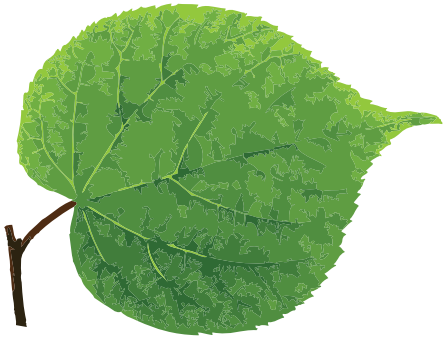

International Conference

Photosynthesis Research for Sustainability

in honor of Vladimir A. Shuvalov

June 2–7 2014
Pushchino, Russia

ABSTRACTS AND PROGRAMME

Institute of basic biological problems
Russian Academy of Sciences

International Conference

“Photosynthesis Research for Sustainability-2014”

in honor of Vladimir A. Shuvalov

June 2–7, 2014
Pushchino, Russia

Abstracts and Programme

Pushchino – 2014

International Conference "Photosynthesis Research for Sustainability-2014: In honor of Vladimir A. Shuvalov"
Eds. S. I. Allakhverdiyev, I. I. Proskuryakov, I. A. Naydov.
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The volume contains abstracts of the lectures and poster presentations at the International Conference "Photosynthesis Research for Sustainability: In honor of Vladimir A. Shuvalov" to be held in June 2–7 2013 in Pushchino, Russia. The experimental and theoretical works covering a wide range of topics, from the primary processes of energy and electron transfer to the physiological aspects of photosynthesis will be discussed at the conference. Considerable attention will be given to discussion of the structural organization of photosynthetic reaction centers, and applied problems of photosynthesis – stress biology, artificial photosynthesis and fuel. The book will be of interest to researchers involved in the study of photosynthesis and other related fields.

THANKS TO OUR SPONSORS

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Additional information is available
on our website:
http://photosynthesis2014.cellreg.org

WELCOME!

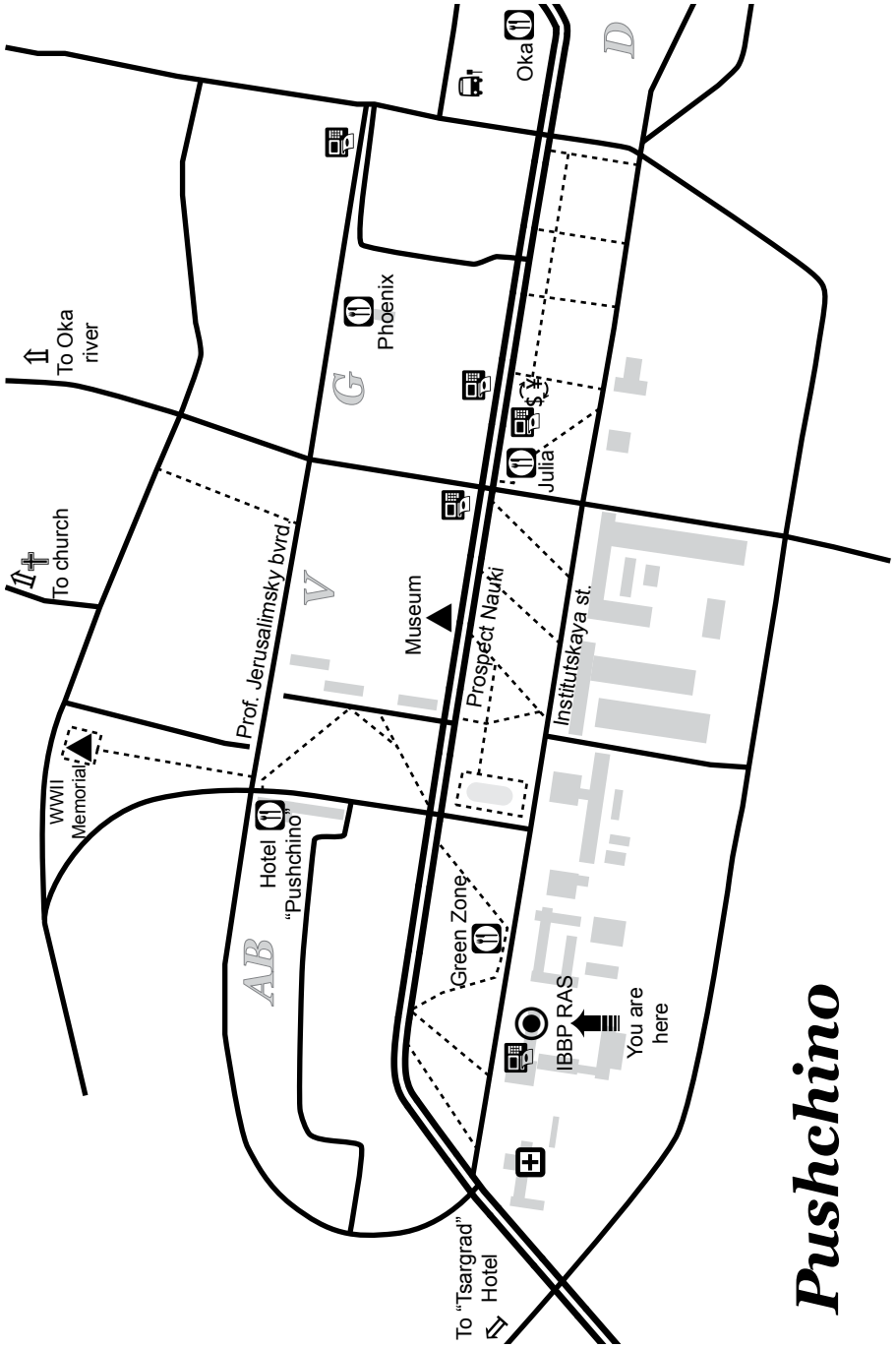
You are most welcome to the International conference "Photosynthesis Research for Sustainability-2014: in honor of Vladimir A. Shuvalov" held in Pushchino.

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

The multidisciplinary nature of this conference is obvious from the list of topics and presented lectures. In total, more than 110 lectures and posters will be presented.

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

Vladimir Shuvalov,
Suleyman Allakhverdiev,
Anatoly Tsygankov



Schedule: Photosynthesis Research for Sustainability-2014

JUNE 1 (SUNDAY)

Arrival, Registration

JUNE 2 (MONDAY – 1ST DAY)

09:00–11:00 – Registration

11:00 – Opening Ceremony
Special events in honor of Professor Vladimir Shuvalov

Prof. Anatoly I. Miroshnikov, Chairman of the Pushchino Research Center
Dr. Ivan Savintsev, The head of the Pushchino town
Dr. Alexey Semenov, President of Russian Society for Photobiology
Prof. Hans van Gorkom *(The Netherlands)*

Lectures

Chairpersons: Masahiko Ikeuchi (Japan), Govindjee (USA), Anatoly Tsygankov (Russia)

12:00–12:40 **S1.1**
Govindjee *(University of Illinois, Illinois, USA)* Primary photochemistry of photosynthesis: A perspective in honor of Vlad Shuvalov

12:40–13:20 **S1.2**
Vladimir A. Shuvalov *(Institute of Basic Biological Problems, RAS, Pushchino, Russia)* Light energy convertor for biosphere

13:20–14:50 (90 min) Lunch

Chairpersons: Jian-Ren Shen (Japan), Alexandrina Stirbet (USA), Kimiyuki Satoh (Japan)

14:50–15:30 **S3.1**
Jian-Ren Shen *(Photosynthesis Research Center, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan)* Possible mechanism of photosynthetic water oxidation based on atomic structure of Photosystem II

15:30–16:10 **S7.1**
Andrey B. Rubin *(Faculty of Biology, Moscow State University, Moscow, Russia)* Mechanisms of regulation of the electron transfer in the primary processes of photosynthesis

16:10–16:50 **S7.2**
Hong Gil Nam *(Department of New Biology, DGIST and Center for Plant Aging Research, IBS, Korea)* Functional transition history of chloroplasts along leaf life span in *Arabidopsis* coordinated by multi-layered regulatory networks

16:50–17:30 **S2.1**
Masahiko Ikeuchi *(University of Tokyo, Tokyo and Japan Science and Technology Agency (JST), CREST, Saitama, Japan)* Engineering of antenna and photosystems in cyanobacteria

18:00 – Let's Get Together

JUNE 3 (TUESDAY – 2ND DAY)

Lectures

Chairpersons: Andrey Rubin (Russia), Victor Nadtochenko (Russia), Alexey Semenov (Russia)

08:30–09:00 **S1.3**
Hans van Gorkom *(Department of Biophysics, Leiden University, Leiden, The Netherlands)* Why plants are not black

09:00–09:30 **S1.4**
Victor A. Nadtochenko *(Semenov Institute of Chemical Physics, RAS, Moscow and Institute of Chemical Physics Problems, RAS, Chernogolovka, Russia)* Primary stages of electron and energy transfer in Photosystem I: Effect of excitation pulse wavelength

09:30–10:00 **S1.5**
Alexey Yu. Semenov *(A.N. Belozersky Institute of Physical-Chemical Biology, Moscow State University, Moscow, Russia)* Free energy and reorganization energy of the primary electron transfer reactions in Photosystem I

10:00–10:30 (30 min) Coffee break

Chairpersons: Tohru Tsuchiya (Japan), Hong Gil Nam (Korea), Alexander Krasnovsky Jr. (Russia)

10:30–11:00 **S1.6**
Mahir Mamedov *(Belozersky Institute of Physical-Chemical Biology, Moscow State University, Moscow, Russia)* Electrogenicity of electron and proton transfer in the protein-pigment complex of Photosystem II

11:00–11:30 **S1.7**
Tatsuya Tomo *(Tokyo University of Science, Tokyo and PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan)* Diversity of chlorophylls in photosynthesis

11:30–12:00 **S1.8**
Seiji Akimoto *(Molecular Photoscience Research Center, Graduate School of Science, Kobe University, and Japan Science and Technology Agency, CREST, Kobe, Japan)* Differences in energy transfer of cyanobacteria grown in different cultivation media

12:00–13:30 (90 min) Lunch

Chairpersons: Hans van Gorkom (The Netherlands), Vyacheslav Klimov (Russia), Seiji Akimoto (Japan)

13:30–14:00 **S8.1**
Barry D. Bruce *(University of Tennessee-Knoxville, Energy Science and Engineering Program, Bredesen Center for Interdisciplinary Research and Education, USA)* Direct solar conversion using thermophilic cyanobacteria

14:00–14:30 **S3.2**
Imre Vass *(BRC, Institute of Plant Physiology, HAS, Szeged, Hungary)* Characterization of singlet oxygen production and its role in photodamage in intact cyanobacteria and microalgae

14:30–15:00 **S2.2**
Julian Eaton-Rye *(University of Otago, Dunedin, New Zealand)* Hydrophilic auxiliary proteins of Photosystem II in the cyanobacterium *Synechocystis sp.* PCC 6803

15:00–15:30 **S3.3**
Takumi Noguchi *(Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan)* Molecular mechanism of photosynthetic water oxidation revealed by infrared spectroscopy with quantum chemical calculations

15:30–16:00 (30 min) Coffee break

Chairpersons: Miwa Sugiura (Japan), Ivan Proskuryakov (Russia), Boris Ivanov (Russia)

16:00–16:30 **S2.3**
Alexander A. Krasnovsky (Jr.) *(A.N. Bach Institute of Biochemistry, RAS and M.V. Lomonosov Moscow State University, Moscow, Russia)* Phosphorescence of triplet chlorophylls

16:30–17:00 **S7.3**
Boris N. Ivanov *(Institute of Basic Biological Problems, RAS, Pushchino, Russia)* The superoxide radical produced in chloroplast thylakoids in the light is reduced in the plastoquinone pool

17:00–18:30 Poster viewing/discussion:

Sections 1–5
Chairpersons: Imre Vass (Hungary), Hong Gil Nam (Korea),
Alexander N. Tikhonov (Russia), Ernst Walter Knapp (Germany)

Sections 6–9
Chairpersons: Julian Eaton-Rye (New Zealand),
Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan),
Kostas Stamatakis (Greece), Hazem Kalaji (Poland)

18:30 – Chamber music

Artists:
The member of Spivakov's orchestra Anastasia Kosarskaya (oboe) and laureate of international contests Vera Kryukova (piano);
Vivaldi, Kirnberger, Mocart, Schubert, Debussi

JUNE 4 (WEDNESDAY – 3RD DAY)

Lectures

Chairpersons: Tatsuya Tomo (Japan), Suleyman Allakhverdiev (Russia), Daisuke Seo (Japan)

08:30–09:00 **S2.4**
Tohru Tsuchiya *(Kyoto University, Kyoto, Japan)*
Molecular genetic analysis of the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina*

09:00–09:30 **S3.4**
Ernst-Walter Knapp *(Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany)* pKa computations of di-manganese model complexes and S1-state EXAFS spectra from DFT optimized Mn-cluster in PS II

09:30–10:00 **S3.5**
Yuki Kato *(Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan)* FTIR spectroelectrochemical study on the influence of Mn-depletion on the redox potential of the non-heme iron and its surrounding structure in Photosystem II

10:00–10:30 (30 min) Coffee break

Chairpersons: Imre Vass (Hungary), Takumi Noguchi (Japan), Arvi Freiberg (Estonia)

10:30–11:00 **S7.4**
Mikhail F. Yanyushin *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Comparison of dendrograms for electron transporting chains components with the common phylogeny of prokaryotes as an aproach to the problem of the origin and the evolution of photosynthesis and respiration

11:00–11:30 **S3.6**
Miwa Sugiura *(Proteo-Science Research Center, Ehime University, Ehime and PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan)* Histidine hydroxyl modification on D2-His336 in Photosystem II of *Thermosynechoccocus vulcanus* and *Thermosynechoccocus elongates*

11:30–12:00 **S3.7**
Vyacheslav V. Klimov *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Bicarbonate requirement for the donor side of Photosystem II

12:00–13:30 (90 min) Lunch

Chairpersons: Suleyman Allakhverdiev (Russia), Marian Brestic (Slovak Republic), Yuki Kato (Japan)

13:30–14:00 **S5.1**
Arvi Freiberg *(Institute of Physics and Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia)* Light harvesting in photosynthetic bacteria acclimated to different levels of light

14:00–14:30 **S4.1**
Ivan Proskuryakov *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow region, Russia)* TR EPR study of singlet-triplet fission of carotenoid excitation

14:30–15:00 **S7.5**
Evgeny Maksimov *(Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, Russia)* The signaling state of orange carotenoid protein

15:00–15:30 (30 min) Coffee break

Chairpersons: Subramanyam Rajagopal (India), Franz-Josef Schmitt (Germany), Alex Ivanov (Canada)

15:30–16:00 **S7.6**
Subramanyam Rajagopal *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)*
Anaerobic and heat induced state transitions in *Arabidopsis thaliana* and its signal mechanism in thylakoid membranes

16:00–16:30 **S5.2**
Vadim Selyanin *(Institute of Microbiology, Academy of Sciences, Třeboň, Czech Republic)* The size and amount of photosynthetic unit in purple bacteria

16:30–17:40 Poster viewing/discussion:

Sections 1–5
Chairpersons: Imre Vass (Hungary), Hong Gil Nam (Korea), Alexander N. Tikhonov (Russia), Ernst Walter Knapp (Germany)

Sections 6–11
Chairpersons: Julian Eaton-Rye (New Zealand), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan), Kostas Stamatakis (Greece), Hazem Kalaji (Poland)

18:00 – Bonfire

JUNE 5 (THURSDAY – 4TH DAY)

Tours

JUNE 6 (FRIDAY – 5TH DAY)

Lectures

Chairpersons: Julian Eaton-Rye (New Zealand), Mi-Sun Kim (Korea), George Papageorgiou (Greece)

08:30–09:00 **S8.2**
Hiroshi Nishihara *(Department of Chemistry, School of Science, The University of Tokyo, Tokyo, Japan)* Photoelectron conversion using combination of bio-components with artificial molecules

09:00–09:30 **S6.1**
Ivelina Zaharieva *(Freie Universität Berlin, Berlin, Germany)*
Manganese oxides as biomimetic water-oxidation catalysts

09:30–10:00 **S7.7**
Franz-Josef Schmitt *(Institute of Physical Chemistry, Technical University of Berlin, Berlin, Germany)* Fluorescence imaging of light induced reactive oxygen species (ROS) in plant cell tissue

10:00–10:30 (30 min) Coffee break

Chairpersons: Hiroshi Nishihara (Japan); Ernst Walter Knapp (Germany), Alexander N. Tikhonov (Russia)

10:30–11:00 **S8.3**
Mi-Sun Kim *(Biomass and Waste Energy Laboratory, Korea Institute of Energy Research, Daejeon, Republic of Korea)*
Photo fermentative hydrogen production in combination with lactate and methane fermentation to maximize the bioenergy recovery from food waste

11:00–11:30 **S8.4**
Hajime Masukawa *(Research Institute for Photobiological Hydrogen Production and Department of Biological Sciences, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa, Japan)*
Photobiological hydrogen production by *Anabaena* PCC 7120 mutants with increased heterocyst frequency

11:30–12:00 **S8.5**
Azat Abdullatypov *(Institute of Basic Biological Problems RAS, Pushchino, Moscow Region, Russia)* Modeling the HydSL-hydrogenase from *Thiocapsa roseopersicina*

12:00–12:30 **S4.2**
Petar H. Lambrev *(Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary)* Energy transfer in plant light-harvesting complex II revealed by room-temperature 2D electronic spectroscopy

12:30–14:00 (90 min) Lunch

Chairpersons: Hazem M. Kalaji (Poland), Vasiliy Goltsev (Bulgaria), Mahir Mamedov (Russia)

14:00–14:30 **S7.8**
Alexander N. Tikhonov *(Faculty of Physics, Moscow State University, Moscow, Russia)* Light-induced regulation of photosynthetic electron transport in chloroplasts

14:30–15:00 **S7.9**
Eugene A. Lysenko *(Institute of Plant Physiology, RAS, Moscow, Russia)* Cadmium uptake into chloroplasts and its impact on chloroplastic mRNAs, proteins, and energy quenching

15:30–16:00 **S9.1**
Alexandrina Stirbet *(204 Anne Burras Ln, Newport News, VA 23606, USA)* Photosynthetic performance indexes based on fast Chl *a* fluorescence induction data: advantages and limitations

16:00–16:30 (30 min) Coffee break

16:30–18:00 – Poster viewing/discussion:

Sections 1–5
Chairpersons: Imre Vass (Hungary), Hong Gil Nam (Korea), Alexander N. Tikhonov (Russia), Ernst Walter Knapp (Germany)

Sections 6–9
Chairpersons: Julian Eaton-Rye (New Zealand), Vasiliy Goltsev (Bulgaria), Tatsuya Tomo (Japan), Kostas Stamatakis (Greece), Hazem Kalaji (Poland)

18:00 – Special Events

1) Young Talents (4 awards/prizes)
2) Best posters (4 awards/prizes)

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

The names of winners will be selected by the committee (see below), according to recommendations of chairpersons of poster sections.

Committee: Govindjee (USA), Julian Eaton-Rye (New Zealand), Jian-Ren Shen (Japan), Tatsuya Tomo (Japan), Imre Vass (Hungary), Suleyman Allakhverdiev (Russia)

19:00 – Banquet

JUNE 7 (SATURDAY – 6TH DAY)

Lectures

Chairpersons: Kimiyuki Satoh (Japan), Govindjee (USA), George Papageorgiou (Greece)

09:00–09:30 **S4.3**
Alexander N. Malyan *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Energy-dependent regulation of chloroplast ATP synthase

09:30–10:00 **S2.5**
Lyudmila G. Vasilieva *(Institute of Basic Biological Problems, RAS, Pushchino, Russia)* Relocation of BChl axial ligands in *Rhodobacter sphaeroides* mutant reaction centers

10:00–10:30 **S4.4**
Anatoly Shkuropatov *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Photosystem II core complexes from spinach with chemically modified pigment composition

10:30–11:00 (30 min) Coffee break

11:00–11:30 **S1.9**
Roman Y. Pishchalnikov *(Prokhorov General Physics Institute, RAS, Moscow, Russia)* Numerical studies of the photosynthetic reaction center femtosecond transient absorption by means of hierarchical equations of motions

11:30–12:00 **S1.10**
Anton Khmelnitskiy *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Femtosecond processes of charge separation in wild type and mutant reaction centers of *Rhodobacter sphaeroides*

12:00 – Closing ceremony

Govindjee (USA), Hans van Gorkom (The Netherlands), George Papageorgiou (Greece), Kimiyuki Satoh (Japan), Yuriy Erokhin (Russia)

Taking photos, all together

The end and Free time

JUNE 8 (SUNDAY)

Departure

Poster Session Schedule

JUNE 03

Section 1

S1.11 Andrei G. Yakovlev, Vladimir I. Novoderezhkin, Alexandra S. Taisova, Vladimir A. Shuvalov, Zoya G. Fetisova
Polarized transient absorption spectroscopy of *Chloroflexus aurantiacus* chlorosomes

S1.12 Larisa Khamidullina, Gusel Akhtyamova, Svetlana Batasheva, Golsoyar Bakirova, Vladimir Chikov
Effect of lighting conditions under plant cultivation on the photosynthetic carbon metabolism in potato with elevated apoplastic invertase activity

S1.13 Peter P. Knox, Evgeni P. Lukashev, Nuranija Kh. Seifullina, Nadezhda P. Grishanova
About the differences in kinetics of redox changes of bacteriochlorophyll and quinone acceptors in purple bacteria RCs

S1.14 Mahir Mamedov, Irina Petrova, Denis Yanykin, Andrey Zaspa, Alexey Semenov
Trehalose effects on Photosystem II complex

S1.17 Anastasia Petrova, Ivan Shelaev, Fedor Gostev, Mahir Mamedov, Victor Nadtochenko and Alexey Semenov
Indication of electron transfer asymmetry in cyanobacterial Photosystem I

S1.18 Andrei Razjivin, Viktor Kompanets, Zoya Makhneva, Andrey Moskalenko, Sergey Chekalin
LH2 complexes: Mechanism of two-photon excitation within 1200–1500 nm range

S1.19 Toshiyuki Sinoda, Daisuke Nii, Tatsuya Tomo, Seiji Akimoto, Min Chen, Hisataka Ohta, Suleyman I. Allakhverdiev
Spectroscopic analysis of chlorophyll *f* containing cyanobacteria

S1.20 Andrei G. Yakovlev, Vladimir A. Shuvalov
Spectral exhibition of electron-vibrational relaxation in P* state of *Rhodobacter sphaeroides* reaction centers

Section 2

S2.6 Parveen Akhtar, Márta Dorogi, Krzysztof Pawlak, Győző Garab, Petar H. Lambrev
Effects of detergents, lipids and trimer-trimer contacts on the pigment excitonic interactions in plant light-harvesting complex II

S2.7 Aleksandr Ashikhmin, Zoya Makhneva, Maksim Bolshakov, Yuriy Erokhin, Andrey Moskalenko
Recovering colored-carotenoid biosynthesis in the cells of the sulfur photosynthetic bacterium *Ectothiorhodospira haloalkaliphila*

S2.8 Maksim Bolshakov, Aleksandr Ashikhmin, Zoya Makhneva, Andrey Moskalenko
Could the LH2 complex from purple photosynthetic bacteria be assembled in the cell without carotenoids?

