations of sodium thiocyanate (NaSCN).

In this work, we show that Purple Carotenoid Protein (PCP), which was obtained by single replacement of tryptophan-288 with alanine, interacts with the phycobilisomes and causes non-photochemical quenching. The effect is comparable to the quenching induced by OCP activated by blue-green light. We used variety of optical methods to compare different forms of OCP and their ability to induce NPQ. Data obtained by Raman spectroscopy suppose that carotenoid conformation is sensitive to the structure of the C-domain. Combination of differential scanning fluorimetry (DSC) and picosecond time-resolved fluorescence anisotropy measurements allowed us to compare the stability of different OCP forms and to estimate relative differences in protein size. We assume that PCP is a promising model of permanently active and stable red form of OCP, which does not require photoactivation to induce NPQ.

Poster 23

Photosystem II of contrasting silver fir provenances in response to heat stress

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Response of tree species to climate changes, including more frequent and intensive heat waves, is hard to predict. Therefore, we tested the response of 11 silver fir provenances originating from contrasting conditions to short-term heat stress. All provenances were sampled in the international provenance plot Hertník (49°13′ N, 21°16′ E; 390 m a. s. l.) in Slovakia. The heat stress was simulated using water bath; seven temperatures ranging from 20 to 48°C were maintained for 30 min and thermotolerance of PS II was assessed via chlorophyll *a* fluorescence. We used the parameters derived from the OKJIP transient well reflecting the changes of the photochemistry performance under the heat stress. As the tested provenances originated from two glacial refugia, the statistical tests were made separately for the eight provenances from the Central Europe line and for the complete dataset. We found the significant relationship between the altitude and PS II thermostability for both datasets. We also observed impacts of the mean temperature and precipitation of origin during the vegetation season in most of the evaluated parameters for the Central European provenances. These trends of average values per provenance indicate that the Central European provenances from the higher altitudes with wetter and colder climates are less prone to heat stress, although differences among the provenances were statistically nonsignificant due to the high variability inside the provenances. Moreover, the obtained results pointed to the different patterns of response to high temperatures between the provenances from different glacial refugia. When, the provenances from Balkan refugium were included to analysis, the impacts of precipitation and temperatures of origin were not confirmed. Balkan provenances show the better values of all measured parameters which indicate their higher resistance against heat. Probably, this is a result of better adaptation of Balkan provenances to high temperatures, since these originate from rather high altitudes but with warmer and drier climate compare to corresponding altitudes in Central Europe.

Poster 24

Investigation of deleterious effects of chromium phytotoxicity on photosynthesis in wheat plant

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Increasing human and industrial activities lead to heavy metal pollution. Heavy metal chromium (Cr) is considered to be a serious environmental contaminant for the biota. Growth parameters were largely inhibited as a result of disturbances in the plant cell metabolism in response to Cr toxicity. The chlorophyll *a* (Chl *a*) fluorescence transient were recorded and analyzed according to JIP test which can quantify PS II behavior using Plant efficiency analyzer (PEA). The plants were daily replenished with normal tap water (control) and with different concentrations of Cr until 40 d. All the measurements were performed with different concentrations of Cr on 25, 35, and 40 d (DAT). Since maximum changes were observed after 25 DAT and leaves started to become yellow after 30 DAT, the data obtained after 25 DAT are presented in this work. Chromium toxicity led to decline in a number of active reaction centres of PS II, rate of electron transport, and change in PS II heterogeneity. Chromium did not cause any change in heterogeneity of the reducing side. Asignificant change in antenna size heterogeneity of PS II occurred in response to Cr toxicity. Chromium seems to have extensive effects on the light harvesting complex of PS II. The work is a significant contribution to understanding the basic mechanism involved in the adaptation of crop plants to stress conditions.

Poster 25

Sll1276, Slr2019 paralog, is essential for acid stress tolerance in *Synechocystis* sp. PCC 6803

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In gram negative bacteria, it has been known that Lipopolysaccharide is the main component of the outer layer of the outer membrane and is important to environmental stress tolerance in *Synechocystis* sp. PCC 6803 (*Synechocystis*).

We have previously reported that Slr2019 which is homologous to MsbA, which is an inner membrane ABC transporter that functions in the initial stages of lipid A export, is important for acid stress tolerance in *Synechocystis*. Slr2019 has three homologous protein in *Synechocystis*. In this study, we aimed that these three protein clarifies participation in acid stress tolerance.

We constructed *sll1276*, *sll1725*, *slr1149*, which is homologous to Slr2019, defective mutant strains. the growth of *sll1276* mutant strain was slower than that of WT under acid stress condition. On the other hand, *sll1725* and *slr1149* mutant strains didn't exhibit significant differences in their growth compared with WT under acid stress condition. The expression of *sll1276* gene increased and the *sll1725* and *slr1149* genes did not increase significantly in WT after acid stress treatment. These results suggested that Sll1276 is important to growth in *Synechocystis*.

Poster 26

Differences of induction curves chlorophyll fluorescence of the applle fruits and of the leaves under the natural development

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Comparison of functional and structural characteristics of leaves and fruits of the apple tree during development is an informative direction of the investigation in the adaptation of plants under the natural development and under the stress conditions.

The induction fluorescence curves of the apple leaf *Malus domestica* included three growth phases (O-J, J-I and I-P) in accordance with the results obtained on other plants. The main difference the induction chlorophyll fluorescence of the fruit surfaces was the existence of the transitions O-K-J at room temperature. According to the literature, K-peak commonly had been seen at the leaf samples after their heat treatment (40°C). In our experiments, the peak K achieved without heat treatment in 800 microseconds interval; its appearance is not accompanied by a decrease in the quantum yield of electron transport in PS2. The values obtained for F_v/F_m parameter in apple fruits were 0.708–0.886 and were similar to the photosynthetic activity of the leaves.

According to transmission electron microscopy in the chloroplasts of cells maturing apple fruits localized in subepidermal layer. In terms of the ultrastructural feature of these chloroplasts are developed membrane system, the visible region of chloroplast contact with each other, numerous paintings fusion and fission. In contrast to the fruit, leaves of apple trees in the cells observed over peroxisomes, which are often part of the "triad": the mitochondria – peroxisome – chloroplast.

Evaluation of plant resistance to environmental factors using methods of chlorophyll fluorescence induction and electron microscopy can be used to study the differences in the apple tree fruits and leaves adaptation under the stress conditions.

Poster 27

Physiological state of selected beech population during peak of growing season

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Beech forests are among the economically most important and most widespread forest communities in Europe. However, due to projected increase in frequency and intensity of heat waves across the most of Europe, there is growing concern over the impact of these events on beech, which is known to be vulnerable to drought. Within the present study we assessed intraspecific physiological variability of five beech (*Fagus sylvatica* L.) population provenances under environmental conditions of Slovakia, during peak of 2015 growing season in terms of provenance plot established under the coordination of the Federal Forest Research Centre, Institute of Forest Genetics in Grosshansdorf, Germany. Since beech is woody plant with a more sensitive response to dry periods, becomes the identification of symptoms and consequences of exposure drought in terms of continued stability and development of beech stands extremely important. We selected beech provenances from distinct climatic origins across the Europe related to longitudinal gradient of altitude (from 55 m a.s.l to 1250 m a.s.l) and assessed their adaptation potential to the local site conditions. Recorded parameters of gas exchange (net photosynthesis [A], stomatal conductance [g_s], water use efficiency [WUE_i]) and parameters of slow and fast kinetics of chlorophyll fluorescence confirmed sensitive physiological response to the actual changes at the plot condition, while the population originally from higher altitudes reflected better ability to recovery following the rainfall event, after dry and heat period.

Poster 28

Influence of a-carbonic anhydrase 4 gene knockout on photosystem II light-harvesting antenna in *Arabidopsis thaliana*

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Carbonic anhydrases (CA) are the enzymes that catalyze the reaction of reversible hydration of carbon dioxide. CAs present in plasma membrane, cytoplasm, mitochondria and chloroplasts of higher plants cells. There are 19 genes encoding CAs in the genome of higher plants, and still remains unclear whether all of these genes are expressed, at least in the photosynthetic cells as well as the physiological role of CAs.

Friso et al. in 2004 using proteomics approaches have found the *At4g20990* gene product coding, so-called, α-CA4 among thylakoid proteins of *Arabidopsis thaliana*. [1] We have confirmed that the *At4g20990* gene was expressed, using primers specific to this gene. The expression level of the *At4g20990* gene depended on light intensity and day length: it was higher in plants grown under 400 μmol photons $m^{-2}s^{-1}$, as against 80 μmol photons $m^{-2}s^{-1}$; this difference was more prominent at long, 16 hours, than at short, 8 hours, day. We used *A. thaliana* plants of two mutant lines, each homozygous for the knocked out gene *At4g20990*, and which differed in the location of the gene knockout insert. These mutant plants differed from wild-type plants (WT) grown under corresponding conditions, only a few large size. The knockout of the *At4g20990* gene decreased non-photochemical quenching and this effect has been more discernible at high illumination for both short and long day plants. Furthermore, the mutation has led to changes in both the expression level of genes encoding the proteins of light harvesting complex of PS II and the level of their translations. The content of PsbS was higher in mutant plants of both lines under all growth conditions. The content of the most other antenna proteins in mutants grown in low light was higher, while in high light it was lower as compared to WT. The data imply that α-CA4 participates in regulation of processes of plant acclimation to irradiance intensity, namely, assisting in biochemical processes leading to energy dissipation in PS II antenna, and affects signals, which are sending to systems of biosynthesis of antenna proteins.

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Poster 29

New antimony(III) complexes as potent inhibitors of photosystem II, carbonic anhydrase, and glutathione reductase

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The usage of specific inhibitors is one of the methods to study enzyme functions. Design, engineering, synthesis and investigation of the molecular mechanism of new chemical inhibitors based on previously obtained data allow getting more sophisticated information about enzymes. Furthermore it may be essential first step for creation of the plants defense means and herbicides as well as possible preparation for the human and animal medicine.

PS II is one of the enzyme systems, determining the growth and productivity of plant. Another important target of inhibitors action is the plant carbonic anhydrases. Glutathione reductase is a major cellular antioxidant enzyme that is widely distributed both in eukaryotes and prokaryotes catalyzing the reduction of oxidized GSSG to the reduced GSH using NADPH as an electron donor.

Creation of universal compounds, which suppress simultaneously several key enzymes and even enzyme systems, is one of the ways to increase the efficiency of plant growth regulation.

In the presented work, new nineteen antimony (III) complexes were examined as possible herbicides. Six of them were synthesized for the first time, and their structures were identified by means of elemental analyses, ^{1}H-NMR, ^{13}C-NMR, FTIR, LCMS, magnetic susceptibility, and conductivity measurement techniques. The most-stable forms of these nineteen antimony (III) complexes were determined by DFT/B3LYP/LanL2DZ calculation method. The effects of these compounds on photosynthetic electron transfer and carbonic anhydrase activity of Photosystem II, and also glutathione reductase from chloroplast were investigated. As was indicated, all antimony (III) complexes have an inhibitory effect on glutathione reductase activity of chloroplast. A number of these compounds also exhibited a good inhibition efficiency of the photosynthetic and carbonic anhydrase activity of Photosystem II.