S2.9 Kostas Stamatakis, Dimitris Vayenos, Christos Kotakis
Integration of Antarctic phaeophyte Kleptoplast in a dinoflagellate host

S2.10 Elena V. Tyutereva, Wolfram G. Brenner, Alexandra N. Ivanova, Katharina Pawlowski, Olga V. Voitsekhovskaja
Changed stoichiometry of the minor antenna and PS2 reaction centres, a possible basis for increased photosynthesis productivity and increased tolerance to light and drought stress in barley

Section 3

S3.8 N. E. Belyaeva, F.-J. Schmitt, V. Z. Paschenko, G. Yu. Riznichenko, A. B. Rubin
Evolution of the Photosystem II redox states by modeling of the electron transfer

S3.9 Imed Hasni, Saber Hamdani and Robert Carpentier
Impact of the interaction of Al^{3+} with the proteins composition of Photosystem II

S3.10 Zhiyong Liang, Ivelina Zaharieva, Oliver Karge, Holger Dau
Suprising glycerol effect on the activation enthalpy of water oxidation in Photosystem II

S3.11 Mohammad Mahdi Najafpour, Mahnaz Abasi, Tatsuya Tomo, Suleyman I. Allakhverdiev
Mn Oxide/Nanodiamond composite: A new water-oxidizing catalyst for water oxidation

S3.12 Shin Nakamura and Takumi Noguchi
Vibrational analyses of the water oxidizing center in Photosystem II using QM/MM calculations

S3.13 László Sass, Zsuzsanna Deák, Imre Vass
In silico photosynthesis: computer assisted simulation of electron transport processes in Photosystem II

S3.15 Sepideh Skandary, M. Hussels, A. Konrad, C. Glöckner, E. Schlodder, J. Hellmich, A. Zouni, M. Brecht
Single molecule spectroscopy on Photosystem II of *Thermosynechoccus elongatus*

S3.16 Masato Yamada, Tatsuya Tomo
Function and characterization of Photosystem II extrinsic protein PSBQ′

Section 8

S8.6 Thitirut Assawamongkholsiri, Alissara Reungsang
Simultaneous bio-hydrogen and microbial oil production by *Rhodobacter sp.* KKU-PS1

S8.7 Barry D. Bruce
Applied photosynthesis: Putting PS I to work

S8.8 Khorcheska A. Batyrova, Anastasia I. Gavrisheva, Anatoly A. Tsygankov
Sustained hydrogen photoproduction by phosphorous-deprived marine green microalgae *Chlorella* C65.

S8.9 Arturo Solís Herrera
Human photosynthesis?

S8.10 Shu Ikehira, Mariko Miyachi, Kyoko Okuzono, Yoshinori Yamanoi, Tatsuya Tomo and Hiroshi Nishihara
Construction of a photochemical system using PS II and a molecular wire equipped with a platinum nanoparticle

S8.11 Tatyana Laurinavichene, Evgeny Shastik and Anatoly Tsygankov
Hydrogen photoproduction by mixed culture of *Rhodobacter sphaeroides* and *Clostridium butyricum*

S8.13 Daria A. Todorenko, T. K. Antal, D. N. Matorin
Study the effect of copper on primary photosynthetic processes in the green microalga *Scenedesmus quadricauda* by using M-PEA-2 instrument

JUNE 04

Section 7

S7.10 Abdelghafar M. Abu-Elsaoud and Allan G. Rasmusson
Does the external mitochondrial NADPH dehydrogenase have a special role in protecting the chloroplasts from bleaching in young leaves of *Nicotiana sylvestris* tobacco?

S7.13 Ulduza Gurbanova, Hasan Babayev, Minakhanym Aliyeva, Yashar Feyziyev, Novruz Guliyev
Effects of drought on mitochondrial NAD-malate dehydrogenase in *Amaranthus cruentus* L. during ontogenesis

S7.14 Shahniyar Bayramov, Minakhanym Aliyeva, Taliya Orujova, Wolfgang Brüggemann
Photosynthetic enzyme activities under drought stress in *Chenopodium album* L.

S7.15 Maria Borisova-Mubarakshina, Boris Ivanov, Tatyana Fedorchuk, Natalia Rudenko, Daria Vetoshkina, Marina Kozuleva, Luca Dall'osto, Stefano Cazzaniga, Roberto Bassi
Identification of the signal messenger for the long-term regulation of the Photosystem II light-harvesting antenna size in high light

S7.16 Marián Brestič, Marek Živčák, Kristýna Kunderlíková, Katarína Olšovská and Suleyman I. Allakhverdiev
Specific responses of PS I and PS II electron transport in leaves of *chlorina* wheat mutants

S7.17 Nina Djapic
Chlorophyll catabolism under low water regime in *Apium graveolens* var. Dulce

S7.18 Tamara Golovko, Igor Dalke, Ilya Zakhozhiy and Olga Dymova
The tolerance of lichen *Lobaria pulmonaria* photosynthesis to excess light and UV(A+B)-radiation

S7.19 Olga Dymova, Mikhail Khristin and Tamara Golovko
The state of pigment-protein complexes in cloroplasts of *Ajuga reptans* summer and winter green leaves

S7.20 Zinaida Eltsova and Anatoly Tsygankov
Continuous cultures of *Rhodobacter sphaeroides*

S7.21 Shahniyar Bayramov, Ulduza Gurbanova, Hasan Babayev, Minakhanym Aliyeva, Novruz Guliyev, Yashar Feyziyev
Effects of temperature and light intensity on photosynthetic enzyme activities in C4 species of *Chenopodiaceae* family in the natural environment

S7.22 Vasilij Goltsev, Stella Dimitrova, Kolyo Dankov, Vladimir Aleksandrov, Vasilena Krasteva, Momchil Paunov, Hazem M. Kalaji, Reto J. Strasser
Application of biophysical luminescence methods for plant phenotyping

S7.23 Farida B. Guliyeva, Samira M. Rustamova, Irada M. Huseynova, Jalal A. Aliyev
Molecular detection of leaf rust resistance genes *Lr26* and *Lr35* in wheat cultivars in Azerbaijan

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Section 1: Primary Processes of Photosynthesis

Lecture S1.1

Primary photochemistry of photosynthesis: a persective in honor of Vlad Shuvalov

Govindjee

Dept. of Biochemistry, Dept. of Plant Biology, and Center of Biophysics & Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
* Fax: 1-217-2447246, e-mail: gov@illinois.edu

I begin by quoting Ciamician (1912) who said: "For our purposes the fundamental problem from the technical point of view is how to fix the solar energy through suitable photochemical reactions. To do this it would be sufficient to be able to imitate the assimilating processes of plants." To imitate it, we must understand it. Vladimir (Vlad) Shuvalov has spent all his life doing just that – particularly the very first steps, the primary photochemistry, the conversion of light energy into chemical energy. He has made fundamental discoveries in these reactions from femtoseconds to picoseconds (i.e., in pt_s scale of 15–12) in the reaction centers, from photosynthetic bacteria, as well as from the photosystems I and II. In this talk, I plan to first present a historical perspective of the early experiments of Eugene Rabinowitch, and Alexander Krasnovsky on photochemistry of chlorophyll in solutions, followed by the pioneering research of Robert (Bob) Emerson, William (Bill) Archibald Arnold, Robert (Robin) Hill, Bessel Kok, Horst Witt, and Louis (Lou) N.M. Duysens on the two-light reaction, two-pigment scheme of photosynthesis. We ask, "Where are we going?" One of the directions is what Ciamician (1912) said "Is it possible or, rather, is it conceivable that …production of organic matter may be increased in general and intensified in special places, and that the cultivation of plants may be so regulated as to make them produce abundantly such substances as can become sources of energy or be otherwise useful to civilization? I believe this is possible."

Lecture S1.2

Light energy convertor for biosphere

Vladimir A. Shuvalov

Institute of Basic Biological Problems RAS, Pushchino, Russia
* e-mail: shuvalov@libro.genebee.msu.ru, shuvalov@issp.serpukhov.su

Primary charge separation at photosynthesis takes place in photochemical Reaction Centers (RC) in photosynthetic bacteria, Photosystem I and II (PS I and PS II) of green plants. Efficiency of the light energy conversion approaches 100% in RC. Primary charge separation dynamics in RC of purple bacterium *Rhodobacter sphaeroides* and its P870 heterodimer mutants have been studied using femtosecond time-resolved spectroscopy with 20 and 40 fs excitation at 870 nm at 293K. Absorbance increase in the 1060–1130 nm region that is presumably attributed to P(A)(δ+) cation radical molecule as a part of mixed state with a charge transfer character P*(P(A)(δ+)P(B)(δ−)) was found. This state appears at 120–180 fs time delay in the wild type RC and even faster in H(L173)L and H(M202)L heterodimer mutants and precedes electron transfer (ET) to B(A) bacteriochlorophyll (anion radical with absorption band at 1020 nm in WT). In core complexes of PS II the subtraction of the $P680^+$ spectrum measured at 455 ps delay from the spectra at 23 ps or 44 ps delays reveals the spectrum of $Pheo_{D1}^-$, which is very similar to that measured earlier by accumulation method. The spectrum of $Pheo_{D1}^-$ formation includes a bleaching (or red shift) of the 670 nm band indicating that Chl-670 is close to $Pheo_{D1}$. According to previous measurements in the femtosecond-picosecond time range this Chl-670 was ascribed to Chl_{D1} as an intermediate electron acceptor [1]. Stimulated emission at 685 nm was found to have two decaying components with time constants of ~1 ps and ~14 ps. These components appear to reflect formation of $P680^+Chl_{D1}^-$ and $P680^+Pheo_{D1}^-$, respectively.

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Lecture S1.3

Why plants are not black

Marcell Marosvölgyi and Hans van Gorkom*

Dept of Biophysics, Leiden University, Leiden, Netherlands
e-mail: vangorkom@physics.leidenuniv.nl

What has long been perceived as a "mismatch" between absorption spectrum of photosynthesis and the available sunlight turns out to be a mistake. Actually, if the energy cost of the photosynthetic apparatus is taken into account, the red absorption band of the chlorophylls may be closely optimized to provide maximum growth power. The optimization predicts a strong influence of Fraunhofer lines in the solar irradiance on the spectral shape of the optimized absorption band, which appears to be correct. It does not predict any absorption at other wavelengths.

Lecture S1.4

Primary stages of electron and energy transfer in Photosystem I: effect of excitation pulse wavelength

A. Semenov[1], I. Shelaev[2], F. Gostev[2], M. Mamedov[1], Vladimir A. Shuvalov[1], Victor A. Nadtochenko[2,3,*]

1 – Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia
2 – Semenov Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, 119991 Moscow, Russia
3 – Institute of Chemical Physics Problems, Russian Academy of Sciences, pr. Akademika Semenova 1, 142432 Chernogolovka, Moscow Region, Russia
* e-mail: nadtochenko@genebee.msu.su

Time-resolved differential spectra of Photosystem I complex were obtained by the "pump–probe" technique with 25-fs pulses with maxima at 670, 700, and 720 nm. The ratio between the number of excited chlorophyll molecules of the antenna and of the reaction center was shown to depend on spectral characteristics of the pump pulses. In all cases, an ultrafast (<150 fs) formation of the primary radical pair $P700^{+}A_{0}$ was recorded. However, on excitation by pulses with maxima at 670 or 700 nm, recording of the charge separation was masked by the much more intensive bleaching at the chlorophyll Q_{y} band due to excitation of the bulk antenna chlorophylls. We show that triggering the charge separation by 25-fs pulses centered at 720 nm allows us to more clearly detect kinetics of formation of the primary and secondary ion-radical pairs. The findings help to explain possible reasons for discrepancies of kinetics of primary stages of electron transfer recorded in different laboratories.

Lecture S1.5

Free energy and reorganization energy of the primary electron transfer reactions in Photosystem I

Alexey Yu. Semenov[1,2,*], Ivan Shelaev[2], Fedor Gostev[2], Wade Johnson[3], Mahir Mamedov[1], Victor Nadtochenko[2]

1 – A.N.Belozersky Institute of Phys.-Chem. Biology, Moscow State University, Russia;
2 – N.N. Semenov Institute of Chemical Physics, RAS, Moscow, Russia;
3 – Department of Chemistry, Susquehanna University, Selinsgrove, PA, USA
* Fax: +7-495-939-31-81, e-mail: semenov@genebee.msu.ru

The electron transfer in PS I involves the special pair chlorophyll P700 as primary donor, A_0 chlorophyll pair(s) as primary acceptor(s) and phylloquinone(s) as A_1 secondary acceptor(s). Our recent femtosecond pump-probe experiment in PS I complexes isolated from cyanobacteria *Synechocystis sp.* PCC 6803 revealed very fast (<100 fs) conversion of delocalized exciton into charge separated state $P700^+A_0^-A_1$; the secondary radical pair $P700^+A_0A_1^-$ was formed within 25 ps (Shelaev et al., Biochim. Biophys. Acta 1797 (2010) 1410–1420). The pump-probe femtosecond flash spectrometry with excitation maximum at 720 nm was employed for the study of kinetics of the primary and secondary ion-radical pair formation in PS I isolated from the *Synechocystis sp.* PCC 6803 *menB* deletion strain containing plastoquinone in the A_1-site. Inactivation of the *menB* gene, which codes for a naphthoate synthase, inhibits the biosynthesis of phylloquinone, and in its absence, plastoquinone-9 binds to the A_1 site. Because plastoquinone-9 binds relatively weakly, it can be displaced by incubation with a wide variety of quinones, including 2,3-dichloro-1,4-naphthoquinone (Cl_2NQ), 1,5-Anthraquinone and 1,2-Anthraquinone (1,2-AQ). The free energy change (ΔG) of the electron transfer from A_0^- to A_1 varied between ~−900 mV for Cl_2NQ-PS I and ~−350 mV for 1,2-AQ-PS I. It was shown that the increase of ΔG from ~−500 mV for the wild type PS I, containing phylloquinone (PhyQ) in the A_1-site, to ~−900 mV for the Cl_2NQ-PS I does not alter the lifetime of the reaction ($\tau \approx 23$ ps), while the decrease of ΔG down to −350 mV (for 1,2-AQ-PS I) results in ~2-fold increase of the lifetime ($\tau \approx 40$ ps). This result shows the lack of essential dependency of the electron transfer rate from the primary chlorophyll acceptor A_0 to the secondary quinone acceptor A_1 on the free energy gap ΔG. It means that formation of $P700^+A_1^-$ occurs in so-called "top regime" at very high electron transfer rate and is located on the plateau of the Marcus dependence of the rate of electron transfer from the ΔG value. The reorganization energy λ of this reaction was estimated to be in the range of 400–500 meV.

Lecture S1.6

Electrogenicity of electron and proton transfer in the protein-pigment complex of Photosystem II

Mahir D. Mamedov

Belozersky Institute of Physical-Chemical Biology, Moscow State University, 119991, Moscow, Leninskie gory, Russia
* Fax: +7-495-939-31-81, e-mail: mahirmamedov@yandex.ru

The light-induced functioning of protein-pigment complex of Photosystem (PS) II is directly linked to electron and proton transfer across the membrane, which results in the formation of transmembrane electric potential difference ($\Delta\Psi$). The major contribution to $\Delta\Psi$ of the PS II reaction center is due to charge separation between the primary chlorophyll donor P_{680} and the quinone acceptor Q_A, accompanied by re-reduction of photooxidized P_{680} by the redox-active tyrosine residue Y_Z. The processes associated with the uptake and release of protons on the acceptor and donor sides of the enzyme, respectively, are also coupled with $\Delta\Psi$ generation.

Generation of $\Delta\Psi$ due to S-state transitions of the water oxidation complex was recently demonstrated in Mn-depleted and reconstituted PS II core complexes incorporated into liposomes. The kinetics and relative amplitudes of the electrogenic reactions in dark-adapted samples during $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_4 \rightarrow S_0$ transitions in response to the first, second and third laser flashes were comparable to those obtained in the intact PS II core particles. These results expand current understanding of the nature and mechanisms of vectorial reactions due to a charge transfer on the donor side of photoactivated apo-WOC-PS II complexes.

Lecture S1.7

Diversity of chlorophylls in photosynthesis

Tatsuya Tomo[1,2,*], Seiji Akimoto[3,4], Suleyman I. Allakhverdiev[5,6]

1 – Tokyo University of Science, Tokyo, Japan
2 – PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan
3 – CREST, Japan Science and Technology Agency (JST), Kobe, Japan
4 – Kobe University, Kobe, Japan
5 – Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia
6 – Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, Russia
* Fax: +81-3-3260-0322, e-mail: tomo@rs.tus.ac.jp

There is a number of diversity in photosynthetic algae. Diversity also exists in the protein composition or pigment species in photosystems. *Acaryochloris spp.* and *Halomicronema hongdechloris* are unique cyanobacteria that differ from the majority of photosynthetic organisms by having chlorophyll *d* and chlorophyll *f*, respectively. Chlorophyll *d* and *f* absorb light with a wavelength up to 30 and 40 nm red-shifted from Chl *a*, respectively. In this study, we isolated Photosystem II reaction center complexes from the cyanobacterium *A. marina* using an anion exchange column chromatography and sucrose density gradient centrifugation. We revealed the molecular species of special pair as chlorophyll *d* by several spectroscopic data, such as circular dichroism, and time-resolved fluorescence. We also analyzed the cyanobacterium *H. hongdechloris* using several spectroscopic methods. We observed rapid energy transfer from Chl *a* to Chl *f* at 77K and up-hill energy transfer from Chl *f* to Chl *a* at 298K.

We will discuss the function of the Chl *d* and Chl *f* in the photosystems.

Lecture S1.8

Differences in energy transfer of cyanobacteria grown in different cultivation media

Seiji Akimoto[1,2,3,*], Kenta Niki[2], Shimpei Aikawa[3,4], Akihiko Kondo[3,4]

1 – Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan
2 – Graduate School of Science, Kobe University, Kobe 675-8501, Japan
3 – Japan Science and Technology Agency, CREST, Kobe 675-8501, Japan
4 – Graduate School of Engineering, Kobe University, Kobe 675-8501, Japan
* Fax: +81-78-503-5705, e-mail: akimoto@hawk.kobe-u.ac.jp

Recently, large-scale cultivation of cyanobacteria in seawater has received much attention owing to the limited freshwater available for large-scale cultivation and the low cost of seawater. It is known that the growth, photosynthetic efficiency, and pigment composition depend on cultivation medium. In the present study, we examined differences in energy transfer processes of cyanobacteria cultivated in different media: the optimum medium for each cyanobacterium and the seawater medium. To cultivate *Arthrospira platensis* cells, SOT medium (the optimum for *A. platensis*) and f/2 (the seawater medium) were used. For *Synechococcus sp.* PCC 7002 cells, modified Medium A (the optimum for *Synechococcus* 7002) and f/2 were used. To observe energy transfer, time-resolved fluorescence spectra were measured with the time-correlated single photon counting method. Pigment compositions and energy-transfer processes in both cyanobacteria grown in f/2 changed from those in the optimum medium; l) relative amounts of phycobilisome to those of chlorophyll decreased, 2) energy transfer from Photosystem (PS) II to PS I was highly inhibited, and 3) energy transfer from phycobilisome to PS II was suppressed.

Lecture S1.9

Numerical studies of the photosynthetic reaction center femtosecond transient absorption by means of hierarchical equations of motions

Roman Y. Pishchalnikov, S. M. Pershin

Prokhorov General Physics Institute, Russian Academy of Sciences, Moscow, Russia
* e-mail: rpishchal@kapella.gpi.ru

Photosynthesis is the most efficient energy conversion process existing on Earth. Light-harvesting antennae and a reaction center (RC) are the pigment protein complexes, which responsible for the sunlight absorption, energy transport and primary chemical reactions. Paradoxically, RCs of higher plants, algae and many bacteria share the same physical and chemical principles of photosynthetic machinery. Depending on type of photosynthetic species, the RC consists of two branches of protein-bound cofactors: chlorophylls or bacteriochlorophylls (BChl), pheophytins or bacteriopheophytins (BPheo) and quinons. The first step of the electron transport chain is a formation of the P^+B^- charge separation state during 3 ps after excitation of a dimer of BChls known as the special pair (SP). Then the P^+H^- state populates within a short time about 0.5 ps [1,3]. Transient absorption spectroscopy allows us to investigate the exciton and the charge separation states dynamics and observe relaxation processes in RCs. The existence of coherent modulations in stimulated emission from the SP excited state (characteristic energies <300 cm^{-1}) is a profound feature of bacterial RC [1]. The nature of such modulations still under debate, but two water molecules located near the special pair in the RC of purple bacteria gave rise to a possible explanation of the excited state absorption at 935 nm and the stimulated emission at 1020 nm [2,3,4].According to the X-ray spectroscopy, water molecules are sited in immediate proximity of the SP, accessory BChl and histidine. The distances between water molecule and the closest neighbors are in a range of 3–7 Å. They are notably larger than the hydrogen-bond length (2.98 Å) in $(H_2O)^2$. With regard to this fact, we consider two molecules in the bacterial RC as a mediator of the electron transport chain. We have been proposed that the influence of water molecules during the first radical pair state formation is strong enough to cause the coherent modulations. The time evolution of the system has been modeled by modern physical approach based on the hierarchy coupled equations for the density matrix [5] which is, actually, the nonperturbative theory (comparing with modifications of Redfield relaxation theories where dynamics of the system usually calculated on the assumption of a weak interaction of the electronic degrees of freedom with the bath). The results of modeling of the stimulated emission and the excited-state absorption are in qualitative correspondence with the experimental data.

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Lecture S1.10

Femtosecond processes of charge separation in wild type and mutant reaction centers of *Rhodobacter sphaeroides*

Anton Khmelnitskiy*, Ravil Khatypov, Anton Khristin, Maria Leonova, Lyudmila Vasilieva, Tatyana Fufina, Vladimir A. Shuvalov

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7-4967-33-05-32, e-mail: hmelan@gmail.com

Despite the advances made in the study of photosynthetic reaction centers (RCs) of purple bacteria, the mechanism of their functioning is not fully elucidated. This is especially true of very early stages of light energy conversion in reaction centers occurring in the femtosecond and picosecond range. Of particular interest are the details of the dynamics of the excited state of the primary electron donor (P), as well as the first charge-separated state $P^+B_A^-$.

Femtosecond spectroscopic measurements of wild type and mutant reaction centers with heterodimeric (bacteriochlorophyll (BChl)/bacteriopheophytin) primary electron donor with 40 fs resolution revealed ultrafast formation of an absorption band at ~1100 nm. The absorption band that appeared at ~120 fs after excitation in wild type reaction centers and almost instantly in heterodimeric ones may be attributed to the formation of excited state of the primary electron donor P* with a charge-transfer character which may determine the direction of the electron transfer.

Studies of mutant reaction centers with increased midpoint potential of the primary electron donor P (from +60 to +260 mV relative to wild type) were also performed by using femtosecond absorption spectroscopy. Formation of a radical anion band of monomeric BChl B_A at 1020 nm was observed in transient absorbance difference spectra of all mutants except a mutant with 3 amino acids substitutions in which midpoint potential of P was highest (+260 mV relative to wild type). Furthermore, formation of $P^+B_AH_A^-$ state in this triple mutant was not detected even at 600 ps delay. These results are interpreted as evidence of a monomeric BChl B_A to be an immediate electron acceptor in mutant RCs, where electron transfer is hampered due to increased energy of the $P^+B_A^-$ state with respect to P*.

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Poster S1.11

Polarized transient absorption spectroscopy of *Chloroflexus aurantiacus* chlorosomes

Andrei G. Yakovlev[1], Vladimir I. Novoderezhkin[1], Alexandra S. Taisova[1], Vladimir A. Shuvalov[1,2], Zoya G. Fetisova[1,*]

1 – Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Leninskie Gory, 119991, Moscow, Russian Federation
2 – Institute of Basic Biological Problems, Russian Academy of Sciences, 142290, Pushchino, Moscow region, Russian Federation
* Fax: +7-495-939-31-81, tel.: +7-495-939-53-63, e-mail: zfetisova@genebee.msu.ru

Isotropic and anisotropic pump-probe spectra of *Cf. aurantiacus* chlorosomes were measured on the fs- through ps-time scales for the B798 BChl *a* Q_y band upon direct excitation of the band at T=293K and T=90K. The shape of the BChl *a* spectra was shown to be independent of the size of the whole chlorosomal antenna. The monomeric nature of BChl *a* was manifested. The anisotropy parameter value *r* was constant within the BChl *a* band at both temperatures and decayed from *r*=0.4 (at 200 fs delay time) to *r*=0.10 at T=293K or to *r*=0.09 at T=90K (at 30–100 ps delay time). The anisotropy decay exhibited a fast component, reflecting the decay of coherences between the one-exciton B798 states that is followed by ps-depolarization due to B798→B798 localized exciton migration. the theoretical dependence of the steady state anisotropy parameter *r* on the angle Θ was presented for the model, in which the BChl *a* Q_y transition dipoles randomly distributed around the normal to the baseplate plane form the angle Θ with the normal. According to the theoretical dependence *r*(Θ), the angle Θ corresponding to the experimental steady state value *r*=0.1 at T=293K was found to equal 35.3°. As the temperature drops to 90K the angle Θ insignificantly increases.

Poster S1.12

Effect of lighting conditions under plant cultivation on the photosynthetic carbon metabolism in potato with elevated apoplastic invertase activity

Larisa Khamidullina*, Gusel Akhtyamova, Svetlana Batasheva, Golsoyar Bakirova, Vladimir Chikov

Kazan Institute of Biochemistry and Biophysics, RAS, Kazan, Russia
* Fax: +7-843-292-73-47, e-mail: larxas@list.ru

We decided to figure how elevated apoplastic invertase activity in potato (cv. Desiree) affects photosynthesis, transport of assimilates and plant productivity. Potato plants were grown at three light levels: 100, 200 and 380 W/m^2. Measuring intensity of 3 min $^{14}CO_2$ assimilation in the light showed that at all levels of illumination the leaves of transformed plants had lower rates of photosynthesis compared to the wild-type plants. Chromatographic analysis of labeled products of 3 min photosynthesis showed the significant differences between B-33 and wild -type plants. The incorporation of ^{14}C into sucrose and aspartate in plants of the different genotypes had the opposite dependence on light. Incorporation of ^{14}C into oligosaccharides was higher in B-33 plants under all light levels. Export of labeled assimilates was lower in B-33 potato plants compared with wild-type plants. In low light, the difference was observed only in ^{14}C transport into roots and tubers in B-33 and wild-type plants. ^{14}C content in high molecular weight compounds was greater in all sink organs of the transformants under low light. Reduced photosynthesis of transformed plants affected their productivity. In a month after planting they had 2–3 times lesser weight of aboveground part than wild-type plants. The difference between the plants of these genotypes decreased with development of plants. So, actions of elevated invertase and low light are unidirectional.

Poster S1.13

About the differences in kinetics of redox changes of bacteriochlorophyll and quinone acceptors in purple bacteria RCs

Peter P. Knox*, Evgeni P. Lukashev, Nuranija Kh. Seifullina, Nadezhda P. Grishanova

Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
* e-mail: knox@biophys.msu.ru

Long time stabilisation of electron in the photosynthetic reaction centre (RC) acceptor part has essential value for effective carrying over of reducing equivalents to a photosynthetic membrane. For studying of mechanisms of this stabilisation informative there is a comparative investigation of kinetics of redox transformations for photoactive bacteriochlorophyll (P) of purple bacteria RC and quinone acceptors (Q_A and Q_B) in their individual absorption bands. The revealed difference in the kinetics of dark recombination of charges photoseparated between P and Q_B, recorded in the P absorption band (600 nm) and the absorption bands of RC ubiquinones (335 nm, 450 nm) in RCs of *Rb. sphaeroides* is apparently due to some polarization and structural changes in the Q_B environment, leading to a “lag” in the return of Q_B absorption to the original dark level as compared to the changes in dimer P band. The substitution H_2O with D_2O not only slowed down the rate of back electron transfer from Q_B^- but also increased the observed difference in the kinetic curves recorded at 600 and 450 nm. These differences disappeared with the addition of 10% (v/v) DMSO to RC preparations (DMSO – strong proton-acceptor agent penetrating the RC structure, modifies the network of hydrogen bonds in RC). Such differences did not register for dark recombination kinetics between P^+ and Q_A^- in control samples of RCs. The differences, however, were shown after isotope substitution and they increased in process of cooling of the RC samples to cryogenic temperatures in the dark. But the differences are not found out for the RC samples cooled on exciting light. It is possible to state the certain conclusions about the hydrogen bonds involved in processes of electrostatic stabilization of electron in the quinone environment in the RC structure.

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Poster S1.14

Trehalose effects on Photosystem II complex

Mahir Mamedov[1,*], Irina Petrova, Denis Yanykin[2], Andrey Zaspa[1], Alexey Semenov[1]

1 – A.N. Belozersky Institute of Physical-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
2 – Institute of Basic Biological Problems, RAS, Puschino, Moscow Region, Russia
* Fax: +7-495-939-31-81, e-mail: mahirmamedov@yandex.ru

Pigment-protein complex of Photosystem II (PS II) catalyzes the light-driven oxidation of water to molecular oxygen and the reduction of plastoqunone to plastohydroqunone. The effects of trehalose on the oxygen-evolving activity, reoxidation of the primary quinone Q_A^- and the stability of PS II complexes were studied. It was shown that trehalose significantly stimulates the steady-state rate of oxygen evolution. The study of a single flash-induced fluorescence decay kinetics demonstrated that trehalose addition does not affect the rate of Q_A^- oxidation, although results in the increase of a relative fractions of PS II reaction centers capable of Q_A^- oxidation. Trehalose prevented PS II complexes from aggregation and inactivation. This disaccharide probably induces a more compact protein conformation due to a change in preferential hydration of PS II

Poster S1.15 *(in absentia)*

Computational study of protons transfer within photosynthetic reaction center of purple bacteria *Rb. Sphaeroides*

Pyotr A. Mamonov

Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
* e-mail: pmamonov@gmail.com

A process of protons capture by photosynthetic reaction centers (RCs) is a crucial step of light energy conversion, performed by photosynthetic apparatus. Captured protons are transferred from RCs surface to the secondary quinone molecule through the network of hydrogen bonded residues. Experimental studies of this process are complicated and give ambiguous results. We use molecular dynamics approach in order to reveal the network of hydrogen bonded residues within the RC of purple bacteria *Rb. sphaeroides* and identify the pathways of protons transfer from solution to the secondary quinone.

The molecular dynamics modeling of the RC surrounded by 150 LDAO molecules and bulk water was performed in order to assess the statistical probabilities of formation of hydrogen bonds involving aminoacid residues of RC and water molecules. Further the Dijkstra algorithm was used to reveal probable pathways of protons transfer between the solution and the secondary quinone molecule.

The results of analysis suggests that the most effective proton transfer pathway between solution and the secondary quinone comprises residues SerL223 and AspL213. According to the results of modeling the latter residue is directly accessible by bulk water. This result contradicts with recently speculated proton transfer pathways within the RC, however this result is consistent with the pathway of secondary quinone protonation proposed in [1].

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Poster S1.16 *(in absentia)*

Participation of apoplastic invertase in the regulation of photosynthesis and transpiration

Alexandr Mikhaylov[1,*], Gusel Akhtyamova[2], Svetlana Batasheva[2], Larisa Khamidullina[2], Olga Timofeeva[1], Vladimir Chikov[2]

1 – Institute of Basic Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia
2 – Kazan Institute of biochemistry and Biophysics, RAS, Kazan, Russia
* Fax: +7(843)2927347, e-mail: mixlove2010@mail.ru

Karpushkin et al. (1981, 1982) suggested that as xylem sap approaches the cell walls of guard cells its concentration increases resulting in elevation of apoplast osmolality and stomatal closure. 16 years later it was confirmed (Lu et al., 1997; Outlaw and Vlieghere-He, 2001) that this effect is associated with sucrose contents. The authors showed that sucrose of guard cells apoplast has a significant osmotic effect on the openness of the stomata. It can be assumed that an increase in extracellular concentration of sugars in the apoplastic liquid when it approaches the stomatal aperture, where the greatest evaporation of water takes place, could be a mechanism that changes the leaf stomatal conductance. In order to test this idea, we studied the gas exchange parameters and assimilation of $^{14}CO_2$ in tomato plants (*Lycopersicon esculentum* L., cv. Moneymaker), in which gene expression of apoplastic invertase in leaves was inhibited by RNA interference (Lin8-RNAi). Analysis of ^{14}C distribution among the products of 3 min $^{14}CO_2$ assimilation showed a decrease in ^{14}C incorporation into hexoses and increase in the glycolate pathway products in Lin8-RNAi plants. Investigation of CO_2 and H_2O gas exchange revealed a minor increase in photosynthesis, transpiration and intra-leaf CO_2 concentration in Lin8-RNAi plants. However, in 30–50 min after the reduction of illumination from 1556 to 771 $\mu mol \cdot m^{-2} \cdot s^{-1}$, photosynthesis decreased in both genotypes, but transpiration decreased in wild-type plants, while it increased in Lin8-RNAi plants. We concluded that apoplastic invertase regulates photosynthesis and transpiration through a change in apoplast osmolality around the stomatal aperture, which controls the functioning of stomata.

Poster S1.17

Indication of electron transfer asymmetry in cyanobacterial Photosystem I

Anastasia Petrova[1], Ivan Shelaev[2], Fedor Gostev[2], Mahir Mamedov[1], Victor Nadtochenko[2] and Alexey Semenov[1,*]

1 – A.N. Belozersky Institute of Physical-Chemical Biology, Moscow State University
2 – N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences
* e-mail: semenov@genebee.msu.ru

Electron transfer asymmetry in photosynthetic reaction centers (RC) is an intriguing question of modern photosynthesis. Cofactors of electron transfer in Photosystem (PS) I, II and bacterial RC are organized in two symmetric structurally very similar branches. In the case of bacterial RC and PS II asymmetry were clearly revealed and examined. It is considered, that generally only one branch of cofactors is involved in electron transfer. However the degree of asymmetry of electron transfer (ET) in PS I is still under debate. It seems that the most important factor, that affects the asymmetry, is the free energy of ET (ΔG). The ΔG values depend on midpoint potentials (E_m) of redox-cofactors in the two branches. One approach to change the Em of electron carrier is mutation of amino acid residue, which binds electron transfer cofactor. In this work, the impact of mutation of asparagine residues $N591_{PsaB}$ and $N604_{PsaA}$ associated with second chlorophyll molecules *Chl2* (or A_{-1}) in the *A* and *B*-branch correspondingly, on the initial steps of electron transfer was studied. The following pairs of cyanobacteria *Synechocystis sp.* PCC 6803 mutants were used: *ANL/BNL*, *ANH/BNH* (where the *A* or *B* notation indicates the subunit in which the mutation is generated). Asparagine residues were mutated to leucines (ANL/BNL) and histidines (*ANH/BNH*). It should be noted, that *Chl2* molecule anchored by *PsaA* (*Chl2A*) refers to the *B*-branch of redox cofactors and vice versa. So *BNL* and *BNH* should be considered as *A*-branch mutants while *ANL* and *ANH* – as *B*-branch mutants. The process of ion-radical pair $P_{700}^{+}A_1^{-}$ formation was studied by differential absorption spectrometry and pump-probe femtosecond absorption flash-spectrometry. Data obtained by steady state differential absorption spectrometry revealed some differences between the PS I spectra of *A*-chain and *B*-chain mutants. The amplitudes of the bleaching at 700 nm for PS I from *BNH* and *BNL* mutants were much smaller than for PS I from *ANH* and *ANL* mutants. On the other hand, the positions of the extremums were very similar in all mutants. These discrepancies indicate that the process of P_{700} oxidation is impaired in the case of *A*-branch mutants. The results obtained by pump-probe femtosecond differential spectroscopy at 500 ps time delay demonstrate that in case of *BNH* and *BNL* mutants, ET to A_1 is impaired, while for *ANH* and *ANL* mutants the ET rates are similar to those from the wild type PS I. These data suggest significant asymmetry of ET in PS I from cyanobacteria in favor of *A*-branch.

Poster S1.18

LH2 complexes: Mechanism of two-photon excitation within 1200–1500 nm range

Andrei Razjivin[1,*], Viktor Kompanets[2], Zoya Makhneva[3], Andrey Moskalenko[3], Sergey Chekalin[2]

1 – Lomonosov Moscow State University, Belozersky Institute of Physical-Chemical Biology, Moscow, Russia
2 – Institute of Spectroscopy, RAS, Moscow, Russia
3 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7-495-939-31-81, e-mail: razjivin@gmail.com

Two-photon excitation of the LH2 complexes of purple bacteria in the range 1200–1500 nm leads to the selective excitation of bacteriochlorophyll molecules (BChl850) of exciton ring aggregate. Monomer molecules (BChl800) are not excited. Several possible mechanisms proposed to explain the two-photon excitation complexes LH2. The report will be a critical analysis of the possible mechanisms of two-photon excitation of LH2 complexes, including our own experimental data.

Poster S1.19

Spectroscopic analysis of chlorophyll *f* containing cyanobacteria

Toshiyuki Sinoda[1], Daisuke Nii[1], Tatsuya Tomo[1,2,*], Seiji Akimoto[3,4], Min Chen[5], Hisataka Ohta[1], Suleyman I. Allakhverdiev[6,7]

1 – Tokyo University of Science, Tokyo, Japan
2 – PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan
3 – CREST, Japan Science and Technology Agency (JST), Kobe, Japan
4 – Kobe University, Kobe, Japan
5 – School of Biological Sciences, University of Sydney, Sydney, Australia
6 – Institute of Plant Physiology RAS, Moscow, Russia
7 – Institute of Basic Biological Problems RAS, Pushchino, Russia
* Fax: +81-3-3260-0322, e-mail: tomo@rs.tus.ac.jp

Chlorophylls (Chls) play important roles in light harvesting, energy transfer, and electron transfer during photosynthesis. Recently, Chen et al. found a more red-shifted Chl, Chl *f*, within the filamentous cyanobacteria *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* using mass spectroscopy and NMR analysis. The Chl content of *H. hongdechloris* varied under different light conditions. When under far-red light (>700 nm), the Chl *f* content increased to ca. 10% of total Chl. When under white fluorescence light, the Chl *f* content decreased negligibly. The photochemical and photophysical functions of Chl *f* are not known. Therefore, we tried to use steady and time-resolved fluorescence spectroscopy for an investigation of intact cells. We observed rapid energy transfer from Chl *a* to Chl *f* at 77K. We discuss the function of the Chl *f* in the two photosystems.

Poster S1.20

Spectral exhibition of electron-vibrational relaxation in P* state of *Rhodobacter sphaeroides* reaction centers

Andrei G. Yakovlev[1,*], Vladimir A. Shuvalov[1,2]

1 – Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Leninskie Gory, 119991, Moscow, Russia.
2 – Institute of Basic Biological Problems, Russian Academy of Sciences, 142290, Pushchino, Moscow region, Russia.
* Fax: +07- 495-939-31-81, tel.: +07-495-939-53-63, e-mail: yakov@genebee.msu.su

Electron-vibrational relaxation in the excited state of the primary electron donor, bacteriochlorophyll dimer P in reaction centers (RCs) of purple photosynthetic bacteria *Rhodobacter sphaeroides* is modeled. A multimode model of three states (ground state P_g, initially excited P_1^* and relaxed excited P_2^*) is used for calculation of the incoherent dynamics of the difference ΔA spectra on a femtosecond timescale for the YM210W mutant RCs. The relaxation processes is described by the step-ladder model. The model shows that the electron-vibrational relaxation in the excited state of P is visualized by the transient red shift of the stimulated emission from P*. The dynamics of this shift is observed as a change of the ΔA spectrum shape in its red-most part within few hundreds of femtoseconds after excitation. As a result, an initial rise of the red-side ΔA kinetics is delayed with respect to the blue-side ones. The time constant of the $P_1^* \rightarrow P_2^*$ electronic relaxation of 54 fs and of the P_g, P_1^* and P_2^* vibrational relaxation of 120 fs used in the model provided the best fit of the experimental time-resolved ΔA spectra and kinetics at 90 and 293K. A possible nature of the $P_1^* \rightarrow P_2^*$ electronic relaxation is discussed.

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

Lecture S2.1

Engineering of antenna and photosystems in cyanobacteria

Hiroaki Yoshino[1], Mai Watanabe[1,2], Masahiko Ikeuchi[1,2,*]

1 – University of Tokyo, Tokyo, Japan
2 – Japan Science and Technology Agency (JST), CREST, Saitama, Japan
* Fax: +81(3)54546641, e-mail: mikeuchi@bio.c.u-tokyo.ac.jp

Photosystems and antennae are assembled in thylakoid membranes as highly organized supercomplexes to mediate efficient energy transfer and electron transfer. To understand their elaborate organization, structural and functional analyses should be focused on a same organism, such as *Thermosynechococcus*. Here, we discuss our combinatorial approaches to engineer photosynthesis genes in this organism for dissection of the antennae-photosystem organization. First, we created phylloquinone (PhQ)-minus mutant (Δ*menD*) in *Thermosynechococcus elongatus*, aiming the elucidation of PS I electron transport and its application to the artificial photosensor. When the mutant was screened only with antibiotics, the segregation was not achieved at all. Then, further screening in the presence of exogenously added PhQ allowed us to obtain the completely segregated mutant. HPLC analysis showed that PS I isolated from the mutant cells was almost free from PhQ. On the other hand, the content of PhQ in PS I of the mutant cells grown in the presence of PhQ was nearly a half of the wild type. Second, we created several mutants of linker proteins, which determine the organization of the phycobilisome supercomplex. Combination of these mutants would allow us to prepare partial complexes suitable for structural analysis.

Lecture S2.2

Hydrophilic auxiliary proteins of Photosystem II in the cyanobacterium *Synechocystis sp.* PCC 6803

Simon A. Jackson, Peter D. Mabbitt, Sigurd M. Wilbanks and Julian J. Eaton-Rye*

Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand
* Fax +64-3-479-7866, e-mail: julian.eaton-rye@otago.ac.nz

Photosystem II (PS II) assembly and repair requires the participation of protein factors that are not present in the final assembled holoenzyme. Several of these factors are common to both assembly and repair processes and these include a number of hydrophilic subunits associated with either the lumenal face of PS II or the cytosolic side (or stromal side in eukaryotes) of the thylakoid membrane. We are currently investigating the roles of four of these proteins in the cyanobacterium *Synechocystis sp.* PCC 6803. The proteins under investigation are Psb27, CyanoP and Ycf48 – all associated with the hydrophilic domain of PS II on the lumenal side of the thylakoid membrane – and two forms of the Psb28 protein that are found on the cytosolic side. Our experiments are ongoing but we will report several novel observations. In the case of Psb27, we have observed that the complete absence of this subunit is better tolerated than forms of the protein carrying targeted mutations. We have also observed that the construction of double mutants lacking Ycf48 reveals otherwise "invisible" phenotypes for strains lacking Psb27, CyanoP and Psb28. Since it is essential that the partially assembled photochemical apparatus in nascent complexes does not "miss-fire" and produce damaging reactive oxygen species, we hypothesize that assembly and repair processes posses a built in "protective redundancy" such that – at least in our examples – a "double hit" is required before the formation of an active holoenzyme is disrupted.

Lecture S2.3

Phosphorescence of triplet chlorophylls

Alexander A. Krasnovsky (Jr.)

A.N. Bach Institute of Biochemistry, Russian Academy of Science and M.V. Lomonosov Moscow State University, Moscow, Russia
* e-mail: phoal@mail.ru

Phosphorescence is light emission, which accompanies direct radiative deactivation of the pigment triplet states. Therefore, this light emission is a basic photophysical property of pigment molecules, which provides direct information on the energy and other properties of the triplet state. In this paper, history of discovery and parameters of phosphorescence of Chls and their magnesium-free analogs are outlined. Phosphorescence was mostly studied at 77K in frozen pigment solutions, model systems, isolated pigment-protein complexes and cells of plants, alga and bacteria. Energy and lifetimes of the triplet states of Chls and pheophytins has been determined. Dependence of these parameters on solvation and aggregation of pigment molecules was investigated and the structures of these species revealed from phosphorescence studies will be discussed. In plant materials, the strongest phosphorescence of protochlorophyll (ide) and Chl *a* appeared in etiolated leaves and greening leaves at the early stage of greening. At this stage, phosphorescence is determined by bulk pigment molecules with the quantum yield of the triplet state population equal to ≥25%. Further greening caused a sharp decrease of the phosphorescence yield. Analysis of the emission and excitation spectra suggests that in mature leaves, isolated chloroplasts and algal cells, phosphorescence is emitted by the triplets of several forms of Chl *a*, which are coupled with the native pigment-protein complexes but are detached from carotenoids. The phosphorescence intensity at 77K correlates with the rates of chloroplast damage at room temperature. The yield of chlorophyll phosphorescence shows the degree of plant protection against photodynamic action of the native pigment apparatus. In particular, measurement of phosphorescence allows for investigation of mechanisms and efficiency of action of photodynamic herbicides. Thus, phosphorescence provides unique information on the photophysics of pigment molecules, organization of the photosynthetic apparatus and mechanisms and efficiency of photodynamic stress in plants. Detailed information is given in recent reviews listed below and refs therein.

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Lecture S2.4

Molecular genetic analysis of the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina*

Tohru Tsuchiya* and Kazuyuki Watabe

Kyoto University, Kyoto, Japan
* Fax: +81(75)7537909, e-mail: tsuchiya.toru.8e@kyoto-u.ac.jp

Chlorophylls (Chls) have a functional role in the charge separation and/or the light-harvesting processes. *Acaryochloris spp.* contains Chl *d* as a major Chl and can utilize far-red light for photosynthesis using Chl *d*. Analyses of photosystems (PSs) purified from *A. marina* revealed its unique properties [1–4]. However, molecular mechanism of photosynthesis driven by far-red light is largely unexplained. To elucidate the unique photosynthetic mechanism including Chl *d* biosynthesis, molecular genetic analysis is one of the effective approaches. Therefore, we developed the transformation system for *A. marina* for the first time [5]. We introduced chlorophyllide *a* oxygenase gene (CAO) into *A. marina*, and the resultant transformant (CAO^+ strain) accumulated a novel Chl species, [7-formyl]-Chl *d* instead of Chl *b*. From the CAO^+ strain, PS II complexes were isolated and analyzed [6]. Analysis of the Chl content of PS II revealed that some Chl *d* molecules were replaced by [7-formyl]-Chl *d* molecules. Furthermore, [7-formyl]-Chl *d* molecules incorporated into PS II functioned as antenna. In contrast, Chl *a* content in PS II was not altered. These results indicated that the presence of indispensable Chl *a* in PS II complexes from *A. marina*. In addition to the introduction of foreign gene, recently, we developed the transposon tagging system for *A. marina*. The potential of molecular genetic analysis of *A. marina* will be discussed.

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Lecture S2.5

Relocation of BChl axial ligands in *Rhodobacter sphaeroides* mutant reaction centers

Lyudmila Vasilieva[1,*], Tatyana Fufina[1], Azat Gabdulkhakov[2], Ravil Khatypov[1], Vladimir A. Shuvalov[1]

1 – Institute of Basic Biological Problems, Pushchino, Russia
2 – Institute of Protein Research, Pushchino, Russia
* Fax: +7-4967-33-05-32, e-mail: vsyulya@mail.ru

Photosynthetic reaction center (RC) of purple bacterium *Rhodobacter (Rba.) sphaeroides* consists of three protein subunits and ten non covalently bound cofactors that are organized in two membrane-spanning branches, A and B. Cofactors are represented by two bacteriochlorophylls (BChls) combined into a special pair P, two monomer BChls, two bacteriopheophytins (BPhe), two quinones, a carotenoid and a non-heme iron atom. The axial ligands, hydrogen bonds and nearby residues that constitute the BChls microenvironment can greatly affect their photophysical and redox properties and can be altered as a result of site-directed mutations. In all known native bacterial RCs central Mg atoms of BChls are pentacoordinated with histidine axial ligands settled perpendicular to the α-side of the tetrapyrrole's plane. We report that in the mutant RCs with substitutions I(L177)H+H(L173)L and I(M206)H+H(M202)L histidine ligands for BChl dimer can be relocated from L173 and M202 positions to L177 and M206 positions, correspondently. This relocation results in considerable blue shifts of the Q_y P band indicating that excitonic interactions in BChl dimer are severely disturbed. Another relocation of axial ligand from one side of the monomer BChl B_A plane to another was shown in the mutant RC I(L177)H+H(M182)L. Computer models based on X-ray structure of the I(L177)H RC were proposed to interpret unusual spectral properties of the mutant RCs with altered positions of histidine ligands.

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Poster S2.6

Effects of detergents, lipids and trimer-trimer contacts on the pigment excitonic interactions in plant light-harvesting complex II

Parveen Akhtar[*], Márta Dorogi, Krzysztof Pawlak, Győző Garab, Petar H. Lambrev

Hungarian Academy of Sciences, Biological Research Centre, 6726 Szeged, Hungary
* e-mail: akhtar.parveen@brc.mta.hu

In photosynthetic membranes fast and efficient directional excitation energy transfer occurs on account of short-range intermolecular excitonic interactions between pigments in the light-harvesting pigment-protein complexes, which give rise to characteristic circular dichroism (CD) signals. CD is highly sensitive to the excitonic interactions and hence to the molecular architecture of photosynthetic membranes. It is well known that detergent extraction of plant light-harvesting complex II (LHCII) from the native membrane or from aggregates brings about significant changes in the excitonic CD spectrum. To elucidate the cause of these changes, e.g. trimer-trimer contacts or detergent-induced structural perturbations, we compared LHCII aggregates, artificial LHCII-lipid membranes, LHCII trimers solubilized in different detergents, trapped in polymer gels in the absence and presence of detergents. By this means we were able to separate the spectral changes specific to protein-protein contacts (at (+)437 nm and (+)483 nm) from those due to detergent-protein interactions (e.g. at (−)495 nm).