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Poster 30

Regulation of photosynthesis by oxylipins generated in allene oxide synthase and hydroperoxide lyase pathways

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Chloroplasts are the site of photosynthesis and also the place where many primary and secondary metabolites are synthesized. Many reactions of biosynthesis of lipid-derived signaling molecules – oxylipins – also occur in these organelles: enzymes of early steps of oxylipin biosynthesis, enzymes of initial reactions of jasmonates biosynthesis and all enzymes of 13-hydroperoxide lyase (13-HPL) branch are localized in chloroplasts. Biosynthesis is initiated by lipases, which cleave free fatty acids from lipid backbone, then lipoxygenases stereospecifically oxidize carbon atom of fatty acid backbone, forming hydroperoxides of fatty acids. Allen oxide synthase (AOS) and 13-HPL are two enzymes competing for the substrate – hydroperoxide of linolenic acid. AOS branch metabolite 12-oxophytodienoic acid (12-OPDA), jasmonic acid precursor, accumulates in chloroplasts in high concentrations and implements biological functions which only partially overlap with jasmonic acid. HPL generates 6-carbon aldehydes and 12-carbon aldoximes from hydroperoxide of linolenic acid. Aldehydes can be further modified spontaneously or enzymatically, isomerized or turned into alcohol, hydroxy- or aceto- derivatives. Very little information is available on the effect of chloroplast-produced oxylipins on photosynthesis. We used different approaches to study the effect of mentioned metabolites on photosynthetic processes, such as oxygen evolution and photochemical activity of photosystem II. To gain information about oxylipins functions in planta we analyzed variable chlorophyll fluorescence parameters of intact leaves of genetically modified *Arabidopsis* plants with altered oxylipin profile. The study was supplemented with analysis of the effect of exogenous oxylipin application on functional activity of PS II in intact plants and isolated thylakoids and thylakoid membranes.

Poster 31

Variation potential influences the resistance of photosynthetic machinery to the thermal stress in pea

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Local damages induce generation and propagation of variation potential (VP) in higher plants. VP influences on different functional processes; particularly, it inactivates photosynthesis, activates respiration and increases resistance of plants to different stressors.

Experimental plants were 14–21 days old pea seedlings (*Pisum sativum* L.). VP was induced by leaf burning and was measured using registration of surface potential. Photosynthetic parameters were measured using a system for photosynthetic analysis including a gas exchange measuring system GFS-3000, a measuring system for simultaneous assessment of P700 oxidation and chlorophyll fluorescence Dual-PAM-100, and a measuring head Cuvette 3010-Dual. Sensitive bioluminescence based ATP determination kit was used for measurement ATP in pea leaves. It was shown that local burning induced VP which decreased CO_2 assimilation rate and quantum yields of photosystem I and II. At the same time VP changed the resistance of the photosynthetic machinery to heating, increasing the resistance of photosystem I (PS I) and reducing the resistance of photosystem II (PS II). Increased PS I resistance was associated with VP-induced inactivation of the dark reactions of photosynthesis; decrease in the resistance of PS II was connected with VP-induced decrease in transpiration. It was also shown that VP stimulated the cyclic electron flow, increased non-photochemical quenching and raised ATP content in pea leaves. We supposed that these responses can participate in VP-induced growth of photosystem I heating resistance. It is probable that changes in heating resistance of photosystem I and II contributed whole-plant resistance to high temperature.

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Poster 32

Rearrangements of photosynthetic antenna units in response to light conditions are regulated by the extent of ROS production

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Plants are constantly faced with changing in light conditions that led to the formation of a large number of the adaptive mechanisms in the photosynthetic apparatus. Light harvesting function in the photosynthetic apparatus of higher plants is performed by the pigment-protein complexes of the photosystem II and photosystem I antenna units. Plants have evolved various mechanisms, which regulate the antenna size of both photosystems, according to environmental light conditions. Depending on the time of plants illumination, short-term response (several minutes) called state transitions and long-term response (several days) can be distinguished. These strategies have different mechanisms while both are strictly regulated by the redox state of the plastoquinone pool (PQ-pool) of the thylakoid membranes. The molecular nature of the signal which carries the information about the redox state of the plastoquinone pool remains unknown in both responses.

In the present study the influence of both the hydrogen peroxide level in leaves and the PQ-pool redox state on the short-term and long-term responses has been investigated.

It has been concluded that: i) STN7 kinase represents a link between the redox state of PQ-pool and both responses to environmental light conditions; ii) hydrogen peroxide, which is produced with the involvement of the PQ pool, influences the redox activity of STN7 kinase, switching the signaling pathways of the short-term as well as the long-term plant acclimation to various light conditions.

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Poster 33

Influence of narrow-band red and blue light on barley chloroplast ultrastructure

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The aim of the study was to investigate effects of narrow-band red and blue light produced by light-emitting diodes (LED) on chloroplast organization. Barley seedlings were grown under various light conditions (red (660 nm) or blue (450 nm) LEDs) and chloroplast ultrastructure was investigated by means of transmission electron microscopy. The control variant were barley plants grown under white fluorescent lamps. Electron microscopic study showed that in the energized condition in the light the chloroplasts formed under narrow-band blue LED light demonstrated the highly organized structure similar to control. By contrast, the chloroplasts formed under narrow-band red LED light possessed a structure partly corresponding to the conditions of low illumination intensity: they had many granal thylakoids and irregular structure of thylakoid membranes. Such structure may correspond to low chloroplast functional activity. After a 6-hour dark period chloroplast ultrastructure in control plants changed significantly whereas the structure of chloroplasts formed under narrow-band blue and red LED light did not manifest any obvious alterations. So, chloroplasts formed under narrow-band blue and red LED light demonstrated reduced lability of inner membrane system as compared to control ones. The results suggest that the features of the chloroplast thylakoid membrane ultrastructure and lability in the plants grown under narrow-band blue and red LED light together with other factors could determine differences in the functioning of the energy-converting chloroplast systems.

Poster 34

Phenotyping of photosynthetic traits in lettuce: the limits and possibilities of chlorophyll fluorescence imaging in drought stress studies

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Chlorophyll fluorescence imaging (CFI) represents the most important tool to measure photosynthetic traits, and therefore, it is involved in numerous phenotyping platforms. CFI is expected to serve as a measure of photosynthetic functions, especially in studies of genotype × environment interactions. In our study, we tested opportunity to apply CFI to recognize drought sensitivity in lettuce genotypes. Eight genetically distinct parental lines of cultivated lettuce (*Lactuca sativa* L.) and one drought resistant wild lettuce (*Lactuca serriola* L.) were cultivated in a growth chamber under limited or non-limited water supply (20% or 70% of available water capacity). At the end of the experiment, plants were exposed to severe drought stress by withholding of irrigation for 3 more days. In parallel with other phenotyping methods applied in the experiment, CFI was recorded regularly in light exposed plants at the actinic light intensity set on the ambient level. Depending on genotypes, total dry mass in drought stressed plants decreased by 20–50% compared to control; the relative plant dry mass decrease (DMD) was used as a measure of drought sensitivity of genotypes. CFI analyses have shown a significant decrease in the apparent electron transport rate, ETR, in all genotypes (having the same trend as the efficient quantum yield, Φ_{PSII}). However, contrary to expectations, the moderate drought stress led to negligible decrease or even a slight increase of ETR, which did not correspond to the observed decrease of photosynthetic performance. It indicates that the electron transport was efficiently re-directed to alternative energy-consuming pathways, such as photorespiration and others. On the other hand, we observed a significant decrease of steady-state fluorescence intensity (Fs), both in moderate and severe drought. Fs decrease can be explained by activation of energy-dissipating processes (non-photochemical quenching) and/or structural changes in the leaves. The correlation analyses indicated a very low correlation between the drought sensitivity indicator DMD and ETR in moderate drought stress ($p=-0.05$), a moderate correlation between DMD and ETR in severe drought stress ($p=0.45$), but the relatively good correlation between DMD and Fs observed in moderate drought ($p=0.65$) and a very high correlation between DMD and Fs observed in severe drought ($p=0.94$). Thus, our results suggest that the steady-state fluorescence signal (Fs) reflected well the effects of water deficit on photosynthetic apparatus and hence it can be used in phenotyping for drought tolerance in lettuce. In contrary, parameter ETR, which is often referred as the most useful parameter for phenotyping, was not sensitive enough. This is an important information emphasizing the need of testing the techniques in individual crops and different stress scenarios.

Sponsor's presentation

Exploring the power of parallel measurements of electron transport, CO_2 and H_2O in plant leaves

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Over the years, increasing population, growing industrialization, expanding agriculture and rising standards of living have pushed up the demand for water while the prospects for increasing the supply are limited. We need to learn to use water more efficiently. There continues to be a significant if not increasing amount of research effort focused on the improvement of water use efficiency in plants of economic importance. At the cutting edge of that research are studies which characterize and evaluate the potential for manipulating CO_2 diffusion within the leaf (mesophyll conductance; gm).

There are several methods for determining gm. All of the methods involve measurements of gas exchange of CO_2 and H_2O. With the introduction of commercial instruments which measure CO_2 and H_2O flux in parallel with estimates of electron transport using fluorometry research in this area has become more common. However there are a number of assumptions and pitfalls to these measurements. The author will discuss some of these assumptions and how the new LI-COR photosynthesis system (LI-6800) was designed to minimize violations of the assumptions.

Section 1.9: Systems Biology of Photosynthesis:

Integration of Genomic, Proteomic, Metabolomic and Bioinformatic Studies

Poster 1

Peculiarities of acetate assimilation in purple non-sulfur bacterium *Rhodobacter capsulatus* B10

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Acetate is assimilated in the majority of bacteria via tricarboxylic acid (TCA) cycle. Many intermediates of this cycle provide sources for biosynthetic needs. Replenishment of oxaloacetic acid pool is achieved via anaplerotic pathways. The most widespread pathway is the glyoxylate cycle, which includes two enzymes, isocitrate lyase and malate synthase, in addition to TCA cycle enzymes. By now, five anaplerotic pathways beside glyoxylate cycle are known: the ethylmalonyl-CoA pathway, the citramalate cycle, the methylaspartate cycle, and two pathways of pyruvate formation, one involving pyruvate synthase and another one (from acetyl-CoA and formate) via reversible formate dehydrogenase along with reversible formate C-acetyl transferase. Pyruvate can be converted to malate or oxaloacetate by C3-carboxylating enzymes. Autotrophic CO_2 fixation pathways (the Calvin-Benson cycle, the reductive (reverse) TCA (RTCA) cycle, the 3-hydroxypropionate bi-cycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, the dicarboxylate/4-hydroxybutyrate cycle can also contribute to replenishment of oxaloacetic acid pool. *Rhodobacter capsulatus* is capable of growing on acetate as the sole carbon source using both glyoxylate cycle and unknown anaplerotic pathway. Analysis of genetic potential for functioning of known metabolic pathways of oxaloacetic acid pool replenishment was carried out for *Rba. capsulatus* B10. The results of the analysis showed presence of all genes necessary for functioning of the ethylmalonyl-CoA pathway and two pathways of pyruvate formation, via pyruvate synthase and via reversible formate dehydrogenase from acetyl-CoA and formate. Among C3-carboxylases, pyruvate carboxylase, two reversible malate dehydrogenases (decarboxylating) and PEP-carboxykinase are present. As for autotrophic CO_2 fixation pathways, all the necessary genes are present for the Calvin-Benson cycle only. This bacterium also possesses all genes of the alternative isoleucine synthesis pathway. Using published microarrays data the expression of the revealed genes necessary for acetate assimilation in the phototrophic cultures of *Rba. capsulatus* was analysed. The results of the analysis are discussed.

This work was supported by the Russian Science Foundation (Project № 15-14-30007).

Poster 2

Integrated model of primary photosynthetic and metabolic processes in algae cells

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Accumulation of experimental data on the structure and functions of cell systems, as well as the development of computer technology induce the formation of "e-cell" models, which include processes of different hierarchical levels, such as gene expression, metabolic reactions, and the reaction of electron transfer in the respiratory and photosynthetic chains. Various mathematical methods are used for different hierarchical levels: differential equations, stoichiometric models, rule-based models etc. To combine different hierarchical levels one meets the problem of integration the processes with different characteristic times usually described by different kinds of models.