The anisotropic CD (ACD) of macroscopically-aligned LHCII was employed to discriminate between excitonic transitions with different polarizations, i.e. found predominantly parallel (face-aligned) or perpendicular (edge-aligned) to the membrane plane. In line with theoretical considerations, the ACD spectra of oriented LHCII in face-aligned position exhibited only some of the bands present in the CD spectra of randomly oriented (isotropic) solution and the amplitudes of these bands were strongly amplified. On this basis, the (+)444 nm and (+)483 nm CD bands could be assigned to excitonic transitions oriented in the membrane plane and the bands at (−)438 and (−)473 nm to excitonic transitions perpendicular to the membrane plane.

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Poster S2.7

Recovering colored-carotenoid biosynthesis in the cells of the sulfur photosynthetic bacterium *Ectothiorhodospira haloalkaliphila*

Aleksandr Ashikhmin*, Zoya Makhneva, Maksim Bolshakov, Yuriy Erokhin, Andrey Moskalenko

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7-4967-33-05-32, e-mail: AshikhminAA@gmail.com

The processes of recovering colored-carotenoid biosynthesis in carotenoid-less cells of the purple sulfur bacterium *Ectothiorhodospira (Ect.) haloalkaliphila* grown with diphenylamine (DPA-cells) have been studied. It has been found that 1) the rate of recovering colored-carotenoid biosynthesis in the lag-phase is far ahead of the growth rate of the cells themselves; 2) several carotenoids (ζ-carotene, OH-neurosporene, neurosporene etc.) act as intermediates in carotenoid biosynthesis; 3) the filling of the "empty" carotenoid pockets in the LH1-RC complexes is faster than that in the LH2 complexes; 4) at the final stage of cell growth, a set of carotenogenetic enzymes from the LH1-RC assembly site synthesizes only one carotenoid, spirilloxanthin, and the same set of enzymes from the LH2 assembly site synthesizes four carotenoids (spirilloxanthin, anhydrorhodovibrin, rhodopin and lycopene). Consequently, there may be at least the two sets of enzymes for LH1-RC and LH2 assembly sites in the intracytoplasmic membrane of *Ect. haloalkaliphila*. In the present study, we discuss the process of colored-carotenoid incorporating into light-harvesting complexes when recovering the carotenoid biosynthesis in the DPA-cells of *Ect. haloalkaliphila*.

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Poster S2.8

Could the LH2 complex from purple photosynthetic bacteria be assembled in the cell without carotenoids?

Maksim Bolshakov*, Aleksandr Ashikhmin, Zoya Makhneva, Andrey Moskalenko*

Institute of Basic Biological Problems, RAS, Pushchino, Russia, 142290
* e-mail: lfbv22@rambler.ru

The cells of *Rhodoblastus (Rbl.) acidophilus* were grown in the presence of the carotenoid inhibitor diphenylamine (DPA). Under carotenoid-limiting conditions (71 μM DPA) the large amount of phytoene is accumulated. Its presence can not be detected spectrally. This precursor binds the LH2 complexes nonspecifically and does not stabilize their structure. DPA reduces carotenoid content in the LH2 complex to ~46%. It is shown that these complexes are heterogeneous in carotenoid composition. A part of the LH2 complexes with 90% carotenoids content survives after heating at 50°C for 15 min. Other part of the LH2 complexes containing about one carotenoid molecule per complex was destroyed by heating. The obtained data reflects the fact that the small part of the LH2 complexes could be assembled in the cells without coloured carotenoids. The obtained data reflects the fact that a small part of the LH2 complexes could be assembled *in vivo* without coloured carotenoids.

Poster S2.9

Integration of Antarctic phaeophyte Kleptoplast in a dinoflagellate host

Kostas Stamatakis[1,*], Dimitris Vayenos[1], Christos Kotakis[1,2]

1 – Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi Attikis, Greece
2 – Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, POB 521, H-6701 Szeged, Hungary.
* Fax: +30-210-651-17-67, e-mail: kstam@bio.demokritos.gr

Photosynthetic dinoflagellates contain a diverse collection of plastid types, a situation believed to have arisen from multiple endosymbiotic events. A novel and abundant dinoflagellate related to the icthyotoxic genera *Karenia* and *Karlodinium* was discovered by Gast et al [1] in the Ross Sea, Antarctica. The dinoflagellate plastid did not share evolutionary history with the plastids of *Karenia* or *Karlodinium*, but was closely related to the free-living haptophyte *Phaeocystis antarctica*, a species that often dominates phytoplankton blooms in the Ross Sea. In the present study, we investigated the fluorescence transient (OJIPS) of the novel dinoflagellate cells compare to the free living haptophyte *Phaeocystis antarctica*, cells induced by continuous strong red actinic light. Plotting the full transients on logarithmic time scale [2, 3], we will compare and discuss how the shape of OJIP provides information for the redox poise of PQ-pool in the dark.

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Poster S2.10

Changed stoichiometry of the minor antenna and PS2 reaction centres, a possible basis for increased photosynthesis productivity and increased tolerance to light and drought stress in barley

Elena V. Tyutereva[1,*], Wolfram G. Brenner[2], Alexandra N. Ivanova[1], Katharina Pawlowski[3], Olga V. Voitsekhovskaja[1,*]

1 – Komarov Botanical Institute RAS, Saint-Petersburg, Russia
2 – Freie Universitaet Berlin, Berlin, Germany
3 – Stockholm University, Stockholm, Sweden
* Fax: +7-812-372-54-43, e-mail: ovoitse@yandex.ru; tuterlena@mail.ru

The identification of physiological, biochemical and molecular genetic determinants of high productivity in barley remains an important task of modern crop physiology. Productivity of the barley mutant *chlorina* 3613 (*clo-f2*^{3613}) lacking functional chlorophyllid-a-oxygenase (Mueller et al., 2012) and, consequently, the chlorophyll *b*-containing antenna, can be enhanced considerably by a special cultivation practice (Tyutereva et al., 2011). As a result, *chlorina* 3613 forms a new, previously unknown phenotype, which we named *cloX*. It is characterized by high tolerance to light and drought stress, and a productivity level comparable to, or higher than, that of the WT. We examined the underlying mechanisms and could show that untreated *chlorina* 3613 exhibited compromised stomata control and a very low level of photoprotection reflected by high singlet oxygen production, high levels of grana degradation and very low levels of NPQ as compared to the WT. Contrary to that, the high productivity of the *cloX* phenotype was based on the restoration of stomata control and the build-up of efficient photoprotection as judged by decreased singlet oxygen production, down to WT levels, restoration of grana, and development of zeaxanthin-independent NPQ. This was achieved by accumulation of: 1) three minor antenna proteins, Lhcb4–6, and 2) not only dimeric but also monomeric PS II RC. Analysis by Affymetrix GenChip®Barley Genome Arrays showed that while 437 genes were differently expressed in leaves of *chlorina* 3613 vs. WT leaves, only 16 genes kept the changed expression pattern in leaves of *cloX* vs. WT leaves. The data suggest that in *cloX*, the minor antenna and the PS II RC efficiently quench triplet and singlet excited chlorophyll states, respectively, leading to an increase in photoprotection and photosynthetic activity, and that the accumulation of the minor antenna proteins (revealed as the site of ABA synthesis; Xu et al., 2012) is responsible for the increased drought stress tolerance of *cloX*.

Section 3: Photosystem II and Water Oxidation Mechanism

Lecture S3.1

Possible mechanism of photosynthetic water oxidation based on atomic structure of Photosystem II

Jian-Ren Shen

Photosynthesis Research Center, Graduate School of Natural Science and Technology, Okayama University, Tsushima Naka, Okayama 700-8530, Japan
* Fax: +81(86)2518502, e-mail: shen@cc.okayama-u.ac.jp

The crystal structure of Photosystem II (PS II) from a thermophilic cyanobacterium *Thermosynechococcus vulcanus* has been solved at 1.9 Å resolution [1], leading to a clear picture of the catalytic center for photosynthetic water-splitting, which is a Mn_4CaO_5-cluster organized into a distorted chair form. This feature of the distorted shape of the metal cluster has suggested a remarkable flexibility in its structure, which would be needed for the structural changes expected to occur during the catalytic water-splitting cycle (S-state cycle). Some of the inter-atomic distances within the metal cluster revealed by the X-ray structural analysis, however, were shown to be slightly longer than those obtained by previous EXAFS studies as well as theoretical studies. Among these, the position of O5 has received particular attention, since it connects Mn1 and Mn4 with bond distances unusually longer compared with typical Mn-O bond distances. This unusual property implied that this oxo-bridge may participate in the O-O bond formation during O_2 release, a proposal also suggested from theoretical studies.

In addition to the structure of native PS II, we have solved the PS II structure with the Ca^{2+} ion substituted by Sr^{2+} at 2.1 Å resolution [2]. The Sr^{2+}-substituted PS II showed an oxygen-evolving activity half of PS II containing Ca^{2+}, and our structural analysis showed that one of the two water molecules bound to Ca^{2+} (designated as W3) was specifically affected by the Sr^{2+}-substitution, leading to an elongation of its bond distance from 2.4 Å to 2.6 Å, as well as a breakage of the hydrogen-bond between W3 and W2, another water molecule bound to Mn4. Based on these results, I will discuss the possible mechanism for photosynthetic water-splitting. I will also show the first structure of eukaryotic PS II we obtained from a red alga.

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Lecture S3.2

Characterization of singlet oxygen production and its role in photodamage in intact cyanobacteria and microalgae

Imre Vass*, Ateeq ur-Rehman, László Sass

Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
* e-mail: Vass.Imre@brc.mta.hu

Singlet oxygen ($^1O_2$) has been implicated as an important mediator of light induced damage of the photosynthetic apparatus. Although various methods (such as EPR and fluorescent spin trapping) are available for detection in isolated photosynthetic systems these sensor molecules are unable to penetrate the cell wall of intact microalgae, which seriously limits the possibility to study the role of $^1O_2$ in photoinhibition *in vivo*. In order to overcome this difficulty we have developed a detection method, which is based on chemical trapping of $^1O_2$ by histidine. This process leads to O_2 uptake during illumination, which can be detected and quantified by commercial oxygen electrodes. By *in vitro* characterization of His-mediated O_2 uptake we have shown that this process is selective for $^1O_2$ trapping and does not interact with H_2O_2, superoxide or hydroxyl radicals. In intact *Synechocystis* PCC 6803 cells addition of His induced O_2 uptake, whose rate was enhanced D_2O, which increases the lifetime of $^1O_2$, and was suppressed by the $^1O_2$ quencher NaN_3. These data demonstrate that exogenous His reaches close vicinity of $^1O_2$ production sites inside the cells. Besides cyanobacteria the method was also tested with green algae and dinoflagellates. By applying the His trapping method we showed that $^1O_2$ production linearly increased with light intensity even above the saturation of photosynthesis. We also studied $^1O_2$ production in site directed mutants of *Synechocystis* PCC 6803 in which the Gln residue at the 130th position of the D1 reaction center subunit was changed to either Glu or Leu, which affect the efficiency of nonradiative charge recombination from the primary radical pair. We found that the D1-Gln130Glu mutant showed decreased $^1O_2$ production concomitant with decreased rate of photodamage relative to the WT, whereas both $^1O_2$ production and photodamage were enhanced in the D1-Gln130Leu mutant. The data are discussed in the framework of the model of photoinhibition in which triplet P680 sensitized $^1O_2$ production plays a key role in PS II photodamage, and nonradiative charge recombination of the primary charge separated state provides a photoprotective pathway.

Lecture 3.3

Molecular mechanism of photosynthetic water oxidation revealed by infrared spectrscopy with quantum chemical calculations

Takumi Noguchi* **and Shin Nakamura**

Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan
* Fax: +81-52-789-2883, e-mail: tnoguchi@bio.phys.nagoya-u.ac.jp

Photosynthetic water oxidation is performed at the Mn_4Ca cluster in Photosystem II. To understand the reaction mechanism of water oxidation, it is essential to monitor the electron and proton transfer processes during the S-state cycle. In this study, we have used infrared spectroscopy in combination with QM/MM calculations to study the mechanism of proton-coupled electron transfer in water oxidation.

Time-resoled infrared spectroscopy revealed early proton release processes before electron transfer at the S_2 and S_3 states, which have an excess positive charge on Mn_4Ca cluster. Fourier transform infrared (FTIR) analysis using D2-K317R mutant, in which the Lys ligand to Cl-1 is replaced with Arg, the $S_3 \rightarrow S_0$ transition is blocked when the sample is partially dehydrated, indicating the involvement of Cl-1 and surrounding water molecules in proton transfer during transition. FTIR difference spectra of Y_Z upon photo-oxidation exhibited a broad positive feature around 2800 cm^{-1} assigned to the N-H band of protonated D1-His190, revealing the presence of a polarizable proton on this His. QM/MM calculations of the Y_Z site showed that one water molecule is significantly moved upon Y_Z oxidation toward D1-His190. We thus propose a novel proton-transfer mechanism via $Y_Z^{\bullet}$, in which Y_Z oxidation induces the rearrangement of the hydrogen bond network and proton hopping from $HisH^+$ to the moved water triggers proton transfer from substrate water to the lumen. This proton transfer mechanism may function in the $S_2 \rightarrow S_3$ transition, which requires proton transfer before electron transfer from the Mn_4Ca cluster to $Y_Z^{\bullet}$.

Lecture S3.4

pKa computations of di-manganese model complexes and S1-state EXAFS spectra from DFT optimized Mn-cluster in PS II

Ernst-Walter Knapp[1,*], Arturo Robertazzi[1], Artur Galstyan[1], Holger Dau[2], Ivelina Zaharieva[2], Petko Chernev[2]

1 – Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany
2 – Physics Department, Freie Universität Berlin, Berlin, Germany
* e-mail: knapp@chemie.fu-berlin.de

Knowledge on the protonation state for the different redox states of the Mn_4Ca complex in PS II is vital to understand the function of this complex during the different states of the Kok cycle. In recent years we have developed procedures to compute redox potentials and pKa values of different compounds accurately with a combination of quantum chemistry and electrostatics [1–5]. For applications on the Mn-cluster in PS II, we have refined this method to compute pKa values of di-manganese model complexes for which measured pKa values are available reaching an accuracy of nearly 1 pH unit.

Up-to-date it is notoriously difficult to relate quantum chemical DFT structures of the Mn-cluster in PS II with the corresponding EXAFS spectra. We show that by optimizing the structure of the Mn-cluster in PS II with high level quantum chemical computations and small adjustments of the resulting optimized structure one can get very precise agreement between measured computed EXAFS spectra based on the optimized structure of the Mn-cluster. This agreement allows to characterize the protonation pattern of the Mn-cluster of PS II in the S1 state.

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Lecture S3.5

FTIR spectroelectrochemical study on the influence of Mn-depletion on the redox potential of the non-heme iron and its surrounding structure in Photosystem II

Yuki Kato* **and Takumi Noguchi**

Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, Japan
* Fax: +81-52-789-28-83, e-mail: yuki.kato@bio.phys.nagoya-u.ac.jp

It has been known that inactivation of the Mn_4CaO_5 cluster by depleting Mn and/or Ca ions from Photosystem II (PS II) induces a positive shift, ca. +150 mV, of the redox potential E_m of the primary quinone electron acceptor Q_A, resulting the suppression of the acceptor side electron transfer from Q_A to the secondary quinone Q_B. This phenomenon has been interpreted in the mechanism of photoprotection. However, the structural rationale behind such a long-range transmembrane (~40 Å) interaction is still unknown.

In the present work, to shed new light on the long-range interaction mechanism between the donor and the acceptor side, we have investigated the influence of Mn-depletion on E_m of the non-heme iron and its surrounding structure by applying an FTIR-spectroelectrochemical technique. Spectroelectrochemical measurements revealed that Mn-depletion shifts $Em(Fe^{2+}/Fe^{3+})$ by ca. +18 mV from +468 mV vs. SHE in intact PS II, which is about ca. 8 times smaller than that observed for Q_A. Comparison of Fe^{2+}/Fe^{3+} difference FTIR spectra indicated that D1-His215, a His ligand to the non-heme iron interacting with Q_B, is structurally modified by Mn-depletion. Fe^{2+}/Fe^{3+} difference spectra in the presence of bromoxynil, a herbicide bound to the Q_B site, further indicated that Mn-depletion also influences the Q_B site. On the basis of these results, a possible mechanism of the long-range interaction is discussed.

Lecture S3.6

Histidine Hydroxyl Modification on D2-His336 in Photosystem II of *Thermosynechoccocus vulcanus* and *Thermosynechoccocus elongatus*

Miwa Sugiura

Proteo-Science Research Center, Ehime University, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan and PRESTO, Japan Science and Technology Agency (JST), 4-1-8, Honcho, Kawauchi, Saitama 332-0012, Japan.
* Fax: +81-89-927-9616, e-mail: miwa.sugiura@ehime-u.ac.jp

The electron density map of the 3D crystal of Photosystem II from *Thermosynechococcus vulcanus* with a 1.9 Å resolution [1] (PDB: 3ARC) exhibits, in the two monomers in the asymmetric unit cell, an until now non-identified uninterpreted strong difference electron density centered at a distance around 1.5 Å from the nitrogen Nd of the imidazole ring of D2-His336. By MALDI-TOF/MS upon tryptic digestion it is shown that in ~20–30% of the Photosystem II from both *Thermosynechococcus vulcanus* and *Thermosynechococcus elongatus* the fragment containing the D2-His336 residue bears an extra mass of +16 Da. Such an extra mass likely corresponds to an unprecedented post-translational or chemical hydroxyl modification of histidine [2].

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Lecture S3.7

Bicarbonate requirement for the donor side of Photosystem II

Vyacheslav Klimov

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7-4967-33-05-32, e-mail: klimov@issp.serpukhov.su

The following possible ways for the involvement of bicarbonate anione in the events within the water-oxidizing complex (WOC) of photosystem II (PS II) are considered:

1) bicarbonate facilitates re-assembly of the water-oxidizing complex from apo-WOC and Mn(II);

2) bicarbonate is an easily accessible base (with an appropriate pK) involved in the removal of protons during the photosynthetic water oxidation;

3) the bicarbonate anions binding to the WOC components is essential for its stability and function.

Poster S3.8

Evolution of the Photosystem II redox states by modeling of the electron transfer

N. E. Belyaeva[1,*], F.-J. Schmitt[2], V. Z. Paschenko[1], G. Yu. Riznichenko[1], A. B. Rubin[1]

1 – Department of Biophysics, Biology Faculty of the M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
2 – Institute of Physical Chemistry, Technical University Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany
* e-mail: natalmurav@yandex.ru

The light induced processes in the Photosystem II (PS II) of oxygen evolving photosynthetic organisms were investigated on a large time scale of 9 orders of magnitude. On the sub-nanosecond time scale thermal dissipation and fluorescence (FL) emission in the antenna compete with charge separation. The electron transfer (ET) is coordinated with proton transfer (PT) on the ns to μs time scale for the donor and up to milliseconds for the acceptor side [1,2]. The time courses of the Chl *a* FL induced by a 10 ns laser flash deliver information on the population and decay of redox intermediates [3]. This allowed an analysis by our multi-scaled PS II model [4] taking into account dissipative processes [5,6].

Our PS II model [4-6] was optimized with a time hierarchic order. Parameters were identified by multiple fittings to data sets of Single Flash Induced Transient Fluorescence Yield (SFITFY) to simulate the patterns measured on *A. thaliana* leaves for different light intensities [5], on spinach and *Chlorella* [6]. ET and coupled PT are specified within confined ranges shown in literature [1,2]. SFITFY fitting of the dark adapted species verified similar multiphasic ns kinetics of $P680^+$ reduction via tyrosine Y_Z [2] for *Chlorella* and spinach. Distinctive time courses of the redox state populations underlying the SFITFY patterns of *Chlorella* and spinach were found on the acceptor Q_B site [6].

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Poster S3.9

Impact of the interaction of Al^{3+} with the proteins composition of Photosystem II

Imed Hasni*, Saber Hamdani and Robert Carpentier

University of Quebec at Trois Rivieres, Quebec, Canada
* e-mail: imed.hasni@uqtr.ca

Aluminum toxicity is one of the major environmental stresses that arise in acid soils. In these soils, the solubilized toxic forms of aluminum, Al^{3+} species, exert their phytotoxicity causing inhibition of the photosynthesis process. This inhibition has been attributed mainly to the impairment of Photosystem II (PSII) activity afterwards the photosynthetic electron transport is inhibited. In order to provide more clarification on the inhibitory action of Al^{3+} in PS II, we have analyzed the interaction of this toxic metal cation with the component of PS II submembrane fractions isolated from spinach. For this purpose, several techniques have been used such as oxygen evolution, chlorophyll fluorescence induction, thermoluminescence, polyacrylamide gel electrophoresis, chlorophyll fluorescence emission spectra at low-temperature (77K) and native green gel electrophoresis. Our results showed that Al^{3+} concentrations above 3 mM caused both the release of the 16, 24 and 33 kDa extrinsic polypeptides and disorganization of the Mn_4O_5Ca cluster associated with the oxygen-evolving complex (OEC) of PS II. This alteration leads to a strong inhibition of the oxygen evolution activity which is completely lost following a damage of the OEC. This is accompanied by a retarded fluorescence induction and a significant reduction of F_v/F_m. The LHCII antenna complex was affected at the same concentrations of Al^{3+}. Indeed, at low temperature (77K), the relative amplitude of the peak at 685 nm of the chlorophyll fluorescence emission spectra was decreased with addition of Al^{3+}. In addition, F_0, the initial Chl fluorescence was increased with Al^{3+} concentrations. This implies a reduction of energy transfer from the antennae complexes to the reaction centers of PS II owing to the dissociation and/or alteration of light-harvesting chlorophyll *a*/*b* protein complexes (LHCII) from the reaction center complex of PS II which was confirmed by the native green gel electrophoresis. We concluded that the interaction of Al^{3+} with proteins subunits of PS II causes both inhibition of oxygen evolution generated by damage of the OEC due to the release of extrinsic proteins and reduces energy transfer from LHCII to PS II core due to alterations of the light harvesting complex. The electron transport activity was inhibited on both donor and acceptor sides of the PS II.

Poster S3.10

Suprising glycerol effect on the activation enthalpy of water oxidation in Photosystem II

Zhiyong Liang, Ivelina Zaharieva, Oliver Karge, Holger Dau*

Freie Universität Berlin, FB Physik, Arnimallee 14, D-14195 Berlin, Germany
* e-mail: holger.dau@fu-berlin.de

Water oxidation in Photosystem II (PS II) is a process of fundamental importance for life on our planet. Moreover, it is a key reaction in future technological systems for production of solar fuel, where the biological process represents an important source of inspiration. Tremendous progress has been obtained, especially in structural characterization of Photosystem II. However, mechanistically water oxidation still is insufficiently understood. Knowledge gaps relate not only to the local redox chemistry at the Mn_4Ca complex of PS II, but also to the pivotal coupling to protonation and protein dynamics at various time and length scales.

In protein crystallography, glycerol is often used as a cryoprotectant. In the ground-breaking PS II model of Shen and coworkers, several protein-internal glycerol molecules are resolved. Now we have investigated how glycerol affects individual reaction steps in PS II employing analysis of time courses (after laser-flash excitation) of delayed Chl fluorescence (10 µs – 10 ms) and of the variable Chl fluorescence yield (100 µs – 500 ms). We used PS II membrane particles from spinach and focused (i) on analysis of the of the oxygen evolution step ($S3 \rightarrow S4 \rightarrow S0 + O_2$) and (ii) on the quinone electron transfer at the acceptor side of PS II.

The deprotonation step before O–O bond formation is found to be strongly affected by glycerol in the aqueous buffer, even at relatively low concentration, while the rate constant of oxygen evolution and the quantum efficiency of S-state transitions is affected only at high glycerol concentration (above 30% volume percent). An Arrhenius analysis reveals that, surprisingly, the activation enthalpy of oxygen evolution is strongly affected (decreased) by using a buffer system with 30% glycerol, whereas the influence on the rate constant at room temperature is negligibly small. With and without glycerol, the activation enthalpies are significantly lower than the energetic barriers obtained by quantum chemical calculations of (e.g.) Siegbahn.

We conclude that the rate of O–O bond formation is determined, inter alia, by the coupling of the local redox chemistry to protonation and/or protein dynamics. The glycerol influence may result from an influence of the protein dynamics relating to increased solvent viscosity or from disturbed protonation dynamics by specific binding of glycerol molecules within PS II.

Poster S3.11

Mn Oxide/Nanodiamond composite: A new water-oxidizing catalyst for water oxidation

Mohammad Mahdi Najafpour[1,2,*], Mahnaz Abasi[1], Tatsuya Tomo[3,4], Suleyman I. Allakhverdiev[5,6,*]

1 – Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran
2 – Center of Climate Change and Global Warming, IASBS, Zanjan, Iran
3 – Department of Biology, Faculty of Science, Tokyo University of Science, Tokyo, Japan
4 – PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan
5 – Institute of Plant Physiology RAS, Moscow, Russia
6 – Institute of Basic Biological Problems RAS, Pushchino, Russia
* e-mail: mmnajafpour@iasbs.ac.ir; suleyman.allakhverdiev@gmail.com

Water oxidation is known as a source for cheap electrons and the reaction may provide electrons for not only for proton reduction but also for providing electrons for other reduction-reactions which are equally important in artificial photosynthesis [1,2].

Carbon is an interesting element that can form various original structures such as C_{60}, carbon nanotubes (CNT), graphene (G), graphene oxide (GO) and nanodiamond (ND). The compounds show exceptional structural and chemical properties [3].

Mn oxide/CNT was shown as promising composites for water oxidation [4]. Many factors such as surface, oxidation state of Mn oxide, dispersion, calcination temperature and crystalinty are important in water-oxidizing activity [4,5]. It is known that compared to other allotropic forms of carbon such as C_{60} and CNT, ND constituted by sp^3 carbons are remarkable inert [6].

Here, we reported nano-sized Mn oxide/nanodiamond composites as water-oxidizing compounds. The composites were synthesized by easy and simple procedures, and characterized by scanning electron microscopy, transmission electron microscopy, X-ray diffraction spectrometry, Fourier transform infrared spectroscopy and atomic absorption spectroscopy. The water-oxidizing activities of these compounds were also considered in the presence of cerium(IV) ammonium nitrate. A Mn oxide/nanodiamond composite reported here shows turnover frequency ~1 (mmol O_2/mol Mn·s).

Regarding the reported results, we concluded that synthesis of amorphous nano-sized Mn oxide/ND composites as efficient water-oxidizing catalysts by the reaction of MnO_4^- and ND is possible. However, other methods such as mixing $CaMnO_x$ and ND or the reaction of MnO_4^- and Mn(II) in the presence of ND cause reduction of Mn oxide to Mn_2O_3 and MnO(OH). The simple van der Waals interactions between ND and these Mn oxides are sufficient to provide a strong enough adhesion for inorganics/nanocarbon. Thus, although ND has fragile structure but using new strategies may cause the application it as a support for heterogeneous catalysts. The results clearly show where amorphous Mn oxide remains intact, good water oxidation is observed by amorphous Mn oxide/ND composite.

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Poster S3.12

Vibrational analyses of the water oxidizing center in Photosystem II using QM/MM calculations

Shin Nakamura* **and Takumi Noguchi**

Graduate School of Science, Nagoya University, Nagoya, Japan
* Fax: +81-52-789-2883, e-mail: snakamura@bio.phys.nagoya-u.ac.jp

Photosynthetic water oxidation is catalyzed at the water oxidizing center (WOC), which consists of the Mn_4CaO_5 cluster and surrounding amino acid residues, in Photosystem II. For understanding of the water oxidation mechanism, it is crucial to clarify the mechanism of proton-coupled electron transfer at the Mn_4CaO_5 cluster and Y_Z as well as the roles of amino acid residues forming WOC. In this study, we have investigated 1) the proton transfer mechanism via Y_Z, and 2) the vibrational structure of the carboxylate ligands to the Mn_4CaO_5 cluster using QM/MM calculations.

1) QM/MM calculations of the Y_Z site showed that upon Y_Z oxidation the proton from Y_Z is trapped at the N_τ of D1-His190 providing a significantly downshifted N-H vibration. It was also shown that upon $Y_Z^\bullet$ formation the surrounding H-bond network is rearranged and one water molecule moves toward His190. A proton transfer mechanism via $Y_Z^\bullet$-$HisH^+$, in which hopping of the N_τ proton to this water triggers proton transfer from substrate water to the lumen, is proposed.

2) Vibrational analysis of the Mn_4CaO_5 cluster with amino acid ligands using QM/MM calculations provided S_2-minus-S_1 infrared difference spectra. Either of the spectra calculated using two isoenergetic S_2 states, in which Mn4 or Mn1 is oxidized, satisfactorily reproduced the symmetric COO^- region of the experimental S_2-minus-S_1 FTIR spectrum. In addition, the ^{12}C-minus-^{13}C double difference spectrum of the C-terminus (D1-A344) (Chu et al., Biochemistry, 2004) was well reproduced by the present calculations. These data indicate the consistency between the experimental FTIR data and the calculated oxidation states of the Mn ions in the Mn_4CaO_5 cluster.

Poster S3.13

In silico photosynthesis: computer assisted simulation of electron transport processes in Photosystem II

László Sass, Zsuzsanna Deák, Imre Vass

Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
* e-mail: Vass.Imre@brc.mta.hu

We have developed a computer model to describe the complex network of electron transport processes in Photosystem II and in the thylakoid membrane. The model is based on a set of differential equations, which are solved by using a Matlab based software. The model provides an excellent tool to simulate electron transport processes under a wide range of conditions and can be used to perform *in silico* experiments, whose predictions can be verified by measuring the abundance and kinetics of various electron transport components. The model helps to get new insight into the understanding of various PS II electron transport characteristics, such as the interpretation of misses in the S-state cycle and the functioning of the Q_A-Q_B quinone electron acceptor complex. Linear electron transport via the Q_A, Q_B and PQ electron acceptors can be studied by measuring the relaxation kinetics of flash-induced variable Chl fluorescence signals. We have recently observed that relaxation of flash-induced fluorescence can exhibit a wave phenomenon showing a transient dip of the fluorescence yield followed by a bump in *Thermosynechococcus elongatus* and other cyanobacteria. We have used our in silico PS II model to interpret this phenomenon in parallel with measurement of the relaxation kinetics in the presence of various electron transport inhibitors and experimental conditions. Our results show that the wave feature of fluorescence decay reflects changes in the redox level of the PQ pool, which are caused by the imbalance of PS II and PS I electron transport and feed back of electrons from the NDH-1 complex to the PQ pool.

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Poster S3.14 *(in absentia)*

Extraction of Mn cations from oxygen-evolving complex by hydroquinone at different pH: correlation between pH-dependent resistance of Mn ions to the action of hydroquinone and oxygen-evolving activity

Boris Semin*, Lira Davletshina, Tatyana Podkovirina, Kirill Timofeev, Andrey Rubin

Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, 119234 Moscow, Russia
* Fax: +7-495-939-11-15, e-mail: semin@biophys.msu.ru

Concentration and time dependence of Mn extraction from oxygen-evolving complex (OEC) of Ca-depleted Photosystem II membranes by hydroquinone (H_2Q) was studied at different pH. We found that H_2Q extracts 3 Mn cations from OEC at pH 6.5. However, this reductant extracts only 2 cations at pH 7.5 and 5.7 that reflects the higher resistance of Mn cations to the action of H_2Q at such pH's. It is interesting that maximum of oxygen-evolving activity of OEC occurs at pH 6.5 and has minimum at pH 7.5 and 5.7. Thus we suppose that pH dependence of oxygen-evolving activity of OEC correlates with the resistance of some Mn cations of OEC to the reduction by H_2Q. Such correlation can be determined by amino acid residues participating in the coordination of Mn cluster which have pK in this pH region. In fact only histidine residues have pK in region of pH 5.5 – 7.5. According to this suggestion and structural model of Kawakami et al (2011) these histidine residues can be represented by D1-His332 (ligand to Mn(1)) and D1-His337 that forms hydrogen bond with μ3 oxo bridge connecting Mn(1), Mn(2) and Mn(3) in Mn_4 cluster of OEC. Possibly protonation/deprotonation of these histidines determine the pH dependence of Mn cluster stability and activity in the OEC. EPR studies have shown that Mn cation which is not extracted by H_2Q at pH 6.5 is in reduced form in the dark and this cation cannot be displaced by Ca^{2+} cation in high concentration. These results imply that it is Mn cation of natural Mn_4 cluster. Under illumination we didn't observe the oxidation of this Mn cation. We suggest that this effect is determined by low ratio between the Mn(II) oxidation rate and Mn(III) reduction rate due to the presence of PsbO polypeptide.

Poster S3.15

Single molecule spectroscopy on Photosystem II of *Thermosynechoccus elongatus*

Sepideh Skandary[1], M. Hussels[1,*], A. Konrad[1], C. Glöckner[2], E. Schlodder[2], J. Hellmich[2], A. Zouni[3], M. Brecht[1,4]

1 – Universität Tübingen, IPTC and Lisa + Center, Auf der Morgenstelle 18, 72076 Tübingen, Germany
2 – Technische Universität Berlin, Max Volmer Laboratorium, Straße des 17. Juni 135, 10623 Berlin, Germany
3 – Humboldt-Universität zu Berlin, AG Biochemie und Strukturbiologie, Unter den Linden 6, 10099 Berlin.
4 – Zurich University of Applied Science Winterthur (ZHAW), Technikumstrasse 13, 8401 Winterthur, Switzerland
* Tel: +49 (7071)2976180, e-mail: Sepideh.Skandary@uni-tuebingen.de

Photosystem II (PS II) is the membrane protein complex of higher plants, green algae and cyanobacteria that uses solar energy to catalyze the electron transfer from water to plastoquinone [1]. The PS II core complex (PSII cc) is composed of the two intrinsic antenna protein subunits; CP43 and CP47, coordinating 13 *chlorophyll a* (*Chl*) and 16 *Chls*, respectively, the *D1D2cyt b-559* reaction center complex, that coordinates 6 *Chl a* and 2 *pheophytin a* molecules, and several additional small subunits.

The spectral properties and dynamics of fluorescence emission of PS II cc are investigated by single-molecule spectroscopy at 1.6K. The emission spectra are dominated by sharp lines (zero-phonon lines, ZPLs) in the range between 680–705 nm [2]. These lines are the result of weak to intermediate electron-phonon coupling and slow spectral diffusion. For several data-sets, it is possible to surpass the effect of spectral diffusion by applying a shifting algorithm. The increased signal to noise ratio enables us to determine the electron-phonon coupling strength (Huang-Rhys factor) with high precision. The Huang-Rhys factors vary between 0.03 and 0.8 in our selected data-sets.

The single-molecule experiments show unambiguously that different emitters and not only the lowest energy trap contribute to the low temperature emission spectrum [2]. The values of the Huang-Rhys factors show no obvious correlation between coupling strength and wavelength position. The existence of one representative value of the electron-phonon coupling for the different emitting states of PS II cc (e.g. F685, F695) seems to be highly questionable based on our experimental findings.

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Poster S3.16

Function and characterization of Photosystem II extrinsic protein PSBQ′

Masato Yamada[1], Tatsuya Tomo[1,2,*]

1 – Tokyo University of Science, Tokyo, Japan
2 – PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan
* Fax: +81-3-3260-0322, e-mail: tomo@rs.tus.ac.jp

The photosynthetic alga has a highly diverse in species. The extrinsic proteins of Photosystem II (PS II) are also rich in diversity. Cyanobacterial PS II contained three extrinsic proteins, such as PsbO, PsbV, PsbU. However, PS II isolated from *C. caldarium*, which is primitive red alga, contained four extrinsic proteins, PsbO, PsbV, PsbU and PsbQ′. The localization and function of PsbQ′ is not clear at present. To reveal the funtion of PsbQ′ subunit, we performed release-reconstitution experiment of PsbQ′ using thermophylic cyanobacteria *Thermosynechococcus elongates* PS II. PsbQ′ was purified by *E. coli* expression system. The results revealed that PsbQ′ subunit was stoichiometrically reconstituted to cyanobacterial PS II. The difference of oxygen evolving activity between normal cyanobacterial PS II and PsbQ′ reconstituted PS II was almost same. However, some differences were shown in the redox potential of Q_A and thermoluminescence. From these results, we discuss the function of the PsbQ' subunit in the PS II.

Section 4: Energy Transfer and Trapping in Photosystems

Lecture S4.1

TR EPR study of singlet-triplet fission of carotenoid excitation

Ivan Proskuryakov*, Irina Klenina, Zoya Makhneva, Andrey Moskalenko

Institute of Basic Biological Problems, RAS, Pushchino, Moscow region, Russia
* Fax: +7(496)7330532, e-mail: pros@issp.serpukhov.su

Time-resolved electron paramagnetic resonance was used to study the properties of carotenoid triplet states populated under their direct excitation in LH2 light-harvesting complexes of purple phototrophic bacteria *Allochromatium minutissimum*, *Rhodopseudomonas palustris*, *Rhodoblastus acidophilus*, and in dry carotenoid films free of bacteriochlorophyll. The obtained results enable a conclusion that the carotenoid triplet states, both in LH2 complexes and films, are populated in the process of homofission of singlet excitation into two triplets, $^1Car^* \rightarrow {}^3Car + {}^3Car$. The ^3Car spectra measured with minimum (130 ns) delay after excitation flash for LH2 complexes and for carotenoid films are different. In a film, a signal with spin-polarization producing mostly absorption (*A*) of microwaves is observed. Time evolution of the signal indicates that it belongs to [TT] complex, the precursor of triplet Car. The fission process in LH2 complexes leads to predominant population of the T_0 spin sublevel of the triplet giving rise to a characteristic pattern of spectral components in emission (*E*) and absorption, *EAAEEA*. One spin sublevel of ^3Car has an increased probability of intersystem crossing to the ground state, independent of the carotenoid configuration. This property after ca. 1 μs after a flash both in LH2 complexes and in dry carotenoid films leads to the shape of the ^3Car signal observed commonly with cw EPR detection (*EAEAEA* polarization pattern). Currently accepted structure of the light-harvesting LH2 complexes of purple phototrophic bacteria does not provide necessary interaction between the Car molecules for singlet-triplet fission to proceed. Using LH2 complexes from purple bacterium *Allochromatium minutissimum*, a drop in the efficiency of ^3Car generation is demonstrated, which correlates with the extent of selective photooxidation of B850 bacteriochlorophylls. A conclusion is made that singlet-triplet fission of carotenoid excitation in LH2 proceeds with a superexchange-type participation of these bacteriochlorophylls. The possibility of singlet-triplet excitation fission involving a third mediator molecule earlier was not considered.

Lecture S4.2

Energy transfer in plant Light-Harvesting Complex II revealed by room-temperature 2D electronic spectroscopy

Kym L. Wells[1], Petar H. Lambrev[2,*], Zhengyang Zhang[1], Győző Garab[2], Howe-Siang Tan[1]

1 – Nanyang Technological University, Singapore
2 – Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary
* Tel. +36-62-599-600 (492), e-mail lambrev.petar@brc.mta.hu

Two-dimensional electronic spectroscopy (2DES), like the pump-probe absorption spectroscopy, is a valuable tool to monitor the excited state dynamics in systems of coupled chromophores. While both methods can identify molecular excited states based on their spectral properties and population dynamics, only 2DES provides separate spectral information about the donor and acceptor molecules involved in energy transfer. This makes it especially powerful in disentangling the energy transfer network in multichromophore systems such as light-harvesting complexes. Pump-geometry 2DES utilizing phase cycling was performed at room temperature on isolated trimeric plant light-harvesting complex II (LHC II). The time-dependent 2D spectra reveal cross peaks representing energy transfer from Chl *b* to Chl *a* and within the Chl *a* exciton manifold, occuring on time scales from <300 fs to >10 ps. Global lifetimes analysis of the 2DES produced 2D decay-associated spectra (2D DAS) whereby separate Chl *b* pools coupled to the bulk Chl *a* exciton states can be distinguished. These energy transfer components occur with lifetimes in the range of 0.3–3 ps. Rapid (0.3 ps) energy transfer from high-energy Chl *b* exciton states to the lowest-energy Chl *a* exciton states is observed. An indirect energy transfer pathway via an intermediate Chl *a*/*b* exciton state is also clearly resolved in the 2D DAS. This intermediate state is rapidly populated from low-energy Chl *b* states and has a long (several ps) lifetime due to weak coupling to the low-energy Chl *a* excitons. The 2D DAS reveal Chl *a* equilibration on a time scale of few ps. A more detailed kinetic scheme was obtained with the help of spectro-temporal model. The experimentally resolved kinetics scheme generally agrees with and adds further details to published structure-based excitonic models and spectroscopic results.

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Lecture S4.3

Energy-dependent regulation of chloroplast ATP synthase

Alexander N. Malyan

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* e-mail: A_Malyan@issp.serpukhov.su

The energy-dependent regulation of chloroplast ATP synthase is underlain by reversible inactivation of its ATPase activity in response to a decrease in transmembrane electrochemical potential of protons. A stroma redox potential-dependent mode of this regulation consists in reduction-oxidation of the γ-subunit disulfide bond which modulates interaction between the "inhibitory" ε-subunit and the catalytic part of the enzyme. Exposure of this bond to a reducing agent (thioredoxin) increases with increasing transmembrane potential. Another regulatory mechanism common for ATP synthases of different biological origin implicates decreasing transmembrane potential and selective ADP binding to one of the three catalytic subunits, which rules out their cooperative functioning necessary to induce the catalytic activity. In turn, tightness of ADP binding is determined by the ADP/ATP ratio at noncatalytic sites of the enzyme. The communication presents a detailed analysis of the regulatory mechanisms of chloroplast ATP synthase activity.

Lecture S4.4

Photosystem II core complexes from spinach with chemically modified pigment composition

Anatoly Shkuropatov*, Maxim Vishnev, Alexey Zabelin, Valentina Shkuropatova, Mikhail Yanyushin, Vladimir A. Shuvalov

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7-496-733-05-32, e-mail: ashkur@mail.ru

The high degree of spectral overlap of the Q_y optical transitions of chlorophylls (Chl) and pheophytins (Pheo) is a major obstacle in identifying the absorption characteristics and functions of the individual pigment cofactors in isolated Photosystem II reaction centers (PS II RCs) as well as in more intact PS II core complexes that contain the native RC together with the integral antenna proteins CP43 and CP47 and the oxygen-evolving complex. In particular, it is very difficult to determine optical transitions of the pigments located in the photochemically inactive cofactor branch, absorption spectra of which are not subjected to appreciable changes in response to the light-induced electron transfer in the RC. A powerful approach to address these problems is selective replacement of the native chromophores with non-natural pigments followed by analysis of spectral and functional consequences of such modifications. We have changed the pigment composition of spinach core complexes by transforming a part of Pheos and Chls within the core complex into their 13^1-hydroxy derivatives, using the selective chemical reaction of the 13^1-keto C=O group of pigments with $NaBH_4$. Thanks to a small but distinct difference in reactivity to $NaBH_4$ between Pheo *a* and Chl *a*, the modified preparations were obtained in which ~25% of Pheo *a* molecules were replaced by 13^1-deoxo-13^1-hydroxy-Pheo *a*, while the amount of 13^1-deoxo-13^1-hydroxy-Chl *a* was relatively low. Spectral, biochemical, and photochemical properties of modified preparations were compared with those of control core complexes. Under the experimental conditions used, the $NaBH_4$-treatment did not lead to significant dissociation of the PS II core complex protein ensemble. The modified preparations retained the ability to primary charge separation, indicating that only the inactive-branch pheophytin $Pheo_{D2}$ was subjected to chemical modification. On the basis of the data obtained, the Q_x optical transition of the native $Pheo_{D2}$ molecule in core complexes was identified at 543 nm at room temperature; in the Q_y region, $Pheo_{D2}$ most probably dominantly contributes to the exciton absorption band at ~680 nm. We also studied the effects of more extensive $NaBH_4$-treatment of core complexes.

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Poster S4.5

New rotation mechanism of the gamma subunit: Mechano-chemiosmotic model

Eldar A. Kasumov*, Ruslan E. Kasumov, Irina V. Kasumova

Research and Production Centre «KORVET», Moscow region, Russia; 142032, Moscow region, Domodedovo, Chulpanovo village, building 1
* Fax: +7(496)792-96-01, e-mail: kasumov_eldar@mail.ru

ATP is a main intermediate of chemical energy in the living organisms. It is mainly synthesized in H^+-F_1F_o-ATPases by utilizing energy equal to energy of one photon either from oxidation of foods or from light via the process of oxidative- or photo-phosphorylation in energy transducing membranes of mitochondria, chloroplasts and bacteria. We propose a mechano-chemiosmotic theory of electron transfer coupling ATP synthesis and cyclic low amplitude swelling-shrinkage. According to this model, an asymmetric contact between dimers of opposite b_6f (or bc_1) complexes inside a thylakoid (or intracristae space) is formed during shrinkage of organelles, which is a mechanical regulator of electron transfer from [2Fe-2S] cluster to heme f or c_1.

The structure of ATP synthase complexes undergoes several changes during their functioning. The details of the molecular mechanism of ATP synthesis are considered in the cyclic shrinkage-swelling. On the basis of structural and other data it is proved that ATP synthase is a pump(Ca^{2+}/K^+, Cl^-)pore–enzyme complex, in which γε-subunits rotate to 360° step by step 30° (30° and 90° rotations of the γε-subunit complex are associated with opening and closing of gate in channels – α-helical bundles of the α- and β-subunits (DELSEED bead) both at synthesis, and at hydrolysis) owing to phosphorylation of positive charged amino acid residues of the N-terminal γ-subunit in electric field. The coiled-coil b_2 subunits are the ropes shortening by phosphorylation of positive charged lysines or arginines and attract the $\alpha_3\beta_3$-hexamer to the membrane in energization. ATP is synthesized during of γε-subunits rotation a back by the destabilization of the phosphorylated b_2 subunits and C- and N-terminal positive residues of γ-subunit under action of Ca^{2+} ions, which pumped over from storage – thylakoid (or intermembrane space) in swelling.

In this model OH^- is transferred into stroma (or matrix) and H^+ slowly (about 8 msec) into thylakoids (or into cristae) during energization (a polarization of membrane , a reduction of cyt f (c_1) and a shrinkage of thylakoids occur). OH^- is exchanged to $H_2PO_4^-$ of outer medium. Because of it regular pH changes of stroma and intra thylakoid space must be registred. In the presence of ADP occur: a synthesis of ATP, a depolarization of membrane, a fast pH jump to about 7, a fast swelling of thylakoids (1 msec), a rapid oxidation of cyt f. Such, the main energy is consumed for the synthesis of ATP, for delivery of phosphate ions in the hexamer with help C-terminal α-helix of γ-subunit as on a lift against the energy barrier, a formation of phosphoryl groups and the release of ATP molecules, synthesized from the hexamer.

Poster S4.6

Effect of narrow-band red and blue light on energy-dependent processes in chloroplasts of barley seedlings

Olga Avercheva*, Elizaveta Bassarskaya, Tatiana Zhigalova

Department of Plant Physiology, Faculty of Biology, Lomonosov Moscow State University, Leninskie gory 1-12, Moscow 119991, Russia
* e-mail: olga.avercheva@gmail.com

Light spectral quality is one of the factors that determine the development and function of different processes in the plant, including photosynthesis. In our previous works we have shown that among the photosynthetic processes studied, the most strongly affected by light quality are energy-dependent processes – non-photochemical chlorophyll fluorescence quenching and photophosphorylation rate.

We studied the functioning of the photosynthetic apparatus in 9-day-old barley (*Hordeum vulgare* L.) seedlings gown with 70 μmol PAR/(m^2·s) and LED light sources with narrow-band light of different spectrum: red (emission maximum 660 nm) and blue (450 nm). Plants grown with fluorescent lamps were used as a control. The main focus of our study were parameters connected to energy accumulation and dissipation on the thylakoid membrane – steady-state proton gradient parameters (assessed with DUAL-PAM-100), electron transfer and photophosphorylation rate (measured in isolated chloroplasts), physico-chemical and functional parameters of isolated CF1 complexes.