In this work we consider the hierarchical model of the plant cell that combines the description of primary photosynthetic and metabolic processes. The sub-model of photosynthetic reaction is presented by sets of differential equations for concentrations of multi-enzyme complexes states. We describe metabolic paths by means of algebraic equations according to Flux Balance Analysis formalism. The model contains both the kinetic block of primary photosynthetic reactions and the flux balanced model of central metabolic pathways based on stoichiometry of metabolic reactions.

In the model NADP reduction connects the photosynthetic reaction block with the metabolic one. The metabolic block is presented by glycolysis, Calvin-Benson Cycle and TCA Cycle and depicts the stationary distribution of central metabolic pathways. NADP stationary influx in Calvin-Benson Cycle is modified by the kinetic block of primary photosynthetic reactions that allows obtaining the series of metabolic flux distributions under different conditions.

The hierarchical model was applied for description of evolution of the metabolic fluxes distribution in algae cells under mineral starvation. The combined hierarchical model thus allows us to see how fast photosynthetic and slow dark metabolic reactions are coupled and study the total response of different cell systems to stress factors.

This work was supported by RFBR grant № 14-04-00326-a.

Section 1.11: Emerging Techniques for Studying Photosynthesis

Lecture 1

Optogenetics, a technology with Russian Scientific roots: Channelrhodopsin and new inhibitory optogenetic approaches

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During the past 10 years, in most optogenetic experiments applicants have employed light-activated ion channels as channelrhodopsins of the green algae *Chlamydomonas* to depolarize cells of interest and to fire action potentials in a sequence that precisely followed applied light trains. For cell inactivation mostly hyperpolarizing light-driven ion pumps were used although these pumps only transport a single ion per absorbed photon. Recently, we engineered a channelrhodopsin in such a way that it specifically conducts chloride (ChloC), which allows to clamp the membrane voltage to the chloride reversal potential [1]. However, the chloride reversal potential greatly varies in different cell species and during development. We have tried to overcome this problem by two-component optogenetic approaches (TCOs). As a proof of principle we combined a proton pump with proton sensitive ion channels such as ASIC2a to trigger with an initial pump currents a larger Na^+-influx into the cell (TCO-ex), in expectation to replace later the ASIC by a K-conducting homologue [2]. In parallel we employed the natural photo-activated adenylyl-cyclases bPAC and were able to activate cAMP-sensitive channels thus promoting Na^+ or Ca^{2+}-influx. In our most recent project we have characterized a rhodopsin-guanylyl-cyclase, RhGC, of the fungus *Blastocladiella emersonii* [3]. This newly discovered member of the rhodopsin family bears a great potential for the activation of cGMP-activated channels (TCO-approach with internal coupling, TCO-in) and preferentially cNG-gated K-channel. Together with cGMP-, voltage- and Ca-reporters our new set of photoreceptors should opens new avenues for multicolor and multimodal activation and imaging in optogenetic experiments for a better understanding of brain function and cellular or evolutionary processes.

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Lecture 2

Small angle neutron scattering studies of photosynthetic membrane structures in cyanobacteria

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Small angle neutron scattering (SANS) gives quantitative and qualitative information on the structure of thylakoid membranes *in vivo* across a range of organisms. There are certain strong peaks that appear in the scattering patterns obtained from differing cyanobacteria under various growth conditions by various groups. These are accepted to be from the thylakoid membranes and the positions of the peaks vary depending on the wavelength of illumination, salinity and other variables. Experimental scattering from cyanobacteria, including from "red-shifted" organisms are compared to other experimental data and various models are fit to these to try and move towards an explanation for the observed scattering.

Lecture 3

Deuteration as a tool to understand photosynthetic membrane organisation in the cyanobacterium *Halomicronema hongdechloris*

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Small angle neutron scattering patterns from whole actively metabolising cyanobacteria are typically a number of peaks superimposed on a decay. Typically these peaks are assigned to periodic spacings of lipids in membranes and changes in peak position are associated with structural changes with the cells [1]. Contrast variation, through the enrichment of the natural abundance hydrogen with deuterium, is a standard technique for enhancing the signal, improving the information content and enhancing the contribution from components in a mixture such as cell in the neutron scattering experiment. Typically it is achieved by providing actively reproducing cell mass with a deuterated carbon source after adapting the cells to deuterated conditions, media and carbon source [2]. Unlike most applications to cell biology photoautotrophs do not require a carbon source and fix carbon from CO_2. The challenges in producing deuterated biomass are quite different.

Here we report on degree of deuteration in biomass grown in up to 50% heavy water, D_2O, from the cyanobacterium *Halomicronema hongdechloris* using Fourier Transform infrared spectroscopy (FTIR). We use the FTIR method [3] and an understanding of the effect of deuteration on the position of infrared peaks for determine the relative amounts of deuterated lipid, protein and nucleic acid in the biomass. The aim is to understand if the peaks in the SANS spectra are due to period spacings of lipids or proteins.

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Lecture 4

Wood structure during pretreatment in ionic liquids

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Cellulose makes up for most of the material in the lignocellulosic's cell wall, and it could provide an abundant source for fuels, materials and chemicals. Mild and selective conversion processes would be desirable for decentralized value-generation from the synthesis power of nature. However, the utilization is still difficult due to the composition and the structure of the biomass' cell wall. Cellulose shows a dense, crystalline structure and the access to these macromolecules is further restricted by lignin and hemicellulose. An efficient conversion hence requires the application of a pretreatment to gain access to cellulosic macromolecules for subsequent conversion processes.

Mechanistic understanding of the pretreatment can likely be gained at the molecular level. However, the cellulose in the cell wall exists in fibrils made of several cellulose chains, which are hold together via intermolecular hydrogen bonds. This regular arrangement forms crystalline structures that are a major obstacle in enzymatic hydrolysis [1]. Hence, molecular analysis needs to be extended by structural analysis to monitor the mechanistic steps of pretreatment.

Ionic liquids proved to be good solvents for the cellulose and the hydrophobic lignin [2], and the high concentrations of acetate at elevated temperatures around 100°C give rise to chemical reactions that constitute the desired pretreatment and improve the enzymatic hydrolysis [3]. Due to the abundance of water in such processes, we systematically studied the effect of water on this pretreatment. Using small angle neutron scattering (SANS), the tissue after the pretreatment was compared to the native wood and a first time-resolved setup was established for this pretreatment.

The crystallinity of the cellulose has decayed at low water concentrations, and the cell structure of the wood is rather destroyed. At higher water contents, the crystallinity is enhanced, and the cell structure is rather preserved, but cellulose fibrils show coalescence. Apart from that, various methods have been applied to support the results and will be presented selectively. Latest kinetic SANS measurements reveal the pretreatment process in more detail.

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Lecture 5

Structure and dynamics of photosynthetic membranes as studied by neutron scattering

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Thylakoid membranes of plants and green algal cells possess remarkable structural flexibility: different short- and long-term regulatory mechanisms are associated with different structural changes in the photosynthetic machinery [1]. In my presentation I will demonstrate how neutron scattering techiques can help to understand the structure and dynamics of these highly organized multi-lamellar photosynthetic membranes. Small-angle neutron scattering (SANS), a non-invasive experimental tool, can provide staistically and spatially averaged structural information of biological systems in aqueous environment without the need of staining or fixation. Neutron spectroscopy techniques, such as elastic incoherent neutron scattering (EINS) can provide insight into the conformational flexibility of biological macromolecules under various temperature and relative humidity conditions.

During the past years we have investigated thylakoid membranes with SANS and EINS. With SANS, we determined characteristic repeat distances and revealed light-induced structural reorganizations during photosynthesis in isolated plant thylakoid membranes and in living unicellular organisms [2–4] and intact leaves [5] with a time resolution of seconds and minutes [6]. We also provided experimental evidence for changes in thylakoid membrane stacking in green algae during state transitions [7]. Our EINS measurements on Photosystem II membrane fragments, revealed a transition due to the detachment of the oxygen evolving complex [8].

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Poster 1

Applying small angle scattering methods to investigate cyanobacterial thylakoid membranes

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Our work focuses on parallel application of small-angle X-ray, neutron scattering and TEM methods on cyanobacteria. Cyanobacteria are considered the ancestors of chloroplasts in higher plants and we hypothesize that a regular, layered cyanobacterial thylakoid ultrastructure has a great advantage of studying thylakoid structure and dynamics *in vivo*, as compared to measurements on isolated thylakoid membranes [1, 2]. We investigate a range of cyanobacterial strains with various thylakoid ultrastructures: layered, radially-arranged thylakoids of wild-type cyanobacterial species and cyanobacterial mutants with different degrees of thylakoid membrane bending, obtained by varying CURT protein [3] expression levels.

A biologically-relevant explanation of thylakoid ultrastructure from pseudo-Bragg peaks was absent in earlier works on scattering from cyanobacteria [4, 5]. Here, all peaks were interpreted as occurring from different repeat distances and were roughly correlated to the dimensions of TEM-observed distances between thylakoid membranes. We aim to create more elaborate cyanobacterial thylakoid ultrastructure models by correlating X-ray and neutron scattering, TEM, spectroscopic and biochemical data. Such models would allow explaining thylakoid ultrastructures *in vivo* and following environment-driven changes of the thylakoid systems.

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PART 2.
HYDROGEN ENERGY FOR SUSTAINABILITY

Section 2.1: Energy for the Future – Hydrogen economy

Lecture 1

Standardization of hydrogen technologies and fuel cells in the Russian Federation

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National hydrogen energy association (NHEA RF) in cooperation with the Technical committee TC 029 "Hydrogen technologies" (GOST R) have developed a plan of implementation of national hydrogen standards by the year 2017. By now, several high-priority standards have been introduced by the Federal Agency of Technical Regulation and Metrology. Below you will find the list of existing national standards.

National hydrogen energy association (NHEA RF) in cooperation with the Technical committee GOST R TC 029 "Hydrogen technologies", a Russian analogue to the ISO/TC197 have implemented 18 national standards harmonized with those of ISO and IEC.

Another circumstance of great importance is that these standards can be applied at customs territory of the Commonwealth of Independent States (Azerbaijan, Armenia, Belorussia, Georgia, Kazakhstan, Kirgizia, Moldavia, Tajikistan, Uzbekistan and Ukraine), and it significantly broadens the geography of their application.

Recently, several national standards have been adopted, such as:
GOST R 55226-2012; GOST R ISO 14687-1-2012; GOST R ISO 14687-2-2013; GOST R 54110-2010; GOST R 54111.1-2010; GOST R 54111.2-2010; GOST R 54111.3-2011; GOST R 54112-2010; GOST R 54113-2010; GOST R 54114-2012. Besides that, new draft regulatory documents, based on international ISO and IEC standards, are being developed now.

Currently, many subsidiaries and representative offices of well-known international companies are actively working in the Russian Federation. These companies are leaders of the world automobile industry, industrial gas production and have huge experience in the usage of hydrogen technologies. However, we did not obtain any significant result in organizing an efficient cooperation between these subsidiaries and municipal and state administration bodies.

At present, there are no hydrogen fuelling stations in Russia and no hydrogen vehicles that are being operated. Although, we currently have all the necessary

normative framework for the development and promotion of hydrogen technologies on the Russian market, including standards for hydrogen vehicles, fuelling stations and autonomous power facilities on fuel cells. The realization of innovative projects in the area of hydrogen technologies could allow us to create a precedent for further compliance of technical regulatory base on hydrogen technologies with international safety requirements and to facilitate the development of hydrogen technologies market in Russia and the Commonwealth of Independent States in the whole and also to overcome possible technical barriers in international trade.

The list of GOST R national standards is presented below, as you probably noticed, some GOST R standards need to be revised. However, this is a temporary factor, which would not affect the promotion of these technologies on the Russian market as the implementation of new international standards is a continuous process.

Designation	**Name**
GOST 3022-80	Hydrogen for industrial use. Specifications
GOST R 51673-2000	Gaseous pure hydrogen. Specifications
GOST R 54110-2010	Hydrogen generators using fuel processing technologies Part 1. Safety
GOST R 54111.1-2010	Fuel cell road vehicles — Safety specifications. Part 1. Vehicle functional safety
GOST R 54111.2-2010	Fuel cell road vehicles. Safety specifications. Part 2. Protection against hydrogen hazards for vehicles fuelled with compressed hydrogen
GOST R 54111.3-2011	Fuel cell road vehicles. Safety specifications. Part 3. Protection of persons against electric shock
GOST R 54113-2010	Compressed hydrogen surface vehicle refuelling connecting devices
GOST R 54114-2010	Transportable gas storage devices. Hydrogen absorbed in reversible metal hydride
GOST R 55226-2012	Gaseous hydrogen. Fuelling stations
GOST R ISO 14687-1-2012	Hydrogen fuel. Product specification. Part 1. All applications except proton exchange membrane fuel cell for road vehicles
GOST R ISO 146687-2-2013	Hydrogen fuel. Product specification. Part 2. Proton exchange membrane (PEM) fuel cell applications for road vehicles
GOST R ISO 13985-2013	Liquid hydrogen. Land vehicle fuel tanks

Designation	Name
GOST R ISO 26142-2013	Hydrogen detection apparatus. Stationary applications
GOST R 55891-2013	Gaseous hydrogen and hydrogen blends. Land vehicle fuel tanks
GOST R ISO 22734-1-2013	Hydrogen generators using water electrolysis process. Part 1. Industrial and commercial applications
GOST R ISO 22734-2-2014	Hydrogen generators using water electrolysis process. Part 2. Residential applications
GOST R 56188.1-2014	Fuel cell technologies. Part 1. Terminology
GOST R IEC 62282-2-2014	Fuel cell technologies. Part 2. Fuel cell modules
GOST R IEC 62282-3-100-2014	Fuel cell technologies. Part 3-100. Stationary fuel cell power systems. Safety
GOST R IEC 62282-3-200-2014	Fuel cell technologies. Part 3-200. Stationary fuel cell power systems. Performance test methods

Table 1. List of GOST R national standards.

The Russian Technical Committee "Hydrogen Technologies" TC029/GOST-R and the National Hydrogen Energy Association of Russia are deeply interested in further development of new ISO and IEC standards and in the improvement of the existing ones, as well as in closer cooperation with these organizations. NHEA is ready to participate directly in the process of development of international standards for hydrogen technologies and fuel cells.

One of the key elements of this cooperation may be a mutually profitable promotion of vehicles and other equipment based on the hydrogen and fuel cell technologies in the Russian Federation.

Lecture 2

Challenges in sustainable algal biofuels production

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With impending climate change and ever decreasing supplies of easily extractable fossil fuel, means to produce renewable and sustainable replacement fuels are being sought. Plants or algae appear ideal since they can use sunlight to fix CO_2 into usable fuel or fuel feedstocks. However, as the world population approaches the 10^9 mark, the use of agricultural land to produce fuel instead of food cannot be justified. Microalgal biofuel production is under intense investigation due to its promise as a sustainable, renewable biofuel that can be produced using non-arable land and brackish or non-potable water. Some species accumulate high levels of TAGs that can be converted to fatty acid esters suitable as replacement diesel fuels. However, there are many technical barriers to the practical application of microalgae for biofuel production and thus a number of significant challenges need to be met before microalgal biodiesel production becomes a practical reality. These include developing cost-effective cultivation strategies, low energy requiring harvesting technologies, and energy efficient and sustainable lipid conversion technologies. The large culture volumes that will be necessary dictate that the necessary nutrients come from wastewaters, such as the effluents from secondary treatment. Economical and energy sparing harvesting will require the development of novel flocculation or floatation strategies and new methods of oil extraction/catalysis that avoid the extensive use of solvents. Recent advances in these critical areas are reviewed and some of the possible strategies for moving forward are outlined with examples drawn from our experience with native Canadian algal strains and with work carried out using Design of Experiments (DOE) and Response Surface Methodology (RSM) approaches for optimization of microalgal growth on synthetic media and amended wastewater. Some recent results from the Advanced Biofuels Development Laboratory (U of M) and from the Life Sciences Research Center (USAFA) will be presented.

Lecture 3

Russian R&D in Hydrogen Energy

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Hydrogen is a universal clean and efficient secondary energy carrier with the highest energy value. Hydrogen can be used for energy storage and converted in electricity in fuel cells with high efficiency without producing any pollution. Russia recognizes "Novel and renewable energy sources, including hydrogen energy" as a Critical Technology in "Energy efficiency and energy saving", which is one of the 8 top priority science and technology development directions in Russia. Renewable energy sector is an important part of sustainable energy development of Russia, since about 2/3 of the Russia's territory with population about 20 million is not covered by Unified Energy System. Subsidies from budgets of all levels for energy supply of Far North territories and remote regions rise up to 600 billion rubles annually to cover a difference between actual and subsidized tariffs on electricity, which can be as high as 30–40 rubles per kWh.

Renewable resources of biomass and organic wastes have a huge energy potential. International Energy Agency estimates that global primary energy demand for bioenergy, excluding traditional biomass, more than doubles from 526 Mtoe in 2010 to nearly 1 200 Mtoe by 2035, growing at an average rate of 3.3% per year.

The Energy Strategy of the Russian Federation up to 2030 supports development of the energy sector based on renewable energy and expanding production and use of new types of fuel derived from various types of biomass. The state policy in the sphere of local energy resources use for the period up to 2030 will provide for the following: restoration and support of development of local energy resources production, establishment of thermal power plants and boiler rooms running on these sources (peat, wastes of forestry and wood processing industries), including in hard-to-reach and remote areas and creation of favorable conditions for energy production on the basis of municipal wastes.

Projects in hydrogen and fuel cell development are supported within the frame of the Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014–2020. The Program is focused in R&D projects, each participant has to have industrial partner (can be foreign) to apply. Ministry for Education and Science in 2015 spent about $50M in "Energy efficiency and energy saving" priority direction.

In 2015 the Russian Government has renewed targets for RES-based generation in the wholesale market: 2.5% of total power production in 2020 and 3972 MW of installed capacity implementation of is planned to put into operation, but bioenergy is not considered as an energy source of wholesale electricity market, and can be used only in isolated energy systems and for distributed generation. Fortunately distributed generation becomes a significant part of Russian energy sector. Annual commissioning of distributed generating capacities today has the same level as commissioning of capacities in Unified Energy System. Thus, there is a big growing market for bioenergy and biohydrogen.

Lecture 4

Microoxic niches within the thylakoid stroma of air-grown *Chlamydomonas reinhardtii* protect hydrogenase and support continuous hydrogen production under fully aerobic environment

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The photosynthetic hydrogen-producing enzyme hydrogenase is common to microalgae such as the model organism *Chlamydomonas reinhardtii*. While for almost half a century the enzyme was considered active *in vivo* only under anaerobic conditions, our results show hydrogenase activity in air-grown microalgae. Hence, the unexpected hydrogenase activity indicates a putative microoxic niche/s at the proximity of the chloroplast thylakoid. Such a locality could be formed by efficient oxygen consumption at the stromal side of thylakoid membrane. Indeed, after careful dissections involving MIMS, isotopes and inhibitors, we found that both chlororespiration catalyzed by PTOX and Mehler reactions catalyzed by Photosystem I (PSI) and Flavodiiron proteins (FLVs) significantly contribute to oxygen uptake rate. Remarkably, while mitochondrial respiration is constant, oxygen consumption in the chloroplast increases in parallel with increasing irradiance and thus protecting hydrogenase upon fluctuating light intensities. Nevertheless, we found that in a transition to high light, the hydrogen production rate is significantly enhanced for a short duration (100 seconds), indicating that hydrogenase functions as an immediate sink for surplus electrons.

Lastly, the main barrier of current engineering efforts toward algal hydrogen production is thought to be the oxygen sensitivity of hydrogenase. Our findings of the microoxic locality at the thylakoid stroma could be used as a platform for the bioengineering of oxygen scavenging mechanisms in the chloroplast. Such a modification, if successful, could results in a revolutionary continuous production of hydrogen in air grown microalgae.

Funding: ISF-iCore 757/12 – ‘Comprehensive understanding and modeling of plant responses to multiple abrupt abiotic stresses and to prolonged climatic changes’.

Lecture 5

Composite oxide supported catalysys for hydrogen production in Anion Exchange Membrane Electrolysis Cells (AEMEC)

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The new trend in the field of hydrogen production by water electrolysis is the development of cells, operating with a polymer anion conducting membrane (AEMEC), which are expected to combine the advantages of alkaline electrolisers, working with liquid electrolyte (using cheaper non-precious metal catalysts) with those of the systems using protonconductive polymer electrolyte membrane (PEMEC) – exhibiting high efficiency, environmental friendliness, and compatability with renewable energy sources. In this study composite nanosized materials based on cobalt are investigated as catalyst for AEMEC. The efficiency of the electrolysis is compared to that in PEMEC using well established platinum and iridium oxide catalysts. Cobalt is deposited by sol-gel method on non-stoichiometric mixture of titanium oxides with common formula Ti_nO_{2n-1} (Magnelli phase titania). Methods of XRD, EDX, and SEM are used to study chemical composition, surface structure and morphology of the synthesized catalysts. Thus obtained electrodes, containing 0.5 mg cm^{-2} catalyst are tested in aqueous alkaline media (25% KOH) as well as in cells with anion exchenge membrane. Common electrochemical techniques of cyclic voltammetry and polarization curves are used to obtain information about the proceeding changes in the oxidation state of Co on the catalyst surface as well as to determine the potentials of oxygen offset and the corresponding current densities. It is found that the composite catalysts under study facilitate essentially the anodic reaction of oxygen evolution, which reaches current densities of 200 mA cm^{-2} at overvoltage of 1.6 V. Long-term potentiostatic tests are performed to study the stability of the catalysts with time. The synthesized composite materials show good durability which allows considering them as promising catalysts for hydrogen production via alkaline water electrolysis.

Lecture 6

Joint Russian-Taiwan project for biohydrogen production and purification

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Research and development in multidisciplinary fields such as a biohydrogen production and purification require a scientific collaboration of different research groups. Productive cooperation, exchange of knowledge and experience allow to tackle challenges and achieve required goals of research groups. The project involves two research groups from Joint Institute for High Temperatures RAS (Russia) and Feng Chia University (Taiwan) and is aimed to solve interdisciplinary problem for biohydrogen production during conversion of different types of organic materials, its purification and storage in the solid phase for use in distributed and autonomous power units.

Joint project is divided into two parts:

- Biohydrogen production via dark fermentation (Taiwanese part)
- Biohydrogen purification and storage (Russian part)

Biohydrogen production includes development of pretreatment methods for sugar recovery from wastes, bioreactor design for biohydrogen production from hydrolizates, experimental investigations of the influence of crucial factors on biohydrogen production rate. Biohydrogen purification and storage includes development new hydrogen absorbing materials meeting the requirements of biohydrogen purification and investigations of their properties, optimization of heat and mass transfer of metal hydride reactors.

During the joint project Taiwanese team developed pretreatment methods based on acid hydrolysis, achieved high biohydrogen production rates in a continuously stirred anaerobic bioreactor and a continuously external circulating bioreactor up to 7 Nl/l/h of H_2, defined optimal values of important factors influencing biohydrogen production rate.

The main achievements of Russian team are preparation and PCT investigations of new AB5-type intermetallic compounds with low equilibrium pressures required for biohydrogen purification, experimental investigations of heat and mass transfer processes in metal hydride reactor during hydrogen extraction from H_2/CO_2 gas mixtures, achieved hydrogen recovery 94% from gas mixture containing 59% H_2 by metal hydrides.