Electron transport rate in plants grown with blue light was higher than in control plants and plants grown with red light. Photophosphorylation rate differed drastically in all plants studied. In plants grown with blue light it was 1.5–2-fold higher than in control plants, in plants grown with red light – about 2-fold lower. The Δψ component of the proton motive force was nearly the same in plants grown with red and blue light and higher than in control plants. The ΔpH component was lower than in control plants in both cases. In plants grown with blue light, the decrease in ΔpH was probably due to higher photophosphorylation rate. In the case with red light-grown plants, the reason for this may be higher membrane permeability to protons or different coupling factor structural and functional state. We discuss possible effects of light quality on physico-chemical properties and function of the ATP-synthase.

Poster S4.7

Double pigment exchange approach to investigate antenna processes in LH1 antenna from *Rhodospirillum rubrum*

Maciej Michalik[1], Heriyanto[1,4], Bożena Boroń[1,3], Anna Susz[1,2], Leszek Fiedor[1,*]

1 – Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387, Kraków, Poland
2 – Faculty of Chemistry, Jagiellonian University, Kraków, Poland
3 – Chełkowski Institute of Physics, University of Silesia, Katowice, Poland
4 – Ma Chung Research Center for Photosynthetic Pigments, Ma Chung University, Malang, East Java, Indonesia
* Fax: +48126646902, e-mail: leszek.fiedor@uj.edu.pl

The photosynthetic unit of purple bacteria is among the simplest found in nature. In *Rhodospirillum rubrum* it consists of a charge-separating reaction center surrounded by a ring-shaped LH1 antenna. LH1 is a transmembrane complex composed of 16 α–β polypeptide subunits, each hosting two bacteriochlorophyll *a* molecules and one spirilloxanthin molecule. Reaction center performs the charge separation across the photosynthetic membrane, which is a starting point of a series of redox reactions necessary for efficient conversion of light energy. In effect, the photosynthetic unit is capable of converting photons to electrochemical energy with quantum yield approaching 100%.

The modular structure of LH1 allows for its *in vitro* reconstitution from detergent dissociated subunits, and a replacement of both of its cofactors by modified bacteriochlorophylls and/or non-native carotenoids. The ground-state properties of Ni-bacteriochlorophyll *a* resemble those of the native pigment, but its excited-state lifetime is dramatically shortened. Its presence in LH1 causes an ultrafast excitation quenching and leads to fluorescence decay correlated to the extent of pigment exchange. The substitution of native spirilloxanthin with spheroidene increases overall efficiency of intracomplex singlet energy transfer, and shifts the light absorption by LH1 in the blue-green spectral region. Such modifications combined with a detailed spectroscopic analysis constitute a convenient tool for studying the mechanisms of assembly and functioning of photosynthetic antenna.

Poster S4.8

Energy transfer in *Anabaena variabilis* filaments during heterocyst differentiation studied by time-resolved fluorescence

Aya Onishi[1,*], Shimpei Aikawa[2,3], Akihiko Kondo[2,3], Seiji Akimoto[1,3,4]

1 – Graduate School of Science, Kobe University, Kobe 657-8501, Japan
2 – Graduate School of Engineering, Kobe University, Kobe 657-8501, Japan
3 – Japan Science and Technology Agency, CREST, Tokyo 102-0075, Japan
4 – Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan
* Fax: +81-78-803-5075, e-mail: 143s208s@stu.kobe-u.ac.jp

Anabaena and many other filamentous cyanobacteria differentiate heterocysts under nitrogen-depleted conditions; phycobiliproteins (PBPs) are degraded during differentiation and heterocysts lack photosystem II (PS II). Therefore, it can be expected that excitation energy-transfer processes also change. To investigate changes in energy-transfer processes of *Anabaena variabilis* cells grown in the standard medium (BG11) and a nitrogen-free medium ($BG11_0$), we measured absorption and fluorescence spectra at 77K. Time-resolved fluorescence spectra (TRFS) were measured with a picosecond time-correlated single photon counting system. In the cells grown in $BG11_0$, the relative amount of PBP to PS I and that of PS II to PS I decreased with the cultivation period, and energy transfer from PBP occurred faster than the cells grown in BG11. In addition, fluorescence kinetics of PS I changed due to the nitrogen starvation; the fluorescence maximum of TRFS just after excitation was located at 735 nm, 740 nm, and 730 nm after the 4-days, 8-days, and 15-days cultivation, respectively. We will discuss energy-transfer processes of *A. variabilis* under the nitrogen-depleted condition.

Poster S4.9

The amine uncoupling of energy transformation in thylakoids of pea chloroplasts

Vera Opanasenko*, Ilya Naydov

Institute of Basic Biological Problems, Russian Academy of Sciences,
Institutskaya str., 2, Pushchino, Moscow region, 142290 Russia
* e-mail: opanasenko45@mail.ru; vera4.opanas@gmail.com

The action of ammonia, methylamine (MA) and imidazole on ATP synthesis with methyl viologen and basal electron transfer from water to ferricyanide were investigated in the absence or in the presence of Palmitic Acid (PA). Low concentrations of these amines have stimulated the synthesis of ATP without PA but inhibited it in the presence of 40 μM PA. In the 7–8 times higher cation concentration of MA than ammonium or imidazole were needed to cause uncoupling.

Dependences of the rate of electron transport on amine concentration were represented as two-three phase curves. Changing the pH of the reaction medium has led to changes in the curves of the simulation. These changes show that the activation of the uncoupling was caused by the increase in the concentrations of amine cations within the lumen. Effect of low concentrations of amine sharply increased in the presence of 40 μM PA.

Induction of nonspecific pores was detected using laser scanning confocal microscopy. In the presence of 2–10 mM MA, there was an increase in the permeability of the thylakoid membrane to sulforhodamine B (a fluorescent dye). Changes of thylakoid system were evaluated on the images of the distribution of chlorophyll fluorescence. It has been shown that at full electron transport uncoupling by amines, thylakoids do not swell, while sulforhodamine B really did in the lumen in the presence of the MA.

It is presumed that phases of amine uncoupling correspond to induction of two unknown PA-stimulated transporters (channels) and nonspecific mechano-sensitive pore. The cations of ammonium and imidazole have in the 7–8 times higher affinity then MA to the gate of the cannels. Under illumination the luminal concentration of MA is enough to induce the pore that can be activated by the increase in the osmotic pressure inside the thylakoids.

Poster S4.10

The induction of chlorophyll *a* fluorescence in cyanobacteria in the absence and in the presence of exogenously added singlet oxygen scavengers

Kostas Stamatakis and George C. Papageorgiou*

Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi Attikis, Greece
* Fax: +30 2106511767, e-mail: gcpap@bio.demokritos.gr

The fluctuations of chlorophyll *a* (Chl *a*) fluorescence that a dark-adapted photosynthetic specimen emits upon continuous illumination reflect the interplay of various photosynthesis-related sub-processes that impact on the photosystem II (PS II) population of excited Chls *a*. The full time trace (or fluorescence induction, FI) of Chl *a* fluorescence (F_{Chla}) consists of two transients: The fast transient (OJIPS) that lasts a few seconds, and the slower transient (SMT) that lasts several minutes. It is generally assumed that after a sufficiently long dark adaptation (e.g., 10 min) the photosynthetic speciment resets to its initial condition and the FI trace can be is reproduced. In this research we question this assumption, considering the very high light intensity (~3 mmol (photons)·m^{-2}·s^{-1}) modern commercial kinetic fluorometers employ in order to excite F_{Chla}. At strong excitation, the PS II repair process is inhibited and photo-oxidative damage does accumulate in PS II (reviewed e.g., in [1]).

In these preliminary experiments, we subjected cyanobacterium *Synechococcus sp.* PCC7942 to consecutive dark-light cycles, both in the absence and in the presence of exogenously added scavengers of singlet O_2 (β-carotene and α-tocopherol) in order to test the reproducibility of the O(JI)PSMT trace of F_{Chla}. Results show that photodamage, which is not repaired by a subsequent 10 min dark rest. Occurs already in the first 10 ms of illumination (i.e., during the OJIP phase of FI). The extent of the photodamage depends on the light dose (intensity × time) and it is mitigated in the presence of singlet O_2 scavengers.

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Poster S4.11

H_2O (D_2O) in purple bacteria reaction center as a modulator of pump-probe kinetics due to spin-conversion

S. M. Pershin, R. Y. Pishchalnikov

Wave Research Center, Prokhorov General Physics Institute, Russian Academy of Science, 38 Vavilov Street, Moscow 119991, Russia,
* e-mail: pershin@kapella.gpi.ru, rpishchal@kapella.gpi.ru

It known that the reaction center from purple bacteria contains the two H_2O monomers. These monomers locate inside the cavity which size is more than enough to free molecules rotation. Shuvalov et al. [1] has observed by pump-probe technique the modulations of reaction center fluorescence and interpreted its as manifestation of H_2O rotation. It is not clear so far why only one-two rotational modes among hundred others have been involved into electron transfer process and discussion. We have proposed the mechanism of coherent modulations of the P* state in the transient absorption spectra of the reaction center isolated from purple bacteria. Two water molecules, located between special pair, B_a, B_b chlorophylls and histidine L173 and M202, are supposed to be the ortho-H_2O and para-H_2O isomers or both identical. It should be noted that the H_2O spin-isomers have the strong quantum differences: ortho-H_2O has magnetic moment and always rotates and in opposite, para-H_2O has no magnetic moment and some of them does not rotate. Ortho-para conversion of H_2O spin-isomers displays by generation or disappearance of the local magnetic field and occurs due to rotational relaxation and in mixed quantum states [2].

The distinctive modulation frequencies we are labeled as the specifically rotational resonances of the ortho-para H_2O when the ortho-para conversion is occured. According to our assumption, the interaction of rotational modes of water isomers and magnetic field scintillation with the charge transfer states is a reason of coherent modulations of kinetics. Note here that the coherent mode of kinetics due to simultaneous exciting by femtosecond pump pulse both H_2O monomers and reaction centers. So its relaxation processes start synchronously. We have modified the system Hamiltonian in order to take into account the rotational modes of ortho-para H_2O. Evolution of the density matrix was calculated in the Liouville space. The relaxation Redfield theory for molecular aggregates was used to model kinetics up to 3 ps. The coincidence of the kinetic calculated curve with the experimental data (Shuvalov et al.) is better than expected.

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Poster S4.12

Molecular mechanism of phycobilisome photoprotection against highly excess light

Pavel Krasilnikov[1], Dmitryi Zlenko[1], Igor Stadnichuk[2,*]

1 – M.V. Lomonosov Moscow State University, Moscow, Russia
2 – A.N. Bakh Institute of Biochemistry RAS, Moscow, Russia
* Fax +7-495-954-27-32, e-mail: stadnichuk@mail.ru

Phycobilisomes (PBSs) function as the antenna of cyanobacterial photosynthetic apparatus. These gigantic particles of about 5000 kDa are the pigment-protein complexes composed of hundreds polypeptide and covalently bound phycobiline chromophore molecules. Excess light absorbed by PBSs is dangerous for cyanobacterial cell due to formation of active oxygen species. In 2004, quite a new photoprotective mechanism of light dissipation into heat known as non-photochemical fluorescence quenching (NPQ) was found in cyanobacterial PBS [1]. Cyanobacteria are the oldest oxygenic photosynthetics which evolutional age is equal to 2.5 Ga; the described effect is therefore the oldest one form of photobiological cell defense. It was demonstrated that the NPQ of cyanobacteria is caused by the chromophorylated orange carotenoid-protein (OCP) [2]. It is known that the OCP simultaneously plays the rolesn of photosensor of blue-green light and an effector of this carotenoid-dependent form of NPQ. In our studies [3], the OCP docking site within the PBS was established by computer docking and molecular dynamic calculations with the use of the crystal structures of OCP and allophycocyanin-composed PBS core. It was shown that the core-membrane polypeptide linker, L_{CM}, besides its central role in excitation energy transfer from the PBS to chlorophyll and as a necessary structural component in settling down of fully assembled PBS to the thylakoid membrane, appears to be the main participant of OCP binding in the photoprotective excitation quenching inside the PBS. The direct interaction of OCP with the L_{CM} was supported by steady state fluorescence spectra studies and fluorescence lifetime measurements. The obtained 3D model of OCP and the PBS interaction allowed us to determine the separation between the carotenoid chromophore of the OCP and the adjust phycobiline chromophore of LCM and their transition dipole moments. Quantum-chemical calculations demonstrate that the L_{CM}-OCP interaction is realized due to the forbidden S_1 state of the OCP-carotenoid within the model of the weak exciton coupling.

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Poster S4.13

Light adaptation of the primitive red alga *Cyanidioschyzon merolae*, proved by time-resolved fluorescence spectroscopy

Yoshifumi Ueno[1,*], Shimpei Aikawa[2,3], Akihiko Kondo[2,3], Seiji Akimoto[1,3,4]

1 – Graduate School of Science, Kobe University, Kobe 675-8501, Japan
2 – Graduate School of Engineering, Kobe University, Kobe 675-8501, Japan
3 – Japan Science and Technology Agency, CREST, Kobe 675-8501, Japan
4 – Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan
* Fax: +81-78-503-5705, e-mail: 147s206s@stu.kobe-u.ac.jp

Photosynthetic organisms adapt to light conditions in various mechanisms. For example, cyanobacteria change the quantity and/or quality of their antenna protein-pigment complexes and energy-transfer processes among antenna complexes and photosystems (PSs) in response to light conditions. *Cyanidioschyzon merolae* is one of the most primordial eukaryotic photosynthetic organisms and its pigment composition is near to those of cyanobacteria; *C. merolae* does not have phycoerythrin. In the present study, we analyzed differences in energy-transfer processes in *C. merolae* cells grown under different light qualities by the picosecond time-resolved fluorescence spectroscopy. As cultivation light, we used blue, green, yellow, red, and white light. White light is used as contol. Blue-light grown cells and green-light grown cells increased the relative amount of their antenna protein-pigment complexes. In green-light grown cells and yellow-light grown cells, the contribution of the energy transfer between PS II and PS I became greater, compared with the control cells. Differences in pigment composition and energy transfer under different cultivation light conditions are discussed.

Section 5: Photosystem I and Bacterial Photosynthesis

Lecture S5.1

Light harvesting in photosynthetic bacteria acclimated to different levels of light

Arvi Freiberg[1,2,*], Kõu Timpmann[1], Manoop Chenchilian[1], Erko Jalviste[1], John A. Timney[3], C. Neil Hunter[3]

1 – Institute of Physics, University of Tartu, Tartu, Estonia
2 – Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia
3 –Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK
* e-mail: arvi.freiberg@ut.ee

We have studied the excitation intensity dependent spectral and kinetic fluorescence response of the chromatophore membranes from *Rhodobacter sphaeroides*, grown under high and low light conditions. The much-expanded (over 8 orders of magnitude) coverage of excitation intensity allowed us to follow the photosynthesis changing from the active to the saturated state. As the low light adapted membrane has more peripheral LH2 complexes, the trapping of the absorbed energy by the reaction centers is delayed owing to an elongated energy transfer path; in the high light adapted membrane sample with lower number of peripheral antennas, the trapping was found to be much faster. By incorporating a kinetic model, we obtained the microscopic rate constants that characterize the energy transfer and trapping process inside the photosynthetic unit as well as the dependence of trapping efficiency on the LH2 to LH1 ratio and on the wavelength of the excitation light. A high efficiency above 80% was observed in most samples and conditions. Within the near infrared absorption band of bacteria the efficiency decreases toward shorter excitation wavelengths; at fixed excitation wavelengths the efficiency declines in correlation with the LH2/LH1 ratio. Our data also detect a small number of disconnected LH2 complexes, particularly in high light adapted samples. The slower energy transfer in low light membranes is offset by having larger peripheral LH2 antenna network, which from the perspective of their ecological habitat facilitates life under low light environments. Moreover, the similarity of the calculated efficiencies for all samples implies a robust mechanism for the primary processes of charge separation in photosynthesis.

Lecture S5.2

The size and amount of photosynthetic unit in purple bacteria

Vadim Selyanin, Michal Kobližek, Dzmitry Hauruseu

Institute of Microbiology, Academy of Sciences, Třeboň, Czech Republic
* Tel: +420-776694400, e-mail: selyaninvv@gmail.com

The photosynthetic apparatus of purple bacteria consists of two types of pigment-protein complexes: the reaction centers and the light-harvesting complexes. The main function of the light-harvesting complexes is to gather light energy and to transfer this energy to the reaction centers for the photo-induced redox processes. The aim of our study was to determine and compare the size of photosynthetic unit in aerobic anoxygenic phototrophs and purple non-sulfur bacteria. We measured the BChl/BPhe ratio in species of bacteria: *Rhodobacter sphaeroides* mutant, *Rhodospirillum rubrum*, *Congregibacter litoralis*, *Roseobacter litoralis*, *Roseococcus sp.*, *Hoeflea phototrophica*, *Rhodovulum sulfidophilum*, *Rubrivivax gelatinosus*, *Roseateles depolymerans*, *Erythrobacter sp.* and purple non-sulfur bacteria *Rhodopseudomonas palustris*. From the ratio of the peak areas of BChl and BPhe in the HLPC chromatograms was calculated the ratio in which these pigments were presented in bacteria. Following results were obtained:

Purple non-sulfur bacteria and aerobic anoxygenic phototrophs contain integral membrane core antenna (LH1) and accessory antenna (LH2) complexes. They are present in different ratios. LH2 are more typical for purple non-sulfur bacteria. LH1 complex consists of 35.2±1.35 molecules BChl *a*.

Anaerobic anoxygenic phototrophs contain 10–100 less photosynthetic complexes per cell when compared to purple non-sulfur bacteria.

In purple non-sulfur bacteria, grown in semiaerobic (typical) condition, there is higher amount (5–10 times) of photosynthetic complexes. In anaerobic anoxygenic phototrophs was not found large difference in concentration between cells, grown in different O_2 conditions.

Poster S5.3

Mechanisms of the Mehler reaction in Photosystem I: Clarification of the role of phylloquinone and ferredoxin

Marina Kozuleva[1,*], Anastasia Petrova[2], Mahir Mamedov[2], Alexey Semenov[2], Boris Ivanov[1]

1 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
2 – Belozersky Institute of Physical-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
* Fax: +7(496)7330532, e-mail: kozuleva@gmail.com

Mehler reaction is a reduction of O_2 by electron carriers of photosynthetic electron transfer chain under light occurring along with $NADP^+$ photoreduction. Electron flow to O_2 diminishes over-reduction of the chain under limited NADPH utilization preventing photoinhibition. Mechanisms of the Mehler reaction are still unknown. Redox cofactors of Photosystem I (PS I) as well as ferredoxin, an acceptor of electrons from PS I, were usually proposed to be major sites of the reaction. The aim of the work was to elucidate the role of ferredoxin and phylloquinone, a quinone cofactor of PS I acting as an intermediate electron acceptor, in O_2 reduction under steady-state illumination.

Using isolated thylakoid membranes from pea (*Pisum sativum*) with added ferredoxin, involvement of both ferredoxin and membrane-bound carriers in O_2 photoreduction was shown. Addition of $NADP^+$ led to a drastic decrease of the share of ferredoxin involvement.

Using isolated PS I complexes from wild type *Synechocystis sp.* PCC 6803 and a mutant strain with blocked phylloquinone biosynthesis (*menB* mutant), in which plastoquinone occupies the quinone-binding site, the involvement of quinone of PS I in O_2 reduction under steady-state illumination was shown. Complexes from wild type and *menB* mutant revealed different character of dependence of O_2 reduction rate on light intensity.

These results are interpreted as evidence of negligible role of ferredoxin and essential role of phylloquinone in the Mehler reaction *in vivo*.

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Poster S5.4

Replacement of the Tyr50 stacked on the *si*-face of the isoalloxazine ring of FAD in *Bacillus subtilis* ferredoxin-$NADP^+$ oxidoreductase modulates its redox properties

Daisuke Seo[*], Erika Nishimura, Hiroshi Naito, Takeshi Sakurai

Division of Material Science, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192, Japan
* Fax: +81(76)2645683, e-mail: dseo@se.kanazawa-u.ac.jp

Ferredoxin-NAD(P)$^+$ oxidoreductases (FNRs, [EC 1.18.1.2], [EC 1.18.1.3]) from the green sulfur bacterium *Chlorobaculum tepidum* (*Ct*FNR) and the low-GC gram-positive bacterium *Bacillus subtilis* (*Bs*FNR) are homologous to the bacterial NADPH-thioredoxin reductase but conserve the two unique aromatic residues stacked on the *si*- and *re*-face of the isoalloxazine ring moiety of the FAD prosthetic group at a distance of 3.5 Å. Similar configuration involving two aromatic residues on the *si*- and *re*-face of the isoalloxazinering moiety have also been found among plastid-type FNR and its relatives. To investigate the role of the *si*-face Tyr50 residue in *Bs*FNR, we performed mutational analysis.

Tyr50 mutants replaced to Trp, Ser and Gly (Y50W, Y50S, Y50G) were successfully expressed in *E. coli* cells and purified to homogeneity, but the preparation of Tyr50Phe mutant was failed. Y50W mutant displayed ~20% NADPH diaphorase activity of wild type level, whereas the activities of Y50G and Y50S mutants drastically decreased to less than 2%. Fd reduction activity of Y50W mutant remained ~14% of that of wild type, but those for Y50G and Y50S mutants decreased to less than 1%. Stopped-flow studies indicated that both Y50G and Y50S mutants as well as Y50W mutant could accept electrons from reduced *Bs*Fd. Redox potentials of these three mutants increased ~50 mV against wild type, which would be one of the reason for the decrease of Fd reduction activity.

Section 6: Artificial Photosynthesis for Hydrogen and Carbon-based Solar Fuels

Lecture S6.1

Manganese oxides as biomimetic water-oxidation catalysts

Ivelina Zaharieva* and Holger Dau

Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany
* Fax: +49-30-8385-6510, e-mail: ivelina.zaharieva@fu-berlin.de

During oxygenic photosynthesis, water is split into protons, electrons (reducing equivalents) and molecular oxygen. The electrons and protons are ultimately needed to reduce CO_2 to carbohydrates; the released protons contribute to formation of the transmembrane proton gradient which drives ATP synthesis. The process of water oxidation also is the anodic process in water electrolysis and is a key reaction in the transition from an energy economy based on fossil fuels to one based on renewable energy sources. However, efficient water oxidation in non-biological systems is kinetically sluggish. Currently there are no efficient catalysts which do not contain precious elements.

In plants and cyanobacteria, the reaction is catalyzed by a Mn_4CaO_5 cluster located in the interior of Photosystem II. The efficiency of the reaction catalyzed by this metal-oxo cluster is high enough to let water oxidation shape the atmosphere on our planet by producing molecular O_2.

This report summarizes recent efforts in developing artificial Mn(Ca)-oxide catalysts for water oxidation. These are amorphous (non-crystalline) and purely inorganic oxide materials able to catalyze water oxidation in the presence of a chemical oxidant or in electrochemical systems. Different synthetic approaches ranging from use of sophisticated molecular precursors to simple water soluble manganese salts and from complicated chemical synthesis routes to one-step electrochemical protocols are compared. Structure of the active catalysts at the atomic level as revealed by X-ray absorption spectroscopy is elucidated and essential structural motifs are identified. Structure-function relations and mechanistic insights as well as surprising similarities to the native paragon, the Mn_4CaO_5 cluster in Photosystem II, are discussed.