Further targets for international collaboration are system integration and development of pilot-scale complex system of biohydrogen production and purification.

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Poster 1

Biohydrogen purification using metalhydride technologies

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Nowadays methods of effective conversion of biomass to methane and hydrogen by microorganisms are widely studied and used in practice. The advantage of these methods is the environmental friendliness and the possibility of organic waste conversion. Development and implementation of distributed and autonomous power systems based on bioreactors for the production of hydrogen, and efficient systems for the solid-state hydrogen storage would reduce the environmental burden due to the conversion of organic waste and provide energy and heat for standalone and distributed consumers. Technical problems of the development of efficient hydrogen storage systems are connected above all with the necessity to arrange efficient heat and mass transfer for the reliable absorption and desorption reaction heat supply or removal. Technical obstacles of system integration *"bioreactor of hydrogen production – purification and solid-state storage system – power unit"* is a key question and should be solved for practical application.

The experimental setup for a study of fundamental processes in a unified system of *"bioreactor of hydrogen production – purification and solid-state storage system – power unit"* is presented. New experimental flow-through reactors RSP-8 and RSP-8I for purification and storage with nominal capacities of 130 st.l. and 100 st.l. respectively was designed and developed.

Experimental investigations of hydrogen separation by a filtration technique from various hydrogen/carbon dioxide gas mixtures are presented. Main reasons of heat and mass transfer crisis origin in a metal hydride bed are identified. Experiments confirmed the possibility of hydrogen purification from carbon dioxide containing gas mixtures by flow-through method. High extraction ratio at low (up to 20 st.l/min) gas flow rate (extraction ratio over 80% at a reactor filling up to 75%) is achieved.

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Poster 2

Self-ignition of pressurized hydrogen diluted by methane

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The fact that the ignition delay of hydrogen can reach values of 10^{-6}–10^{-5} s certainly suggests that the storage and operation of compressed hydrogen can be highly explosive, even when there are no external ignition sources. The ignition of hydrogen occurs not only in preliminary mixed mixtures: the ignition of gases is possible at the contact surface of fuel and oxidizer. One of such cases is self-ignition of hydrogen at a pulse discharge from a vessel under high pressure into air.

One method for prevention of the ignition of hydrogen is the using an admixture of other combustible gases. Wherein, the ignition delay of a binary mixture is several times higher than the delay of ignition of pure hydrogen. The aim of the present paper is the experimental determination of the ignition delays of the pulse jet of a binary mixture of hydrogen with methane, depending on the initial pressure of the hydrogen-methane mixture.

A mixture of hydrogen with methane was preliminary prepared in a vessel of 40 liters. The initial pressure of the mixture was varied from 3 to 15 MPa. During the discharge of the binary mixture ignition delays were measured relative to the moment of a breaking of the diaphragm. Ignition delays were determined by a photomultiplier tube.

Ignition delays of self-ignition of the binary mixture of hydrogen and methane were determined experimentally as a function of the initial pressure and concentration of methane. It was shown, that the adding 18% (mol.) of methane leads to a 6-fold increase of ignition delays. Estimation with using the quasi-one dimensional equations of gas-dynamics showed that the effect of impurities on methane ignition delay is mainly thermodynamic nature.

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Poster 3

Scale effect in LaNi5-based alloys used in bio-hydrogen purification and storage

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Research Metal hydrides are considered as convenient and safe hydrogen storage for PEM fuel cells and large scale metal hydride storage systems are needed for practical applications. An accuracy of data on PCT diagram of a hydrogen absorbing material is crucial to obtain adequate simulation results and thus to a system design. Scaling up from laboratory samples to metal hydride reactors has shown a difference in properties. Changes in capacity and rates of hydrogen sorption and desorption at a system level may include: heat transfer during hydrogen uptake and release, local temperatures within the material test bed, decrepitation and/or agglomeration of the materials, interlocking of grains and resulting forces generated with material expansion, gas channeling effects, special variations in hydrogen content.

It is well known that the hydrogen absorption is always connected with large unit cell expansion (up to 25%) due to interstitial occupation of hydrogen atoms. This expansion not only leads to the dispersion of materials to powders with average particle size o 1–10 lm, but also induces elastic strains in particles as well as in their contact points. In the systems consisting of large number of particles, additional elastic strains may emerge due to the collective elastic particle interactions.

For the first time the dependence of thermodynamic parameters of hydrogen desorption process on sample scale (weight) was discovered experimentally for the desorption isotherms of 100 g and 500 g samples of $LaFe_{0.1}Mn_{0.3}Ni_{4.8}$. The scale effect leads to decreasing of the equilibrium pressure for the larger sample, the pressure difference is ΔP^{scale}=−0:15±0:03 MPa at 100°C, and is in the range ΔP^{scale}=−0:5 to −0:3 MPa at 150°C. In our opinion the scale effect is determined by elastic strains due to the mutual influence of particles while phase transition from hydride to solid solution and back in large-scale fine-disperse beds of AB5-type compounds. Scale effect has no influence on the behavior of pure hydride phase and is absent for small samples.

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Poster 4

Detonation mitigation in hydrogen-fueled spark ignition engine by adding low-energetic components

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Nowadays one of the developed approaches is the use of hydrogen as an additive to widespread fuels, such as gasoline, methane, in internal combustion engines [1, 2], whose aim is the improvement of effectiveness of these fuels and reduction of carbon oxides emissions. In the present paper opposite approach is investigated, that was also studied in [3, 4]: hydrogen is supposed to be the main fuel that could be promising for energy production in regions distant from the mainstream power supply systems where hydrogen produced by electrolysis is becoming a preferential accumulator for sustained energy storage. However, the use of hydrogen as a fuel is challenged by its low detonability limit. In the perspective of the detonation exclusion when engine runs on hydrogen the present paper analyses the use of three different small additives increasing the detonability limit: methane as a widespread fuel, water steam as combustion product, and air excess.

For the determination of lower limits of additives of mentioned substances to hydrogen providing the absence of detonation the 2D modelling of combustion of corresponding fuel in spark ignition engine was performed. Simulations were conducted with the use of detailed kinetic reaction mechanisms. It was received that when the premixed hydrogen-air mixture contains less than 1.0% of methane, 2.0% of water steam, or 3.0% of air excess combustion ceases to be detonative (the mixture content is stated as follows: 70.5% (vol.) of air – x% of additive – (29.5−x)% of hydrogen). The amounts of additives providing the most optimal combustion, i.e. characterized by the maximum in-cylinder pressure and its corresponding crankshaft angle not exceeding 6 MPa and 20° after top dead center correspondingly, were also found: (2.5–3.0)% of methane, (3.1–5.4)% of water steam, (4.2–7.3)% of air excess.

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Section 2.2: Elevating Climate Change

Poster 1

Torrefaction technology

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Energy utilization of wood waste (or other plant origin biomass) is a promising direction of development. The first step on the way of increasing the biomass energy density is its granulation. The granulated biomass has a high bulk density and a higher heating value (against the feedstock). The main drawback of initial pellets is their high hygroscopicity. Pellets require quite stringent conditions of storage and transportation. These disadvantages can be eliminated by using the new pretreatment technology – torrefaction.

Torrefaction is a fuel thermal pyrolytic pretreatment at relatively low temperatures about 200–300°C. Torrefied pellets are non-hygroscopic and have a higher combustion heat, approaching by the main characteristics to coal.

A growing number of investigations in the field of energy production are aimed at replacing coal in order to reduce harmful emissions. One of the most promising coal substitutions is biomass. But at the partial coal replacement a number of problems associated with the fuel supply in existing coal-fired furnaces are appeared. Power consumption for grinding of biomass has a significant effect on the maximum share of the replaced coal. Heat pretreatment of raw materials will significantly reduce electricity consumption for grinding of the final product.

This paper presents developed in JIHT RAS scheme of industrial unit with biomass torrefaction reactor. Torrefaction reactor capacity is 200 kg/h by feedstock. The distinctive feature and the main advantage of JIHT RAS technology is using of combustion products of gas generator station as a heat transfer agent. The final product since its high consumer characteristics can be recommended as a primary (or partially substitute) fuel in coal-fired boilers without their major modifications.

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Poster 2

Unconventional and renewable energy resources for sustainable arctic development

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An interest towards Arctic region development in Russia is related to its resource availability, including oil and natural gas, new transit routes such as Northern Sea Route (NSR), and new development opportunities opened by the climate changes. Russian Arctic development is a strategic priority, where energy and transportation sectors play a key role. It is a complex issue that demands advanced technical solutions, individual planning approaches to an efficient energy supply, and ecological safety priority.

Sustainable Arctic development is being restrained by the challenges in energy supply of isolated distanced consumers. At the present time this region has decentralized energy system based on diesel power stations and fuels delivery. Arctic energy supply optimization is targeted on the governmental level. It is based on the increased use of renewable and unconventional energy sources, including the local ones, and corresponding energy infrastructure development. Renewable energy resources play considerable role in the Arctic region social and economic development, and in providing national security.

Due to the fact that Arctic infrastructure (including NSR support infrastructure) are going to be intensively developed with governmental support in the nearest future and will respectively demand stable energy supply, it is clear that previously functioned methods are not efficient. This is why the search for new urgent solutions for safe and efficient energy supply with optimal use of local renewable energy resources, e.g. wind and solar, is needed. This will give the opportunities to decrease fuel dependency and maximize energy and economic effects. It will be only possible when the equipment is climate adapted in order to minimize energy losses in the extreme climate conditions. However, the challenges of availability, accessibility and efficiency of renewable energy resources in the Arctic region of Russia are still left unsolved. Energy demand analysis of the Arctic region based on scale, properties and demand features is crucial in order to understand the renewable energy development potential.

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Section 2.3: Biological hydrogen production

Lecture 1

First principles design of water-soluble photochemical proteins engineered for solar energy conversion in living cells

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We are designing and testing entirely novel photochemical proteins, maquettes, incorporated into the genome of living cells to provide a self-sustaining way to convert solar energy into useful chemical fuels. By intercepting the energy of absorbed light early enough to avoid competing functions and energy diversions normal to natural photo-systems, these manmade photochemical proteins promise maximized engineering efficiencies solely directed to fuel production. Maquette design combines first-principles protein folding and cofactor assembly with first-principles molecular electron tunneling engineering drawn from detailed analyses of natural photo-systems. Design and constructions is making progress toward photochemical maquettes engineered to operate at optimal energy conversion in water-soluble proteins that can operate in the aqueous compartments of living microorganisms. While there are significant hurdles still to surmount, early developments are promising. Maquettes have been proven to integrate with the *in vivo* machinery of cofactor biogenesis and ligation of bilins, hemes and chlorophyll. They are compatible with membrane transport by either Tat or Sec transporters. Maquettes have also been fused with natural light-harvesting proteins (biliproteins) to support multi-cofactor light energy transport. Maquettes are readily expressed in high yield and production is scalable.

Lecture 2

Hydrogen production by *Rhodobacter sphaeroides* mutants without LH2 complex

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Rba. sphaeroides are capable to grow in a wide range of conditions and dispose of different organic substrates. This bacteria can produce hydrogen under anaerobic light conditions with nitrogen limitation. The rate of the process is close to the practical significance using organic acids. Using the mutant strains with reduced amount of pigment can increase the biomass in photobioreactor (PBR) without self-shading effect.

The aim of this study was to compare the influence of light intensity on the growth and hydrogen production of *Rba. sphaeroides* parental strain and their mutant lacking peripheral antenna. Obtained results showed that the growth rates of turbidostat cultures of parental strain and the mutant increased with the increase of light intensity with the presents of lactate, succinate and mix of lactate and acetate, as a carbon source. The maximum rate of growth (μ_{max}) of parental strain (2.4.1), growing with lactate, was almost 2.5 times higher than for mutant without LH2 complex (DBCΩ). However, DBCΩ achieved μ_{max} at lower light intensity (I) than 2.4.1. With the presence of lactate and acetate the growth rate of the mutant started to increase later, but it achieved maximum earlier than for 2.4.1. The both strains exhibit higher growth rates in the presence of succinate. DBCΩ can grow faster than the 2.4.1 in this case, but mutant needs a much higher light intensity for that.

The H_2 production by chemostat cultures of *Rba. sphaeroides* strains limited by ammonium increased with the increasing of biomass content, which was determined by adding of $(NH_4)_2SO_4$ up to certain limit. Mutants without LH2 can produce hydrogen in cultures with higher cell concentration than parental strains. Maximum H_2 production was also higher for the mutants.

Short-term experiments of H_2 production shown that max rate of the process per volume of cell suspension depends on cells concentration. Mutants cells suspensions with high biomass concentration produced hydrogen with much higher rate than parental strains but they demand higher light intensity for that. The parental strains did not produce H_2 in dense suspension.

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Lecture 3

Constraints in the scale-up of photobiological hydrogen production with microalgae

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Biological hydrogen production is being evaluated for use as a fuel since it is promising substitute for carbonaceous fuels owing to its high conversion efficiency and high specific content. The basic advantages of biological hydrogen production over the other green energy sources are that it does not compete for agricultural land use, and it does not pollute, as water is the only by-product of the combustion. These properties make hydrogen a suitable fuel for the future. Among several biotechnological approaches, photobiological production carried out with the microalga *Chlamydomonas reinhardtii* has been investigated in the recent years. It was found that sulfur-deprived *C. reinhardtii* CC124 cultures grown in laboratory photobioreactors (PBRs) equipped with an improved mixing system, the light conversion efficiency can reach 1.6% of PAR (photosynthetically active radiation), and up to 3.2% of PAR which is remarkably high, with the D1 protein mutant L159I-N230Y which is considered one of the highest worldwide H_2 producers. However, the use of solar light is mandatory to scale-up photobiological hydrogen production to an industrial level. A number of PBR designs have been proposed, mostly in the mass culture of microalgae for biodiesel production, while information on the scale-up of H_2 production, in particular outdoors, is still very limited. Results of experiments conducted with sulfur-deprived cultures of *C. reinhardtii* CC124 in a 50-L tubular PBR have demonstrated the hydrogen production under direct sunlight is feasible, but the output per unit of volume was about 18–20% of that produced in the laboratory. The strong inhibition of PS II (F_v/F_m) by solar light was considered the main reason of the reduced H_2 output. To circumvent this problem, which is actually a great drawback in algal biotechnology, we have designed and devised a new photobioreactor (volume 110 L) which allows a more uniform distribution of light on the reactor surface. To reach this goal the tubular reactor was immersed in a light scattering silica nanoparticle suspension. The reactor performance was assessed with *C. reinhardtii* CC124 under sulfur starvation. The use of nanoparticles allowed a dilution of the incident light irradiance by a factor of four, which strongly reduced the risk of oversaturation of the photosynthetic electron transport chain. Hydrogen production with the 110-L PBR was investigated using a direct inoculum of sulfur-limited cultures having a residual sulfate concentration below 1.0 mg L^{-1}. The total amount of hydrogen gas

collected was about 3 litres, with a light conversion efficiency of 0.05% of solar light. Recently, a first attempt to produce hydrogen with the cyanobacterium *Synechocystis* PCC 6803, via an indirect light driven process, was investigated in the outdoor tubular photobioreactor (50 L). The rate of hydrogen production achieved was 0.05 mL H_2 $L^{-1}h^{-1}$. The production of hydrogen in the dark was sustained by fermentation of carbohydrates accumulated during the nitrogen starvation of cells previously grown under solar light. Recently, another novel photobioreactor (1300 L) was devised and constructed by our group, for the outdoor cultivation and hydrogen production with *Synechocystis* PCC 6803. The reactor was designed to promote the *"light dilution effect"*, which should enable the cells to use solar light with higher efficiency, and reduce the risk of photoinhibition. The biomass yield of the culture reached about 25 g/m^2/day. One of the serious problems that arose during the cultivation of *Synechocystis* outdoors was the susceptibility of the cells to predation by various type of protozoa and other class of algae (e.g. Chrysophyceae). Practical methods for preventing contamination in large-scale PBR have been successfully tested.

Lecture 4

Temperature-sensitive PSII: toward a sustainable bioreactor for photosynthetic hydrogen production

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Photosynthesis is one of the most important life-sustaining reactions on our planet. It provides the energy required for the survival of all life forms, and it underlies the accumulation of fossil fuels, the main source of energy for sustaining modern human lifestyles. Solar energy is the most abundant energy source on our planet and drives photosynthesis by exciting Photosystem II and Photosystem I. Certain microalgae and cyanobacteria possess the ability to capture solar energy trough photosynthesis and transfer it to the energy carrier, H_2. H_2 is a valuable fuel, because its combustion produces only one by-product: water. However, the establishment of an efficient biophotolytic H_2 production system is hindered by three main obstacles: (1) the hydrogen-evolving enzyme, [Fe,Fe]-hydrogenase, is highly sensitive to oxygen; (2) energy conversion efficiencies are not economically viable; and (3) hydrogen-producing organisms are sensitive to stressful conditions in large-scale production systems. This study aimed to circumvent the oxygen sensitivity of this process with a cyclic hydrogen production system. This approach required a temperature-sensitive PSII mutant that responded to high-temperatures by reducing oxygen evolution. To that end, we randomly mutagenized the green microalgae, *Chlamydomonas reinhardtii*, to generate mutants that exhibited temperature-sensitive photoautotrophic growth. The selected mutants were further characterized by their ability to evolve oxygen and hydrogen at 25 and 37°C. We identified four candidate mutants for this project. They were characterized by spectroscopy and by biomolecular means under conditions relevant for the proposed cyclic system. Finally, we demonstrated that these mutants could function in a prototype hydrogen-producing bioreactor. These mutant microalgae represent a feasible approach for a large-scale hydrogen production plant.

Lecture 5

Acclimation of *C. Reinhardtii* to magnesium deficiency: establishment of anoxia at high photosynthetic activity

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Sulfur deprivation is a widely used method applied to sustain hydrogen photoproduction in green algae *C. reinhardtii*. It induces sharp decline in photosynthetic reactions, in particular, PS2 activity promoting culture transition into anaerobic conditions and activation of oxygen-sensitive Fe-Fe hydrogenase. Recently Mg-deprivation has been proposed as an alternative method sustaining hydrogen evolution during longer period and at higher PS2 activity as compared with S-deprivation [1]. However, the mechanisms that ensure long-term hydrogen photoproduction in Mg-deprived cells with active O_2-evolving PS2 activity remain unclear. In the present work we performed a comparative analysis of the main chloroplast proteins and photosynthesis/respiration rates at different stages of Mg- and S-deprivation in *C. reinhardtii*. Immunoblotting analysis revealed that the main photosynthetic complexes (Rubisco (Rbcl), $Cytb_6f$ (CytF), PS II (PsbA)) remained at relatively high level during 120 h of Mg- deprivation whereas S-deprivation induced rapid decrease in their content already at 24 h of incubation. This finding was consistent with the relatively high values of light-saturated net O_2 rate and PS II activity attaining 60–70% of those in control (non-starving cells) up to 120 h of Mg deprivation. PS I (PsaD) remained quite stable under both types of deprivation. Comparison of dark O_2 consumption in Mg- and S-deprived cells revealed only minor differences between them. Therefore we assume that dark photosynthetic reactions, and respiration alone is rather not sufficient to ensure Mg-deprivation stimulated transition into anaerobic conditions at light. This suggests the involvement of alternative pathways for efficient O_2 removal operating in the chloroplast in light. Water-water cycles including Mehler reaction and flavodiiron proteins (FlvA/B, [2]) could be feasible candidates.

This research was supported by Russian Foundation of Basic Research (projects 14-04-00302 and 15-54-78014).

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2. M. Jokel et al, 2015, Plant Cell Physiol., 56(8):1598–607

Lecture 6

Harnesing photosynthesis for solar energy conversion and hydrogen generation: building a bio-generator

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Photosynthetic organisms, membranes and complexes are attractive starting materials for solar energy conversion (SEC). Our overall goal is to develop methods to perform SEC using these materials in simple, inexpensive and totally non-polluting fashion. We have previously shown (Larom et al., PNAS, 2010) that a mutation in PS II in the cyanobacterium *Synechocystis* sp. PCC 6803 (Syn), results in a strain that has the enhanced ability to transfer electrons from water to electron carriers or to modified gold electrodes (Larom et al. Photosyn. Res. 2015). Here we show how the remarkable photocatalytic activity of the photosynthetic apparatus leads to overall water splitting with oxygen and hydrogen production in Bio-Photo-Electro-Chemical (BPEC) cells via the simplest and cleanest of deposition processes and without the need for sacrificial electron sources. With plant thylakoids, electrons are shuttled by FeCN to a transparent electrode, 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 with the thylakoid membranes and a Si photovoltaic module achieves overall water splitting with solar to hydrogen conversion efficiency of 0.3% (Pinhassi et al. PLOS One 2015 and submitted). With cyanobacterial cells, following a brief treatment that does not kill the cells, electrons are transferred directly to a graphite electrode, utilizing endogenous electron carriers. The current produced can be used for hydrogen production at low additional bias for significantly longer durations than the plant thylakoids.

Lecture 7

Multipurpose technology of natural gas pyrolysis for the hydrogen energetics

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Hydrogen using in energy purposes is able to greatly reduce the concentration of harmful substances in atmosphere. However, existing energetic hydrogen production technologies are associated with significant emissions of carbon dioxide into the environment. This factor is a prerequisite to the development of the new environmental friendly hydrogen producing technologies. One of the potential utilization ways is considered in injecting carbon dioxide into the oil or natural gas exhausted well. This practice cannot be regarded as rational, since it leads to disruption of the natural balance. As it shown in last investigations, injection of carbon dioxide leads to the passivation of the natural catalyst system. This fact may lead to a breach of the natural gas and oil formation process.

The energetic hydrogen production can be rational and economically valuable only if all end-products (including by-products) will have industrial application. The solution of ecological problem of increased harmful substances concentration problems should become multipurpose. At JIHT RAS there is proposed a new integrated technology for biomass waste and natural gas processing with hydrogen and carbon-carbon composite production. The carbon material can be used as high-energy ecological friendly fuel and as raw materials for industrial processes. The developed technology is an example of the new approach implementation to solve the problem of reducing the harmful impact of the fuel-power complex on the balance of nature.

This work was supported by Leading Scientific School (SS-8406.2016.8).

Poster 1

Ferredoxin–hydrogenase fusion protein successfully diverts the photosynthetic electron flux towards hydrogen production *in vivo*

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Hydrogen photo-production in green algae, catalyzed by the enzyme [FeFe]-hydrogenase (HydA), is considered a promising source of renewable clean energy. Yet, a significant increase in hydrogen production efficiency is necessary for industrial scale up. We have previously shown that a major challenge to be resolved is the inferior competitiveness of HydA with NADPH production, catalyzed by ferredoxin-$NADP^+$-reductase (FNR). In this work, we explored the *in vivo* hydrogen production efficiency of the fusion protein Fd-HydA, in which the electron donor Ferredoxin (Fd) is fused to HydA and expressed in the model organism *Chlamydomonas reinhardtii*.