Poster S6.2

Oxygen evolution by manganese complexes in the presence of chemical oxidants

Mohammad Mahdi Najafpour[1,2,*], Atefeh Nemati Moghaddam[1], Małgorzata Hołyńska[3], Sayed Habib Kazemi[1,2], Amir Nasser Shamkhali[4], Emad Amini[1], Mostafa Ghaemmaghami[1], Davood Jafarian Sedigh[1], Rahim Mohamadi[1], Sasan Zaynalpoor[1], Katrin Beckmann[5] and Warwick Hillier

1 – Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan 45137-66731, Iran
2 – Center of Climate Change and Global Warming, Institute for Advanced Studies in Basic Sciences (IASBS), Iran
3 – Fachbereich Chemie and Wissenschaftliches Zentrum für Materialwissenschaften (WZMW), Philipps-Universität Marburg, Hans-Meerwein-Straße, D-35032 Marburg, Germany
4 – Department of Applied Chemistry, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, P. O. Box: 56199-11367, Iran
5 – Research School of Biology, Canberra, ACT 0200, Australia
* Tel.: +98-241-4153201, Fax.: +98-241-4153232,
e-mail: mmnajafpour@iasbs.ac.ir

Water oxidation is bottleneck for water splitting into H_2 and O_2. Thus, Mn compounds as efficient, cheap and environmentally friendly water oxidizing compound were proposed as water oxidation in artificial photosynthetic systems. A Mn compound is also used by Nature for water oxidation. Here, eighteen Mn complexes with N-donor and carboxylate ligands have been synthesized and characterized. The reactions of oxygen evolution in the presence of oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) and cerium(IV) ammonium nitrate catalyzed by these complexes are studied and characterized by UV-visible spectroscopy, Fourier transform infrared spectroscopy, electron paramagnetic resonance spectroscopy, and electrochemistry. Some of these complexes evolve oxygen in the presence of oxone as a primary oxidant. CO_2 and MnO_4^- are other products of these reactions. Proposed mechanisms for oxygen evolution are also discussed.

Poster S6.3 *(in absentia)*

Magnesium deprivation as an alternative approach to sustain hydrogen photoproduction in green microalgae

A. A. Volgusheva*, T. K. Antal, G. P. Kukarskikh, O. G. Lavrukhina, T. E. Krendeleva

Lomonosov Moscow State University, Moscow, Russia
* Fax: +7-495-939-11-15, e-mail: volgusheva_alena@mail.ru

Hydrogen is considered as a promising clean fuel in future because its oxidation by molecular oxygen releases a lot of energy with water as the only by-product. Some green algae can use solar energy to perform water biophotolysis that involves photosynthetic electron transport and activity of the specific enzymes [Fe,Fe]-hydrogenases. The activity of algal hydrogenases is strongly repressed by molecular oxygen, therefore a temporal separation of a photosynthetic (aerobic) stage of oxygen production from hydrogen synthesis (anaerobic) stage is necessary to induce hydrogen evolution. Depletion of sulfur is a widely used approach to alter algal metabolism in a way that sustains hydrogen production under constant illumination.

Here we report that Mg-deprived *C. reinhardtii* cells may produce essential amounts of hydrogen. Thus, maximum rate of H_2 evolution calculated on the Chl basis was about 17 μmoles $H_2 \cdot (mg\ Chl)^{-1} \cdot h^{-1}$ that exceeds more than two times the rates usually attained in S-deprived cells of wild type. Partly this effect of Mg deficiency is explained by Chl bleaching in the absence of Mg which is an important component of the Chl molecule. The bleaching is absent in sulfur deprived cells. Study the mechanisms of hydrogen production upon Mg deficiency revealed some similarities with S-deprived cells, including: 1) decrease in PS II photochemistry and increase in the content of Q_B-non-reducing centers; and 2) starch accumulation during aerobic phase and its degradation during H_2 production stage. Similar to S-deprived cells, in Mg-deprived cells DCMU, an inhibitor of PS II, suppressed H_2 photoproduction by about 80%, suggesting a major involvement of PS II as a primary electron donor to the hydrogenase.

During cultivation in the bioreactor under Mg-deficiency, the establishment of anaerobic conditions occurred when PS II activity was about 70% from the initial level, whereas much stronger PS II inactivation is observed in S-deprived cells at this stage. This result indicates that the balance between photosynthetic oxygen production and respiration is essentially modified in Mg-deprived cells that allows to maintain hydrogen photoproduction at the relatively high PS II activity. Remaining high PS II activity during anaerobic phase can be responsible for high hydrogen output in Mg-deprived cells. Low Chl content provides high efficiency of light energy conversion.

Poster S6.4 *(in absentia)*

Towards design rules for building photosynthetic systems

Neal Woodbury

Center for Innovation in Medicine, The Biodesign Institute, Arizona State University, USA
* e-mail: nwoodbury@asu.edu

A great deal is now understood about how light powers the photosynthetic apparatus and provides energy for subsequent reactions. In recent years, we have begun to explore the possibility of assembling complexes of photosynthetic components and enzymes based on man-made scaffolds organizing biological components. This has involved incorporating artificial light harvesting components into reaction centers, attaching these to DNA nanostructures and creating multi-enzyme systems for using redox energy to process molecules. Several of these projects will be described and the possible future of this kind of directed assembly will be explored.

(Presented by Neal Woodbury in collaboration with the laboratories of Hao Yan, Dmitry Matyushov and Nils Walter)

Section 7: Regulation of Photosynthesis and Environmental Stress

Lecture S7.1

Mechanisms of regulation of the electron transfer in the primary processes of photosynthesis

Andrey B. Rubin

Faculty of Biology, Moscow State University, Moscow, Russia
* e-mail: rubin@biophys.msu.ru

Lecture S7.2

Functional transition history of chloroplasts along leaf life span in *Arabidopsis* coordinated by multi-layered regulatory networks

Hye Ryun Woo[1], Daehee Hwang[1,2], Hong Gil Nam[1,2], Pyung Ok Lim[1], and collaborators

1 – Department of New Biology, DGIST
2 – Center for Plant Aging Research, IBS, Korea

Leaves harvest light energy and fix CO_2, being the only practical source of foods on earth. Leaves, during their lifespan, undergo developmental and physiological shifts. At the very initial stage, they form the photosynthetic machinery by utilizing energy and material sources from the other parts of plants. Shortly after the initial stage, leaf can harvest energy and store the energy in various forms in leaves. At a later stage, leaf exports the energy and material harvested during the growth season to the other parts such as seeds and dies. Thus, leaf is an effective energy system with energy harvest, storage, and export ability, and has to coordinate its age-dependent functional transition.

Here, we used strand-specific total RNA-seq and smRNA-seq to understand the structure and dynamics of multi-dimensional (RNA type, organellar, and time dimensions) transcriptomes of *Arabidopsis* leaf during the entire lifespan. Integrative analysis revealed temporal coordination of age-dependent expression patterns of various types of regulators and identified dynamic multi-layered regulatory networks formed by these regulators. Major new findings include the followings. 1) Coordinated transition of chloroplast function is a key constituent in leaf lifespan programs. 2) Half of the transcriptome at the mature stage leaf came from chloroplast genome and showed the most dramatic change across leaf lifespan. In contrast, the nuclear and mitochondrial transcriptomes were relatively constant till death. 3) Expression of nuclear- and chloroplast-encoded genes for photosynthesis complex and chloroplast ribosomal complexes were tightly coordinated, implying that plants utilize coordinated transcriptional programs as a means of inter-organellar communication between nucleus and chloroplasts. 4) Coordination of gene expression between chloroplast and nuclear genomes is tightly controlled by multiple layers of regulations at the transcriptional as well as post-transcriptional regulations. 5) The constituents of the photosynthetic complex and their regulatory modes are constantly changing along the life span.

Lecture S7.3

The superoxide radical produced in chloroplast thylakoids in the light is reduced in the plastoquinone pool

Boris Ivanov*, Sergey Khorobrykh, Maria Borisova-Mubarakshina, Marina Kozuleva

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7(4967)330532, e-mail: ivboni@rambler.ru

Using the ESR detectors of different lipophilicity, the production of superoxide radical in the course of photosynthetic electron transport was shown to take place not only at outer surface of thylakoid membrane but also within the membrane, and just intramembrane production increased with increase in light intensity. The study of oxygen reduction in the isolated PS I complexes revealed that increase of superoxide radical production with increase in light intensity occurred owing to the processes in this photosystem. It was found that some superoxide radicals being generated due to O_2 molecules reduction in the thylakoids in the light did not appear outside the thylakoid membrane, and their amount increased with increase in the actinic light intensity. At the same time, an increase in hydrogen peroxide production in the thylakoids with increase in light intensity was mostly due to H_2O_2 formation within the thylakoid membrane. The calculated relative flow of electrons participating in such H_2O_2 formation also increased, reaching 60% of all electrons, which reduced oxygen up to hydrogen peroxide. The PQ-pool contribution to a total oxygen reduction depended on light intensity in the same way as the production of intramembrane hydrogen peroxide. The characteristics of the first rapid phase of the PQ-pool oxidation after thylakoid illumination, namely its contribution to a total size of fully oxidized pool as well as the apparent rate constant both correlated with the rate of superoxide radical production in the light.

All data imply the proceeding of reaction of superoxide radical with plastohydroquinone within the thylakoid membrane and the production of H_2O_2 there due to this reaction. In particular, PQ-pool oxidation after illumination can be simulated, only including this reaction. The reaction is very thermodynamically favorable owing to the large difference between the E_{m7} values of the redox pairs $PQ^{\bullet-}/PQH_2$ and $O_2^{\bullet-}/H_2O_2$; the calculated equilibrium constant is higher than 10^9. The vigorous reaction of superoxide radical with reduced quinone was shown in octanol media.

The reaction of superoxide radical with plastohydroquinone within the thylakoid membrane both scavenges reactive oxygen species capable to initiate reaction of lipid peroxidation and produces H_2O_2 molecules signaling about redox state of PQ-pool.

Lecture S7.4

Comparison of dendrograms for electron transporting chains components with the common phylogeny of prokaryotes as an aproach to the problem of the origin and the evolution of photosynthesis and respiration

Mikhail F. Yanyushin

Institute of Basic Biological Problems, Russian Academy of Science, Institutskaya 2, 142290 Pushchino, Russia
* e-mail: yanyushin@yandex.ru

During the progress of life different proteins emerged at different times. It seems possible to find out the point of the emergence of a protein and its subsequent evolution comparing the dendrogram for it with total phylogeny based on the dendrogram for universal molecular clocks. Here, concatenated amino acid sequences of nine universal proteins not prone to lateral gene transfer are used to construct a tree for a representative set of prokaryotes.

Complexes that oxidize quinols and reduce periplasmic mobile electron transporters take central positions in electron transporting chains. There are two types of these complexes: the well studied *bc1(b6f)*-complex and an alternative one non homologous to the *bc1*. The of *bc1*-operons are present in main bacterial phyla (Proteobacteria, Actinobacteria, Bacilli, Cyanobacteria) and some minor ones (Chlorobi among them). The clusters on the dendrogram for *bc1*-operons are congruent to the structure of the phyla on the common phylogeny. But there are some mixed clusters that simultaneously comprises the *bc1*-operons from several bacterial phyla.

The alternative complexes are homologous to three-subunit molybdopterin-containing oxidoreductases. There are operons that gradually acquire genes encoding additional subunits: five-heme cytochrome c, one-heme cytochrome c, duplicate of the membrane subunit, and one more membrane subunit. These operons can be found in the genomes of bacterial phyla that do not contain *bc1*-operons (Chloroflexi among them) or contain those that enter to the mixed clusters.

Clusters on the dendrogram of different types of cytochrome oxidase correspond to the clusters on the dendrograms of the two types of quinol:periplasmic e-transporter oxydoreductases. This may reflect the co-evolution of functionally bound complexes.

Only Cyanobacteria and Chlorobi contain the operons of photoreaction centers in all their sequenced genomes. But only a little portion of Proteobacteria and Chloroflexi have operons of photoreaction centers and the clusters in the dendrogram are intermixed due to lateral gene transfers. This corroborates the hypothesis that operons encoding photosynthetic apparatus firstly emerged in the phylum that gave rise to Cyanobacteria and later were transferred to other phyla.

Lecture S7.5

The signaling state of orange carotenoid protein

Evgeny G. Maksimov[1,*], E. A. Shirshin[2], N. N. Sluchanko[3], D. V. Zlenko[1], G. V. Tsoraev[1], V. V. Fadeev[2], V. Z. Paschenko[1] and A. B. Rubin[1]

1 – Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
2 – Department of Quantum Electronics, Faculty of Physics, M.V. Lomonosov Moscow State University, 119992 Moscow, Russia
3 – A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, 119071 Moscow, Russia
* e-mail: emaksimoff@yandex.ru

Water-soluble phycobiliprotein antenna complexes allow cyanobacteria effectively absorb light in the spectral region where absorption of chlorophyll is insignificant. In parallel with increase of absorption mechanism, this requires creation of special protection from excess of excitation in phycobiliprotein-containing antenna complexes. This mechanism is called nonphotochemical quenching of phycobilisome fluorescence. A specific 35 kDa water-soluble carotenoid protein, the orange carotenoid protein (OCP), plays an essential role in this process. Recent studies have shown that OCP acts as a light intensity sensor; absorbing blue-green light OCP goes from the orange (OCP^O) to the red (OCP^R) form that can interact with the phycobilisome and effectively quench its excited states, thus reducing the amount of excitation energy being transferred to chlorophyll of the reaction centers.

In order to study the mechanism of OCP photoactivity this protein was isolated from cyanobacterium *Arthrospira maxima* grown under intense illumination. A set of both steady-state and time resolved optical methods was applied to investigate $OCP^O \rightarrow OCP^R \rightarrow OCP^O$ conversion which allowed us to determine corresponding rate constants. To describe time-courses of OCP spectral changes we propose a model, which explains temperature dependence and dependence on the intensity of blue-green illumination observed in experiments. $OCP^O \rightarrow OCP^R$ photoconversion is associated with conformational changes of both – carotenoid chromophore and protein accompanied by small changes in entropy of the system, this fact allows us to use methods of molecular dynamics to investigate the conformation of OCP in different stages of its photocycle.

Lecture S7.6

Anaerobic and heat induced state transitions in *Arabidopsis thaliana* and its signal mechanism in thylakoid membranes

Subramanyam Rajagopal, Sreedhar Nellaepalli, Sireesha Kodru

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad, India 500046
* e-mail: srgsl@uohyd.ernet.in

The mechanism of state transitions involved in the functional optimization of photosystems is well documented under low light conditions at optimal temperatures. However, when the temperature enhanced to 40°C or low and changed from aerobic to anaerobic, the alterations in the mechanism of state transition are poorly understood. The present study deals with the effect of heat (40°C) and anaerobiosis on state transitions and the factors responsible for state transition in *A. thaliana*. Dark aerobic and 40°C condition caused full expression of Chl *a* fluorescence transient (O-J-I-P) rise. Upon exposure to dark-anaerobic and heat condition the shape of the transient is completely altered. Rise in F_O and F_J was observed due to the loss of oxidized PQ as the PQ pool becomes reduced resulting in decreased electron flow during anaerobic condition. When leaves were recovered back to dark aerobic condition, the altered OJIP transients were resumed back, indicating the recovery is reversible. Post illumination transient studies have shown that increase in F_O' was due to the non-photochemical reduction of PQ pool under anaerobic and heat conditions. The non-photochemical reduction of PQ pool (the Q_o site of Cyt b_6/f was occupied by PQH_2) by anaerobic and heat conditions would activate STN7 kinase and induce LHCII phosphorylation. Further, 77K fluorescence emission studies indicated that the phosphorylated LHCII is migrated and associated with PS I supercomplex increasing its absorption cross-section. Furthermore, evidences from *crr2-2* (NDH mutant) and *pgr5* mutants (deficient in non NDH pathway of cyclic electron transport) have indicated that NDH is responsible for non-photochemical reduction of the PQ pool and transition to state II (LHCII phosphorylation) in anaerobiosis.

However, in heat induced state transition the signal operation seems to be different than light and anaerobic conditions. The role of NDH and cyclic electron transfer on non-photochemical reduction of PQ pool was studied in the mutants *crr2-2* and *pgr5*. In *pgr5* mutant, non-photochemical reduction of PQ pool was observed indicating the involvement of alternative electron transfer routes apart from cyclic electron transfer contributing to PQ pool reduction at dark 40°C. In chlororespiratory mutant *crr2-2*, the non-photochemical reduction of PQ pool and LHCII phosphorylation was observed signifying the reduction of PQ pool is independent from NDH mediated pathways which are operative under dark. Further, antimycin A inhibitor studies in wt and mutants revealed its inhibitory action on non-photochemical reduction of PQ pool affecting both LHCII phosphorylation and migration to PS I leading to state I transition.

Lecture S7.7

Fluorescence imaging of light induced reactive oxygen species (ROS) in plant cell tissue

Franz-Josef Schmitt[1,*], V. D. Kreslavski[2,3], G. Shirshikova[2], C. Keuer[1], S. K. Zharmukhamedov[2], A. Jajoo[4], S. I. Allakhverdiev[2,3], T. Friedrich[1]

1 – Institute of Physical Chemistry, TU Berlin, Berlin, Germany
2 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
3 – Institute of Plant Physiology, RAS, Moscow, Russia
4 – School of Life Science, Devi Ahilya University, Khandwa Road, Indore, India
* e-mail: schmitt@physik.tu-berlin.de

UV-radiation, high irradiance, higher or lower temperatures, high salt concentration and toxic compounds like polyaromatic hydrocarbons (PAHs) lead to generation and accumulation of reactive oxygen species (ROS) in animal and plant cells. We investigated formation, decay and the functional role of ROS in both oxidative degradation and signal transduction during exposure of oxygen-evolving photosynthetic organisms to UV-A radiation. ROS generation by naphthalene (Naph), a lipophilic polyaromatic hydrocarbon (PAH) was studied with fluorescence microscopy employing the ROS (mainly H_2O_2) sensitive dye dichlorofluorescein. Under high light illumination Naph treated leaves of Arabidopsis thaliana showed the spread of ROS waves over several hundreds of μm across the tissue with a period time of 20 min. which might be caused by the induction of ROS due to the destruction of the Chl pigments or development of oxidative stress in various membranes due to joint effects of UV-A and Naph which is effectively absorbed by membrane lipids.

The acute effects of three typical polyaromatic PAHs: Naph, phenanthrene (Phen) and fluoranthene (Flu) in detached leaves of 3-week-old pea plants showed that the damage of PS II depends strongly on the water solubility of a given type of PAHs, their concentration and exposure time. During short-time exposure the compound with highest water-solubility, Naph revealed the strongest effect. The polyphasic chlorophyll *a* fluorescence induction (OJIP) test revealed that damage firstly occurs on the acceptor side of photosystem II (PS II). During long-time exposure the compounds with low water-solubility (Phen, Flu) revealed the strongest effect as the corresponding PAH accumulates in the thylakoid membranes especially when the solution is oversaturated containing a solid phase. The reduction of PS II activity at the presence of naphthalene was accompanied by transient generation of H_2O_2 as well as swelling of thylakoids and distortion of cell plasma membranes, which was indicated by electron microscopy images.

In a future approach the concomitant imaging of the concentration of PAHs in the cell tissue, mainly membranes, the concentration of ROS and the activity of the PS II is desired. We present a multi-parameter setup based on to a Nikon TI Eclipse microscope that combines time resolved fluorescence microscopy, anisotropy microscopy and spectrally resolved imaging by splitting the detector optically into two independent areas and additionally synchronizing the acquired photon stream with a switch able filter wheel.

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Lecture S7.8

Light-induced regulation of photosynthetic electron transport in chloroplasts

Alexander N. Tikhonov[1,*], Alexey E. Frolov[1], Vasily V. Ptushenko[2], Boris V. Trubitsin[1], Alexey V. Vershubskii[1]

1 – Faculty of Physics, Moscow State University, Moscow, 119991, Russia
2 – Institute of Physical-Chemical Biology, Moscow State University, Moscow, Russia
* e-mail: an_tikhonov@mail.ru

In this communication, we summarize results of our experimental and theoretical studies of the mechanisms of feedback regulation of photosynthetic electron transport in higher plant chloroplasts. We consider the following questions: 1) the nature of the rate-limiting step in the chain of the intersystem electron transport; 2) pH-dependent mechanisms of electron transport control in chloroplasts, and 3) the role of alternative pathways in regulation of photosynthetic electron transport and sustainability of photosynthetic apparatus. The data obtained show that the rates of plastoquinone (PQ) reduction to plastoquinol (PQH_2) in PS II and its diffusion to the *b6f* complex do not limit electron transport between PS II and PS I. Analysis of experimental and quantum chemical data demonstrates that the rate-limiting step in the intersystem chain of electron transport is determined by PQH_2 oxidation at the Q_o site of the *b6f* complex, which is accompanied by the release of two protons into the thylakoid lumen. The feedback control of PQH_2 oxidation is governed by the intrathylakoid pH: the lumen acidification causes deceleration of PQH_2 oxidation, impeding the intersystem electron transport. Results of our experimental studies of pH-dependent regulation of electron transport are described within the framework of a mathematical model, which includes the key stages of the linear electron transport and cyclic electron flow around PS I, transmembrane proton transport, ATP synthesis, and pH-dependent regulatory processes (down-regulation of the intersystem electron transport and activation of the Benson–Calvin cycle). The model describes the feedback mechanisms of electron transport control and adequately reproduces a variety of experimental data on induction events observed in intact chloroplasts under different experimental conditions, including a complex kinetics of P_{700} photooxidation, CO_2 consumption and photorespiration at different concentrations of atmospheric CO_2 and O_2. Along with the down-regulation of the intersystem electron transport associated with the lumen acidification ($pH_{in}\downarrow$), the model predicts the attenuation of PS II activity due to significant light-induced alkalization of the narrow partition between adjacent thylakoids of grana ($pH_{gap}\uparrow$).

Lecture S7.9

Cadmium uptake into chloroplasts and its impact on chloroplastic mRNAs, proteins, and energy quenching

Eugene A. Lysenko[1,*], Alexander A. Klaus[1], Natallia L. Pshybytko[2], Victor V. Kusnetsov[1]

1 – Institute of Plant Physiology, RAS, Moscow, Russia
2 – Institute of Biophysics and Cell Engineering, NASB, Minsk, Belarus
* Fax: +7(499)9778018, e-mail: genlysenko@mail.ru

Photosynthesis have to be protected from Cd, however, small portion of cadmium invades into chloroplasts. Magnitude of this portion remained unclear. We studied accumulation of Cd in highly purified intact chloroplasts of barley (C3) and maize (C4). Seedlings of both species accumulated equal amount of Cd and its growth was retarded in a similar manner. Barley accumulated Cd less in their leaves but more in their chloroplasts as compared to maize. Our data filled the gap between drastically different data obtained previously and make obvious that portion of leaf cadmium penetrated into chloroplasts can vary in very broad range: 4.5–336 ng Cd/mg Chl and 0.02–14.3%. Ratio of Cd atom to chlorophyll molecules in chloroplasts is typically one-to-thousand(s) and never exceed one-to-hundred. Therefore Cd cannot impact substantially light absorption through substitution of Mg in antennae chlorophylls. Photochemistry (F_v/F_m, Φ_{PSII}, qP) was not influenced by Cd but non-photochemical quenching was reduced much in barley but not in maize. The difference was specific to cadmium – copper decreased NPQ in a similar manner in both species. Fall of NPQ was due to its fast relaxing component (qE), and small but significant rise of its slow relaxing component (qI) was recorded. Hence Cd can change NPQ up and down simultaneously and the sum depends on which value is more; if both changes are equal their sum will be next to zero mimicking absence of any changes. In chloroplasts Cd didn't influence mRNA level, but content of some photosynthetic proteins was diminished: slightly in leaves of barley and heavily in leaves of maize. Probably, we firstly analyzed Cd impact on a level of photosynthetic proteins in C4-plant and revealed its severe reduction. In all analyzed C3-species the Cd impact on content of photosynthetic proteins was mild or absent.

Poster S7.10

Does the external mitochondrial NADPH dehydrogenase have a special role in protecting the chloroplasts from bleaching in young leaves of *Nicotiana sylvestris* tobacco?