We show that once the synthetic gene Fd-HydA is expressed in *C. reinhardtii*, Fd-HydA efficiently diverts the electron flow to hydrogen production, thus supporting the previous observations made *in vitro*. In conclusion we show that Fd-HydA has a ~4.5-fold greater photosynthetic enzyme activity (PEA) than that of the native HydA *in vivo*.

Poster 2

Photosynthesis and hydrogen photoproduction by the cyanobacterium *Anabaena* sp. 7120 and its mutants with modified nitrogenase under different light and temperature

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Photobiological production of H_2 by cyanobacteria is an attractive source of renewable energy because these microorganisms have simple needs: simple nutritional requirement with readily available sunlight for energy and water as the electron donor. The main H_2-producing enzyme in cyanobacteria is nitrogenase (N_2ase). In *Anabaena*, the enzyme activity is induced during the late stages of heterocyst differentiation and then declines in a rather short time under ambient air or N_2, because its product (ammonia) meets the nitrogen nutritional requirements of cells and consequently suppresses de novo N_2ase synthesis. Mutants with amino acid substitutions presumed to be located in the vicinity of the FeMo-cofactor of nitrogenase (dc-Q193S and dc-R284H) that express N_2ase activity even under high N_2 atmosphere had been created from *Anabaena* sp. strain PCC 7120ΔHup as the parent. We studied the effects of temperature and light intensity on H_2 production by the parental strain and the two mutants dc-Q193S and dc-R284H.

The photosynthetic O_2 evolution rates of the parental strain and their mutants as a function of light intensity were studied by an O_2 electrode for 4 min. The highest O_2 evolution rates were achieved at the temperature 35°C. Optimal temperature for the growth of the parental strain and their mutants was 27°C. Maximum photosynthesis rate was the highest with PCC7120ΔHup at all measured temperatures. Transition of cultures to the stationary phase was accompanied by a decrease of photosynthesis rate. Data on the influence of the light intensity and the temperature on H_2 production under varied gas phase are discussed.

This work was supported by Russian Foundation for Basic Research (15-54-50032).

Poster 3

Advantages of mixed carbon fermentation in biological hydrogen production by *Rhodobacter sphaeroides*

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Biological hydrogen (H_2) production is considered as one of the promising ways to generate effective, ecologically clean and renewable energy from various organic substrates, and it can make a significant role in the development of energy technology. Nowadays, the interest of H_2 production by various bacteria is mixed carbon fermentation, mainly because of the cheap and effective carbon sources like glycerol or industrial wastes. Batch photofermentation experiments were carried out to investigate the effect of the composition of the mixed carbon (succinate, glucose, glycerol and acetate) on growth and H_2 production by *Rhodobacter sphaeroides* MDC6521 from Armenian mineral springs. Initial pH, temperature and light intensity were optimized for maximal H_2 production based on our previous research [1]: they were pH 7.0, 28°C, and 2000 Lx, respectively. The results obtained show that in a medium containing glucose in addition to succinate (15 mM) bacterial growth was inhibited and no H_2 production was detected. pH measurements suggest that the metabolism of glucose might create a low pH in the medium, which inhibits the further growth of bacteria. Addition of acetate (15 mM) supported bacterial growth, but didn't show significant difference in H_2 yield compared to control (15 mM succinate only). However, glycerol (15 mM) addition had a positive effect both on bacterial growth and H_2 production. Even though the H_2 production yield (3.6 mmol/L) in the medium containing 15 mM succinate and glycerol didn't reach the value obtained with 30 mM succinate only (6.3 mmol/L), further experiments will be performed to obtain the optimal succinate/glycerol ratio to get the maximum H_2 yield. Combination of carbon sources (succinate, acetate, glycerol, etc.) with different nitrogen sources and other culture condition beneficial for H_2 production can enhance H_2 yield in *R. sphaeroides* an increase the efficiency of transformation of different organic sources.

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Poster 4

Hydrogen photoproduction by Hup− mutant of *Rubruvivax gelatinosus* RL2 under microaerobic conditions

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Mutants of phototrophic bacteria deficient in Hup hydrogenase are considered to be promising for H_2 photoproduction systems due to the absence of H_2 consumption. The aim of the present research is to find out the conditions where the elimination of Hup hydrogenase is beneficial to photobiological production of H_2 in *R. gelatinosus* RL2. In growing cultures of *R. gelatinosus*, no significant difference was found in H_2 photoproduction under anaerobic conditions (Ar) between Hup− mutant and the parental strain. It means that H_2 consumption does not play significant role under these conditions. Under microaerobic conditions, however, the mutant strain produced much more H_2 than the parental strain. In the parental strain, the H_2 production rate gradually decreased to zero with time and H_2 consumption started instead (typically in 2–6 days). Short-term experiments demonstrated that after air addition to H_2-producing cultures, H_2 consumption by parental strain took place under both light and dark, while O_2 consumption, only under dark. Thus, we propose that the principal electron acceptor for H_2 consumption in the parental strain is O_2 in the dark, and N_2 in the light. We added N_2 to the H_2-producing cultures grown under Ar, or, alternatively, cultivated them initially under 50%Ar+50%N_2. In both cases, the noticeable H_2 consumption took place in only the parental strain but not in the mutant. To summarize, the H_2 production by the parental strain appeared to be more sensitive to a trace of air due to H_2 recycling. Hence, the Hup− mutant is promising under microaerobic conditions. In the presence of N_2 or air, the advantage in H_2 production of Hup− mutant over the parental strain becomes more apparent. It follows that N_2 is not optimal to provide anaerobic conditions for a maximal H_2 production by Hup-positive strains.

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Poster 5

Two-stage thermal conversion of biomass into hydrogen-containing gas mixture

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There is high interest to the problem of development of hydrogen production systems based on the renewable energy source – biomass. Experimental investigations of two-stage process of thermal conversion have shown that wood, peat and agricultural waste can be used for obtaining hydrogen-containing gas mixtures.

A method for the two-stage pyrolytic processing of various types of biomass into hydrogen-containing gas mixtures is a combination of pyrolysis of a feedstock and the cracking of the volatile pyrolysis products on the charcoal. The two-stage thermal conversion of biomass is performed sequentially in two reaction volumes with specific temperature conditions. Whole the process is carried without access of oxidant. During the first stage biomass is heated up to 600–700°C in hot wall reactor (the first reaction volume). There occurs the formation of pyrolysis products: charcoal and volatile. They are moved into the second reaction volume – cracking reactor. Here the charcoal is heated up to 1000°C. During the second stage volatile are blown through the thick charcoal bed. The destruction of complex molecules of volatile (tars) on the surface of carbon residue is occurred.

According to the researches, biomass processing allows to obtain a gas mixture which contains of about 45–50% (vol.) of hydrogen (H_2), 40–50% of carbon monoxide (CO) and about 5–10% of other gases (CO_2, CH_4 and other). Gas mixture contains no tar and its calorific value is about 10–12 MJ/m^3.

The work was supported by the Ministry of Education of the Russian Federation and performed under Federal Target Program “Research and development on priority directions of scientific-technological complex of Russia for 2014-2020”, grant agreement from 27.10.2015 № 14.607.21.0134.

Poster 6

Novel approaches to simultaneously combat the oxygen sensitivity of hydrogenase and its poor electron acceptance

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Biological photoproduction of hydrogen is considered to be an ultimate carbon-free source of energy, yet, its commercialization is hindered by two major problems: [FeFe]-Hydrogenase's (HydA) sensitivity to oxygen and poor electron transfer to HydA due to a competition with other processes. In a recent study we found that a *Chlamydomonas reinhardtii* clone (P6) expressing the Ferredoxin-Hydrogenase (Fd-Hyd) fusion enzyme, shows an increased hydrogen production rate. To understand the mechanism of this improvement, we compared the phenotype of clone P6 to its parental wild-type clone D66. We found that upon a transition from dark anaerobic induction to light, D66 ceased hydrogen production prior to oxygen accumulation. In contrast, P6 had a prolonged hydrogen production, thus indicating that a competition for electrons rather than oxygen accumulation inhibits hydrogen production in D66. After the initial transition to light, P6 sustained a steady electron transfer rate (ETR) with minimal oxygen accumulation enabling it to keep producing hydrogen from water photolysis. To gain further insight of the prolonged hydrogen production, we examined the hydrogen accumulation under sulfur deprivation. Under these conditions, we found that although the cellular content of Fd-Hyd enzyme in P6 was lower than the content of HydA in D66, P6 accumulates much more hydrogen.

Poster 7

The *in vitro* enhancment of [FeFe] hydrogenase activity by [Fe] superoxide dismutase

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H_2 producing micro-algae such as *Chlamydomonas reinhardtii*, express the strictly anaerobic enzyme FeFe-hydrogenase (HydA). In this study, we examined whether superoxide dismutase (SOD), an antioxidant, can protect HydA under aerobic conditions. We conducted *in vitro* assays using purified enzymes, to analyze the activity of HydA in the presence or absence of SOD. We observed that SOD enhances HydA activity both in the presence and absence of oxygen, showing that the SOD effect on HydA activity is not strictly oxygen dependent. Furthermore, we found that SOD boosts Ferredoxin-$NADP^+$-oxidoreductase (FNR) $NADP^+$ reduction but not NADPH oxidation i.e. diaphorase activity. Thus, suggesting a mechanism involving proton transfer rather than electron transfer. Based on these findings, we constructed a HydA-SOD fusion protein that further boosted hydrogen production by HydA. The HydA-SOD photosynthetic activity was enhanced by 300%, reaching 700 μmol H_2 (mg [chl] hr)$^{-1}$, which is the fastest photosynthetic rate ever reported for an algal HydA.

Section 2.4: Hydrogenases

Lecture 1

Oxygen diffusion pathways through HydSL hydrogenase from *Thiocapsa roseopersicina*

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Oxygen diffusion through HydSL hydrogenase from *Thiocapsa roseopersicina* was studied by molecular dynamics simulations in YASARA Structure program. The active site of the enzyme was substituted to oxygen molecule. The simulations revealed two possible pathways of oxygen diffusion. One of these pathways led through the small subunit, whereas another one was located between small and large subunits.

An interesting obstacle for oxygen molecule motion was observed in the large subunit of the enzyme. Alignment of this enzyme with an oxygen-sensitive hydrogenase from *Desulfovibrio vulgaris* Miyazaki showed a possible determinant of relative oxygen resistance of *Thiocapsa roseopersicina* hydrogenase. It could be an asparagine residue which is bulkier than a threonine residue present in correspondent position in *Desulfovibrio* hydrogenase.

The work was supported by Russian Foundation for Basic Research (grant number 14-04-01676).

Lecture 2

2D-Tailoring of [FeFe]-Hydrogenases for applications

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The active centers of biocatalysts have evolved to provide perfect preconditions to facilitate specific chemical reactions. The reversible H^+-reduction activity of [FeFe]-hydrogenases is exceptionally high, requires no over-potential, is achieved at standard biochemical conditions and does not depend on the availability of rare metal compounds. Their high catalytic capacity is a product of the complex interplay between catalytic cofactor and protein scaffold [1] rendering [FeFe]-hydrogenases highly interesting targets for applied research. In the photofermentative metabolism of green algae like *Chlamydomonas reinhardtii* the H_2 production activity of [FeFe]-hydrogenases is coupled to the linear photosynthetic electron transport [2] thus being a perfect blueprint for bioinspired photohydrogen production. However, due to metabolic constraints such as the presence of competitive redox enzymes like the FNR photohydrogen evolution in the algal cell does not exploit the full capacity of the hydrogenase. Site selective manipulation of the contact interface on the shared electron mediator PetF enables to redirect the main electron flow from FNR towards the hydrogenase [3]. *In vitro*, simple organic dye molecules can replace PSI as photosensitizer leading to extraordinary high photohydrogen production rates even under ambient light. However, less beneficial features including the native oxygen sensitivity have to be overcome either by establishing protective conditions [4, 5] or by enzyme design approaches before applications based on [FeFe]-hydrogenases as biocatalysts become feasible. While protein manipulation is a standard technique in enzyme design which helps us in multiple ways to understand structure-function relationships within the protein part such as proton transfer and adjustment of catalytic bias, the active cofactor (H-cluster) has long been presumed untouchable. The H-cluster consists of a standard [4Fe4S]-cluster coupled to a unique [2Fe2S]-moiety coordinated by highly unusual ligands which include two CN, three CO and one azadithiolate group. In an interdisciplinary approach combining synthetic chemistry and biochemistry we have developed a spectrum of techniques for the de novo design and site specific manipulation of the complete inorganic H-cluster of [FeFe]-hydrogenases targeting either one or both cluster parts to study the influence of individual constituents on catalytic performance and other enzymatic features [6, 7].

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Lecture 3

Fluorescent proteins as biosensors for studying the activity of hydrogenases and intracellular pH

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Genetically encoded fluorescent biosensors exhibit optical properties responding to molecules in the local environment. In particular, the green fluorescent protein (GFP) and its variants are used to study a large variety of cellular processes. To exploit the potential of GFP for sensing, GFP-based *in vivo* sensors have been developed by targeted mutations and directed evolution. The optical properties of these biosensors change due to binding of protons, oxygen, water molecules and/or cofactors or are influenced by electron transfer.

The determination of pH in the cell cytoplasm or in intracellular organelles is of high relevance in cell biology, for example, to study the capacity of proton transporters or ΔpH induced non-photochemical quenching. We used the pH-sensitive dual-emission GFP for the quantitative analysis of pH in mammalian cells. The time-course of intracellular acidification was used to determine the transversal H^+ diffusion coefficients in cell membranes [1].

The fluorescent biosensor Peredox responds to the local NAD^+/NADH concentration ratio. Peredox was constructed by combining the circularly permuted GFP derivative T-Sapphire with the bacterial NADH-binding repressor protein T-Rex [2]. The biosensor Frex consists of a circularly permuted yellow fluorescent protein inserted into a tandem dimer of the Rex protein. Frex responds to the absolute NADH concentration.

We applied Peredox to monitor the metabolic state of the "Knallgas" bacterium *Ralstonia eutropha* which expresses four hydrogenases. One of these enzymes, the so-called soluble hydrogenase, links the reversible oxidation of H_2 to the reduction of NAD^+: $NAD^+ + H_2 \leftrightarrow NADH + H^+$. *In vivo* studies on this enzyme together with biotechnological approaches directed towards the cellular production of H_2 benefit from knowledge about the concentration ratio of the NADH/NAD^+ redox couple and the pH.

Our results suggest that the cytoplasm in aerobically grown *R. eutropha* cells is in a more reduced state than in mammalian cells. However the application of Frex showed a clear increase of the NADH concentration in *R. eutropha* in hydrogen atmosphere. We provide a detailed spectroscopic characterization of both NADH sensors.

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2. Y. P. Hung et al., Cell Metab., 14, 2011

Poster 1

Peptides for immobilizing HydSL hydrogenase from *Thiocapsa roseopersicina*

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Large peptide libraries were screened in Autodock Vina program package in order to find proper agents for immobilization of HydSL hydrogenase from *Thiocapsa roseopersicina*. An initial screening of homopentapeptides showed the strongest affinities for Q5, R5, W5 and Y5 in case of alpha-helical peptide conformations docked on truncated enzyme model and for H5, W5, Y5 in case of coiled peptide conformations docked on. In subsequent round of docking, 8000 tripeptides and all tetra- and pentapeptides consisting of amino acids from the following sets, {Q, R, W, Y} and {H, W, Y}, were screened. Enzyme-peptide complexes revealed by molecular docking were thoroughly analyzed in terms of affinities of the peptides, distances from peptides to predicted sites of methyl viologen binding and specificity of interaction of the peptides with the small subunit of the hydrogenase. The best peptides showed affinity values below −10 kcal/mol.

The results of molecular docking are planned to be validated by inhibitory analysis in solution and used in immobilization of this enzyme on an electrode surface.

The work was supported by Russian Science Foundation (grant number 15-14-30007).

Poster 2

Long-term storage of *Thiocapsa roseopersicina* BBS

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Thiocapsa roseopersicina BBS is a purple sulfur bacteria of the Chromatiaceae family. It is a representative of the group of microorganisms in which multiplicity of hydrogenases had been identified. HynSL hydrogenase is a bidirectional enzyme, which belongs to the group of [NiFe]-hydrogenases. It shows high thermal stability (temperature optimum at about 80°C), and resistance to oxygen, sulfide, and carbon monoxide. Outstanding stability brings this enzyme into the focus of biotechnological interest. Preservation of stains possessing useful characteristics is the basic element for the development of the applied and fundamental microbiology and biotechnology. The aim of this work was to select methods for long-term storage of *T. roseopersicina* BBS.

Lyophilization is one of the most economical and popular methods for long-term storage of microorganisms. This method has broad application to bacterial cultures, but it is considered that purple sulfur bacteria cannot be preserved this way. There is a lack of evidence about lyophilization of phototrophic bacteria in the literature nowadays. We used 10% skimmed milk and 7.5% sucrose as protective agents. After freeze-drying, the number of viable organisms decreased from $(11\pm0.2)\times10^8$ to $(2\pm0.5)\times10^8$ colony forming units (CFU) per mL. The accelerated storage test has shown that it's possible to store freeze-dried *T. roseopersicina* BBS for more than 10 years at +4°C.

Liquid nitrogen storage is one of the best methods for preserving many different species of microorganisms. However, because of its relatively expensive cost, there have been many studies on the effect of higher temperatures on survival of various groups of microorganisms. The temperature −70°C appears to be sufficiently low to preserve most organisms. Freezing at −80°C was used for long-term storage of *T. roseopersicina* BBS cells. It was shown that the rate of cooling has no significant effect on cell survival. After freezing without cryoprotective agents, reduction in the viability of cells was less than 10-fold. In samples with 5% DMSO, there weren't any changes in viability. After a 6 month storage at −80°C with 5% DMSO, the number of viable organisms decreased from $(3,3\pm0,2)\times10^{10}$ to only $(1,1\pm0,6)\times10^{10}$ CFU×mL^{-1}.

To conclude, we can say that both developed methods are appropriate for long-term storage of *Thiocapsa roseopersicina* BBS cells.

The work was supported by a Russian Science Foundation grant (14-50-00029).

Poster 3

Inactivation of termostable HydSL hydrogenase from *Thiocapsa roseopersicina* by cyanide

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The effect of cyanide on activity and structure of *Thiocapsa roseopersicina* hydrogenase was studied using infrared spectroscopy with Fourier transform. The spectra of hydrogenase in oxidized or reduced state with or without inhibitor were registered for the first time. No difference was found in spectra of Ni-Fe active center after cyanide addition. Furthermore, data indicated that cyanide inhibited hydrogenase activity in non-competitive manner towards hydrogen or carbon monoxide. It suggested that cyanide did not interact directly with Ni-Fe active center of hydrogenase. From the other hand, the appearance of ferricyanide in the enzyme solution after prolonged incubation with cyanide, as well as discoloration of the enzyme in the visible region of the spectrum were indicative of the destruction of iron-sulfur clusters which leaded to loss of enzymatic activity.

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Our primary goal is to offer You a wide variety of high-quality products as well as perfect service. Many years of experience allow us to offer You most beneficial solutions for lower prices.

LabInstruments Co. represents in Russia **LI-COR Biosciences Company** (USA). LI-COR Biosciences manufactures equipment for environmental studies: field and lab CO2, H2O, CH4 gas analyzers, ecomonitoring systems (GHG), meteorological stations (Biomet), wide range of sensors of meters, portable photosynthesis systems, soil gas flux systems and many others.

GHG / Biomet monitoring systems

Portable photosynthesis systems

Soil gas flux systems

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Founded in 1998, **Fitosila LLC** successfully operating in the market for over 15 years. The company has grown from a small trading enterprise to one of the largest manufacturers of traditional medicine in the Russian market. The main range comprises products based on medicinal plants and fats extracted from wild animals. The first production line has been started in 2002, and since then the company continues to expand its product portfolio and improves the quality of the goods.

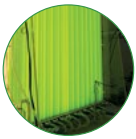
Years of cooperation with several research institutes, such as Kirov's Research Institute of Hunting, Pushchino's State Research Institute «Research Institute of Fundamental Biology Problems» of Russian Academy of Sciences, and Independent Non-profit Organization «Soyuzekspertiza», allowed the **«Fitosila»** company to develop and implement a large number of unique technologies. These inventions include a technology for preservation of therapeutic properties of animal fats through industrial processing; the regulatory framework for fatty acid composition of animal fats and oil-based products to help identification of counterfeit goods. The company also has its own laboratory facilities, which allows strict quality control for both raw materials and consumer products.

The **«Fitosila»** company exercises careful usage of natural resources, therefore all raw materials are received from licensed hunting farms and individual hunters.

In addition to the production of cosmeceuticals and oil-based remedies, the **«Fitosila»** company is a full-cycle enterprise producing medicinal herbs from cultivation to packaging thus avoiding import of raw materials which in turn has a positive influence on the price and quality of consumer products.

The future plans of the **«Fitosila»** company include the further development of product portfolio and conducting numerous clinical trials of traditional remedies followed by the implementation of the results into practice.

Our innovations



Photobioreactor

The photobioreactor is the unique pilot product — the oxygen generator for home and office. Usage of the generator allows people to breathe fresh and clean air in polluted city environment.

The nursery of hibernating animals

In order to preserve population of badgers in wildlife, the **«Fitosila»** company sponsored establishment and provides continuos support for the nursery of hibernating animals in the Republic of Khakassia.

Fat-processing technology and equipment

To avoid quality loss during industrial processing of animal fats, the **«Fitosila»** company has developed and implemented the unique technology that helps to avoid loss of beneficial properties of animal fats in oil-based products.

Trade marks

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SPEZLABPROEKT company specializes in supplying of analytical instruments and laboratory equipment. **SPEZLABPROEKT** is an exclusive representative of the **Heinz Walz** company in Russia. **Heinz Walz** is a world leader in the manufacturing of sophisticated scientific instruments for photosynthesis researches: **plant gas exchange** and **chlorophyll fluorescence**.

Heinz Walz instruments are widely used in biology, botany, plants physiology, plants selection, agronomics, plant growing, biotechnologies, environmental monitoring and many other related areas. **Heinz Walz** proposes the equipment both for field and laboratory use.

Design of **Heinz Walz** instruments is a result of a close cooperation with the skilled scientists, who are an experts in photosynthesis and related areas. This ensures **Heinz Walz** to remain the world leader in production of the world class scientific equipment.

Heinz Walz provides a wide choice of PAM (Pulse Amplitude Modulation) fluorometers for the measurements of the level of a chlorophyll fluorescence, which is a primary characteristic of the photosynthesising activity of plants, as well as high-performance systems for measurement of plants gas exchange (GFS-3000) which can be equipped by or connected to various PAM fluorometers for simultaneous acquisition of gas exchange and chlorophyll fluorescence data.

Heinz Walz also offers dew point measuring systems, gas coolers, cold traps, gas mixing units, light meters & data loggers, Quantum sensors for PAR measurements, lighting units and more. Custom gas exchange chambers as well as other custom solutions are available upon request.

SPEZLABPROEKT provides the full range of services consisting in delivery, installation, and service of laboratory equipment. Our skilled experts are always glad to help with a right selection of instruments and formation of a particular set of equipment, optimally corresponding to the user's needs. We are ready to deliver your orders all over Russia and CIS countries. Our engineers provide assembly and installation service if necessary. We perform as well service, warranty and post warranty maintenance of the various equipment.

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