Abdelghafar M. Abu-Elsaoud[1,2,*] and Allan G. Rasmusson[2]

1 – Dept. of Botany, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt
2 – Dept. of Biology, Lund University, Lund 22362, Sweden
* Fax. +46-46-222-4113, e-mail: Abdoacacia@gmail.com

NADH and NADPH and their oxidised forms NAD and NADP are central mediators of reductant energy between metabolic processes in cells. Chloroplasts under stress can become overly reduced, yet it has been suggested that surplus reduction can be exported to the cytosol as NADPH, which may be oxidised by the mitochondria. In the current study we investigated short-time high light exposure of *Nicotiana sylvestris* lines overexpressing and suppressing *NDB1*, which encodes the external mitochondrial NADPH dehydrogenase. Plants were grown under 16/8 h day length and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light. After 14 days, light was switched to high light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$). PAM-imaging parameters, growth parameters, pigment composition, NADPH/$NADP^+$ and photosynthetic rate were monitored. The sudden increase in light intensity lead to yellowing of young leaves specifically in the NDB1-suppressed line. Clear marginal yellowing "chlorosis" of young tobacco leaves after 6 days of exposition to high light were recorded as two large spots on sides of midrib. Chlorophyll *a*, chlorophyll *b* and carotenoids showed significant difference among studied tobacco genotypes after exposure to HL. Significant variations were also observed (paired sample T-test for marginal vs. central leaf ETR; p=0.0001*) among the PAM-imaging parameter of both NDB1 overexpressor and suppressor lines for both marginal site and central site of tobacco leaves. We hypothesise that the suppression of NDB1 leads to an increased NADPH reduction level, which has detrimental consequences for the chloroplast of margins in young leaves. This is also suggested by previous reports showing changes in this parameter upon stress, and by that NADPH is involved in many stress tolerance and defence systems (e.g. antioxidant enzymes), but a dynamic link has not been directly evidenced. Chloroplast overreduction may thus induce damages to the photosynthetic machinery, e.g. degradation of proteins and pigments.

Poster S7.11 *(in absentia)*

Photosynthetic gas exchange of wheat varieties under water deficit

Jalal A. Aliyev

1 – Department of Plant Physiology and Biotechnology, Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan, Baku, Azerbaijan
2 – Department of Fundamental Problems of Biological Productivity, Institute of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan
* tel.: +994-12-538-1164, +994-12-510-2433, e-mail: aliyev-j@botany-az.org

Under water stress active photosynthetic function of different assimilating organs, mainly of an ear, together with an optimal architectonics of plants plays a crucial role in yield formation. Drought as a negative environmental factor adversely affected the photosynthetic gas exchange in wheat, reducing its rate by 30–40% in the short-stemmed varieties and by 35–45% in long-stemmed ones. Lower leaves were more affected by drought than the upper leaves. The greatest decrease in the photosynthetic rate during ontogenesis in short-stemmed varieties occurred at the flowering and grain formation, and in the long-stemmed ones – at the flowering and milk ripeness stages. In intensive varieties the critical period of water deficit was the end of flowering and grain formation, in extensive ones it started from the beginning of flowering and covered the whole following period of ontogenesis. Leaf area began to decrease starting from the earing and flowering stages, and at the end of ontogenesis its area shortened by more than half. The ear surface area at the end of ontogenesis decreased in short-stemmed varieties by 32% and in long-stemmed ones by 23%. Alterations in the correlation between assimilating and consuming organs in different wheat genotypes under drought led to a change in photosynthetic rate. Variation of the source potential with the removal of 7-layered leaves increased the rate of the 8-layered leaves in the short-stemmed varieties under normal irrigation and water deficit on average by 19 and 21%, in long-stemmed ones by 36 and 28%. After removal of 8-layered leaves, these parameters changed to 22 and 28% in short-stemmed and 37 and 23% in long-stemmed genotypes. The decrease of the ear acceptor force led to a decrease of the rate of photosynthesis of leaves in the control and stressed variants on average to 15 and 9.5% in intensive types, and 18 and 12.5% in extensive ones, respectively. In drought-tolerant intensive wheat variety in canopy more than 60% of grain yield and protein synthesis occurred due to ear photosynthesis. Together with high photosynthetic activity and attractive force of the ear it constituted the basis of high yield. The rate of photorespiration was to some extent in inverse correlation with the water supply. With increasing tolerance of varieties to water stress or with strengthening of the drought, the photorespiration rate decreased to a large degree in the ear elements.

Poster S7.12 *(in absentia)*

Effect of drought stress on yield and yield components of durum and bread wheat genotypes

Tofiq I. Allahverdiyev

Research Institute of Crop Husbandry, Ministry of Agriculture of Azerbaijan Republic, Pirshagi, Sovkhoz-2, Baku AZ1098, Azerbaijan
* Fax: +994125516130, e-mail: tofig_1968@mail.ru

Drought stress is one of the most widespread environmental stresses affecting wheat yield worldwide including in Azerbaijan. The aim of this research was to study the effect of soil water deficit on yield and yield components of six durum wheat (Garagylchyg 2, Vugar, Shiraslan 23, Barakatli-95, Alinja-84, Tartar) and seven bread wheat (Gobustan, Giymatli-2/17, Gyrmyzygul-1, Azamatli-95, Tale-38, 12nd FAWWON№97, 4th FEFWSN№50) genotypes grown under irrigated and rain-fed field conditions. Plant height, number of spikelet per spike, spike length and width decreased slightly, however, spike weight, number and weight of grains per spike, exposed peduncle decreased significantly under the influence of drought. The decrease in the height of cultivars was more expressed among bread wheat genotypes. Long exposed peduncle was detected in genotypes Vugar, Shiraslan-23, Gobustan, Azamatli-95, 4th FEFWSN№50, short exposed peduncle was detected in genotypes Giymatli-2/17, Gyrmyzygul-1. Number of spikes per m^2 was higher among bread wheat genotypes than durum wheat. A strong reduction of grain number and grain weight per spike were detected in genotypes Garagylchyg 2, Alinja-84, Giymatli-2\17, Azamatli-95, Tale-38, 4th FEFWSN№50. Profound decrease of 1000 kernel weight was observed in genotypes Tale-38, 12nd FAWWON№97 and 4th FEFWSN№50. We found an increase of harvest index under rain-fed condition. More reduction of grain yield was observed in genotypes Vugar, Shiraslan-23, Barakatli-95, Alinja-84, Gobustan, and Tale-38. Less reduction of grain yield was observed in genotypes Gyrmyzygul-1, Tartar, Azamatli-95. There were positive correlation between grain yield and plant height, spike number per m^2, aboveground biomass.

Poster S7.13

Effects of drought on mitochondrial NAD-malate dehydrogenase in *Amaranthus cruentus* L. during ontogenesis

Ulduza Gurbanova, Hasan Babayev*, Minakhanym Aliyeva, Yashar Feyziyev, Novruz Guliyev

Institute of Botany Azerbaijan National Academy of Sciences, 40 Patamdar Highway, AZ 1073 Baku, Azerbaijan
e-mail: babayev_hg@yahoo.co.uk

In this study we identified differences in mitochondrial NAD-ME (NAD-MDH) isolated from various leaf tissues of C4 plant *Amaranthus cruentus* L. under drought. Plants were grown in field conditions and after appearance of the second leaf drought was induced by ceasing watering, while control plants were well watered. Drought caused an important rearrangement in isoenzyme content of both mesophyll (M) and bundle sheath (BS) cells. In control and drought exposed plants three (63, 68, and 72 kDa) and two (63 and 72) constitutive isoforms of the enzyme, respectively, were observed in mitochondria of M cells at pre-anthesis. Inductive isoform of 57 kDa was found in both control and drought exposed plants at anthesis, which disappeared in grain ripening phases. In BS cells of control plants three constitutive isoforms of NAD-MDH with molecular weights of 68, 72 and 77 kDa remained in mitochondria till the end of the vegetation. Whereas in drought exposed plants only the isoform of 68 kDa existed at pre-anthesis. Inductive isoform of 63 kDa appeared at anthesis and another inductive isoform of 72 kDa was formed in the grain ripening phase. Only one isoform of 72 kDa remained at the end of the vegetation in both M and BS mitochondria. The observed enzyme isoforms consisted of two 28–38.5 kDa subunits. Isoforms of 63, 68 and 72 kDa were common for both tissues, while 77 kDa isoform was localized only in BS cells. The activity of the mitochondrial enzyme 1.5–2 times exceeded that of the enzyme localized in cytosol. Being very active NAD-MDH showed some activity till the end of amaranth ontogenesis. The isoforms localized in chloroplasts had the lowest activity. In general, the reactions catalyzed by mitochondrial isoforms follow Michaelis-Menten kinetics. The observed change in the number of isoenzyme spectrum and localization of NAD-MDH isoforms in subcellular fractions of amaranth leaf assimilating tissues is the sign of adaptation and probably a part of the adaptation mechanism.

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Poster S7.14

Photosynthetic enzyme activities under drought stress in *Chenopodium album* L.

Shahniyar Bayramov[1,*], Minakhanym Aliyeva[1], Taliya Orujova[1], Wolfgang Brüggemann[2]

1 – Institute of Botany Azerbaijan National Academy of Sciences, 40 Patamdar Highway, AZ 1073 Baku, Azerbaijan
2 – Department of Ecology, Evolution and Diversity, University of Frankfurt, Max von Laue Str. 13, 60438 Frankfurt, Germany
* e-mail: sbayramov@hotmail.com

Drought stress is considered to be one of the main environmental factors that strongly limit growth and yield of plants worldwide. We examined the effects of drought stress on water status of a cosmopolitan weed C3 plant *Chenopodium album* leaves. For this purpose Calvin cycle enzyme activities were determined and compared to well-watered plants (control). Drought stress had obvious effects on the Rubisco initial activity and activation state. The Rubisco initial activity was variable on the first 0–3 days of drought. Afterwards, it declined gradually, reaching minimum on the 5th day of drought. Rubisco total activity showed the same trend as the initial activity. The other two Calvin cycle enzymes NADP-glyceraldehyde phosphate dehydrogenase (NADP-GAPDH) and Phosphoribulokinase (PRK) also showed decreased rates under drought stress. 50% decline in relative water content resulted in 3-fold decrease in the Rubisco initial and total activities relative to controls. Similar results were obtained for NADP-GAPDH and PRK. In contrast, the initial and total activities of another key enzyme of the Calvin cycle, stromal fructose-1,6-bisphosphatase (sFBPase), increased significantly in stressed plants compared to controls. However, upon re-watering, the sFBPase initial activity and activation state rapidly reached near control levels. These results indicated that Calvin cycle enzymes contributed to the limitation of photosynthesis in drought exposed *Chenopodium album* leaves.

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Poster S7.15

Identification of the signal messenger for the long-term regulation of the Photosystem II light-harvesting antenna size in high light

Maria Borisova-Mubarakshina[1,*], Boris Ivanov[1], Tatyana Fedorchuk[1], Natalia Rudenko[1], Daria Vetoshkina[1], Marina Kozuleva[1], Luca Dall'osto[2], Stefano Cazzaniga[2], Roberto Bassi[2]

1 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
2 – Dipartimento Scientifico e Tecnologico, Universita di Verona, Italy
* Fax: +7(496)7330532, e-mail: mubarakshinamm@gmail.com

The main long-term strategy of the photosynthetic organisms to acclimate and survive under light conditions is the regulation of the light-harvesting antenna size of Photosystem II (LHCII). Higher plants possess the ability to increase the LHCII size in the shade or to reduce the antenna size in high light. In the present study using barley plants we gain insight into the signal nature for the regulation of the LHCII size under high light illumination. It was shown that the LHCII size was not changed in high light under conditions minimizing the hydrogen peroxide content in barley leaves. In contrast, a decrease of the antenna size was observed in low light in the presence of elevated hydrogen peroxide concentration. It has been concluded that the hydrogen peroxide molecule produced in the light triggers the photosynthetic apparatus response for the LHCII size regulation in high light.

Poster S7.16

Specific responses of PS I and PS II electron transport in leaves of *chlorina* wheat mutants

Marián Brestič[1,*], Marek Živčák[1], Kristýna Kunderlíková[1], Katarína Olšovská[1] and Suleyman I. Allakhverdiev[2,3]

1 – Slovak University of Agriculture, Nitra, Slovak Republic
2 – Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia
3 – Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Russia
* Fax: +421(37)6415-494, e-mail: marian.brestic@uniag.sk

Antenna mutants represent a unique tool to study photosynthetic processes running at the level of the thylakoid membrane. In our experiments, we examined high light responses of chlorophyll *b*-less mutants of spring wheat (*Triticum aestivum* L.) in comparison to wild type (WT) *in vivo*. The fast chlorophyll fluorescence kinetics confirmed the lower apparent antenna size, but values of other parameters derived from OJIP-transient were at the level similar to WT. Whereas the chlorina mutants had almost normal CO_2 assimilation rate, the simultaneous measurements of chlorophyll fluorescence and P700 absorbance indicated altered electron and proton transport, resulting to lower trans-thylakoid pH-gradient, leading to lower NPQ. The measurements of electrochromic bandshift at 520 nm confirmed lower proton motive force associated with lower transthylakoid proton gradient. As a result of insufficient regulation of linear electron transport, the acceptor side of Photosystem I (PS I) was more reduced, creating conditions for enhanced oxidative damage due to reactive oxygen species. The shift of balance between PS II and PS I redox poises indicates lower PS I to PS II ratio in chlorina mutant compared to WT. Our results also suggest that chlorina mutant of barley had lower capacity to increase the rate of cyclic electron flow around PS I, which makes this mutants more susceptible to environmental constraints.

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Poster S7.17

Chlorophyll catabolism under low water regime in *Apium graveolens* var. Dulce

Nina Djapic

University of Novi Sad, Technical Faculty "Mihajlo Pupin", Djure Djakovica bb, Zrenjanin, Republic of Serbia
* e-mail: nidjapic@gmail.com

Apium graveolens var. dulce was grown to the full development stage. Then, the plants were left under low water regime. The low water regime induced the chlorophyll decomposition in *Apium graveolens* var. dulce leaves. The chlorophyll catabolite content was analyzed by gas chromatography-mass spectrometry. The results obtained permitted the proposition of the chlorophyll catabolic pathway in Apium graveolens var. dulce leaves with the regard to the abiotic stress.

Poster S7.18

The tolerance of lichen *Lobaria pulmonaria* photosynthesis to excess light and UV(A+B)-radiation

Tamara Golovko, Igor Dalke, Ilya Zakhozhiy and Olga Dymova*

Institute of Biology, RAS, Syktyvkar, Russia
* Fax: +7(8212)240163, e-mail: dymovao@ib.komisc.ru

Lichens are a resistant self-regulating association between autotroph photobiont and the heterotroph fungal partner, mycobiont. The folious epiphytic lichen *Lobaria pulmonaria* is frequently occurred in the old taiga forests in the European North-East of Russia. Lichen is settled on the bark of deciduous and coniferous trees at the height of 2–3 m. Our aim was to study the photosynthetic responses of lichen thalli to light stress. The primary photobiont in *Lobaria* thalli is the green alga. The alga's cells (~5 μm in diameter) form layer, 20–30 μm thick, under the upper cortical layer of the fungal hyphae. In nature the rate of CO_2 net-uptake in some thalli reached 6 μmol/m^2·s. At the same time CO_2 evolution was recorded in other thalli. It could be associated with their partial desiccation. In the laboratory on the hydrated thalli the clear dependence of CO_2 gas exchange and electron transport rate (ETR) in PS II on the light was showed. Non-photochemical quenching of Chl fluorescence (qN) increased significantly under excess light. The xanthophyll cycle (XC) is an irradiance-triggered conversion of violaxanthin to zeaxanthin (Zx) that is able to dissipate the excess absorbed energy in the heat. The conversion level of XC pigments (DEPS) in the *Lobaria* photobiont increased by 5 times as the PAR intensity increased from 100 to 1000 μmol/m^2·s. The DEPS value was 1.5–2 times higher in winter thalli as compared to the summer ones. Therefore, it is possible to suggest that the Zx-depended mechanism of energy dissipation contributes greatly into photoprotection of photobiont cells in the thalli. Maximal photochemical quantum yield of PS II (F_v/F_m) in the air-dried thalli was extremely low (0.05) and increased to 0.55–0.65 in 5–10 min after short-term hydration. The results indicate the functionality of PS II RCs of the photobiont in desiccated thalli. The wet thalli treated by UV(A+B)-radiation (daily rate near 5 KJ) during week retained a high potential photochemical activity of PS II (F_v/F_m=0.70±0.01) and ability to photochemical quenching of Chl fluorescence (qP=0.83±0.02 at PAR 180 μmol/m^2·s). The rate of CO_2 net-uptake in treated thalli did not differ much from the control. Photosynthetic responses of lichen demonstrate the tolerance of autotroph photobiont to photoinhibition and the ability of thalli to resist the effect of direct sun rays during the sunflecks.

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Poster S7.19

The state of pigment-protein complexes in cloroplasts of *Ajuga reptans* summer and winter green leaves

Olga Dymova[1,*], Mikhail Khristin[2] and Tamara Golovko[1]

1 – Institute of Biology, RAS, Syktyvkar, Russia
2 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow region, Russia
* Fax: +7(8212)240163, e-mail: dymovao@ib.komisc.ru

Changes in pigment-protein complexes (PPCs) organization in the chloroplasts of *Ajuga reptans* leaves were studied to show the adaptive reactions of photosynthetic apparatus (PSA) to overwintering in natural habitats (54°50′N, 37°37′E). To characterize the PPCs state the methods of gradient sucrose centrifugation, low temperature fluorescence of the photosystems (PS I, PS II), and native green gel electrophoresis (PAGE) were used. The existence of aggregated (PSII-LHCII and PSI-PSII-LHCII supercomplexes) and trimeric with monomeric forms of the light-harvesting complexes (LHCII) within the thylakoid membranes of leaf chloroplasts was observed in summer (July). The value of the ratio (F695/F685) is equaled to 1.11, characterizing the high level of aggregation. Strong macro-aggregation of the PPCs provides maximum of photosynthesis and the ability of PSA to response on a short-term increase in light intensity (sunflecks) under the forest canopy. In late autumn (November) the bugle leaves lost the PSII-LHCII supercomplex. The chlorophyll (Chl) content decreased by 30% and deepoxidation state of the xanthophyll cycle pigments declined greatly, from 65% up to 6%. A decrease in lutein and β-carotene contents was observed as well. The LHCII were reorganized mainly into monomeric forms of the LHCII PSII during winter. Earlier it was shown by us that bugle plants was able to photosynthesize after melting of the snow cover in early spring due to Chl accumulation (Dymova, Golovko, 2001) and to recover PPCs organization in overwintering leaves. In spring (April) free pigments and LHCII PSII trimer and monomer were in the chloroplasts. The ability to aggregate LHCII PSII monomer *in vitro* is observed. Immunoprecipitation revealed 22 kDa PsbS protein in the isolated thylakoid membranes of the chloroplasts in summer green leaves. Small amounts of the PsbS from overwintering leaves were found. The PsbS subunit of the PS II may play a crucial role in xanthophyll-dependent nonphotochemical quenching of excess absorbing light energy, thus contributing to defense mechanism against photoinhibition. Our results demonstrate the presence of complementary mechanisms involved in PSA maintenance in overwintering leaves of herbaceous plants.

Poster S7.20

Continuous cultures of *Rhodobacter sphaeroides*

Zinaida Eltsova* **and Anatoly Tsygankov**

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* e-mail: z.eltsova@gmail.com

Purple non-sulfur bacterium *Rhodobacter sphaeroides* ATCC 2.4.1 is anoxygenic photosynthetic bacterium with very versatile metabolism. It can grow under photoheterotrophic and photoautotrophic anaerobic conditions, under chemoheterotrophic aerobic and anaerobic conditions. Furthemore, it can grow under diazotrophic conditions possessing very high nitrogenase activity. Under nitrogen limitation it can produce molecular hydrogen which is very attractive for biotechnological systems of hydrogen production. But process should be optimized for maximum hydrogen production. Continuous cultivation is very useful tool for the optimization.

We studied the influence of light intensity on the growth rate of *Rba. sphaeroides* in turbidostat culture using lactate or succinate as organic electron donor for anoxygenic photosynthesis. Maximum growth rate (0.21 h^{-1}) as well as the light intensity for the half of maximum growth rate (appr. 10 $W \cdot m^{-2}$) was the same for lactate and acetate. Addition of carbon dioxide to turbidostat cultures with succinate did not infuenced on the growth rate under saturating conditions. With the decrease of light intensity bacteriochlorophyll *a* content in cells increased. After changes of light intensity the steady state conditions were reached less than for 3 h, which is much lower than usual 5–7 times of doubling for any light intensity. It was observed that very fast alteration of pigments content leads to fast changing of growth rate. This is an indirect confirmation the rapid adaptation possibility for the bacteria to changes in light intensity.

When continuous turbidostat cultures were transferred to ammonium-limited chemostat cultures oscillations of biomass concentration, redox-potential of the culture, and hydrogen producing activity were observed. The steady state conditions were reached very slowly (more than 10 times of doubling). When ammonium-limited chemostat cultures were supplied with the medium containing 4 mM NH_4^+ instead of 2 mM NH_4^+, biomass concentration, redox potential of the medium and hydrogen production were also oscillated but with lower amplitude than after the transition from turbidostat cultures to ammonium-limited chemostat. Possible reasons of oscillations are discussed.

Poster S7.21

Effects of temperature and light intensity on photosynthetic enzyme activities in C4 species of *Chenopodiaceae* family in the natural environment

Shahniyar Bayramov, Ulduza Gurbanova, Hasan Babayev, Minakhanym Aliyeva, Novruz Guliyev, Yashar Feyziyev*

Institute of Botany Azerbaijan National Academy of Sciences, 40 Patamdar Highway, AZ 1073 Baku, Azerbaijan
* e-mail: feyziyev-y@botany-az.org

The effect of temperature and light intensities on activities of characteristic enzymes of C3 and C4 cycle has been studied in C4 species of *Chenopodiaceae* family during intensive vegetation period in summer. Diurnal changes in activities of phoephoenolpyruvate carboxylase (PEPC) and aspartate aminotransferase (AATase) in NAD-ME type *Atriplex tatarica* indicated a positive correlation between the rising temperature and enzymatic activities. In contrast, any significant change did not occur in acitvities of these enzymes in NADP-malic enzyme type plants, such as *Noaea mucronata*, *Salsola dendroides*, *Salsola australis* and *Salsola paulsenii* under the same conditions. Activities of NADP-glyceraldehyde phosphate dehydrogenase and fructose-2,6 bisphosphatase, which are redox regulated enzymes of the Calvin cycle, increased in parallel with the rising environmental temperature in *Atriplex tatarica*. However, in NADP-malic enzyme type species increasing temperature did not cause marked changes in the enzyme activities. A pronounced increase in the NAD-malatdehydrogenase activity was found only in *Atriplex tatarica* under heat stress. Activities of the enzymes were also studied related to the leaf age. Thus, activities of PEPC and AATase appeared to be higher in young leaves compared to middle-aged and old ones only in NAD-malic enzyme type *Amaranthus cruentus* L. Whereas, there was not any relation between the enzyme activities and the age of the leaves in NADP-malic enzyme type *Salsola dendroides* and *Salsola paulsenii*. Thus our results show much more increases in the activities of the studied enzymes in NAD-ME type relative to NADP-ME type species with rising temperature.

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Poster S7.22

Application of biophysical luminescence methods for plant phenotyping

Vasilij Goltsev[1,*], Stella Dimitrova[1], Kolyo Dankov[1], Vladimir Aleksandrov[1], Vasilena Krasteva[1], Momchil Paunov[1], Hazem M. Kalaji[2], Reto J. Strasser[3]

1 – Department of Biophysics and Radiobiology, Faculty of Biology, St. Kl. Ohridski University of Sofia, 8 Dragan Tzankov Blvd., Sofia 1164, Bulgaria
2 – Department of Plant Physiology, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
3 – Bioenergetics Laboratory, University of Geneva, CH-1254, Jussy-Geneva, Switzerland
* e-mail: goltsev@biofac.uni-sofia.bg

Plant phenotyping requires adequately detailed information on the important features of plants. The light absorbed, scattered or emitted by plants is a suitable carrier of essential information for the physiological state of plant tissues. Photosynthesis is the most important bioenergetic process in the plant cell and at the same time it is highly responsive to the plant environment. So, it could be one of the best testing systems reflecting the plant's state and its stress behavior.

The present study discusses the possible experimental approaches to describe a plant's *in vivo* state by three types of signals tightly bound to photosynthetic reactions: i.e. prompt chlorophyll fluorescence, delayed chlorophyll fluorescence and light reflection at 820 nm. Correlation between the different characteristics of these signals and structural and functional properties of the light phase of photosynthesis as well as the main algorithms for data processing are shown. The experimental approaches allowed the authors to extract the important functional and structural information from a combination of the above-mentioned signals. Examples of the application of these methods on some environmental stressors such as drought, high and low temperatures, and deficiencies in micro- and macro-elements in the nutrient solution are presented.

Poster S7.23

Molecular detection of leaf rust resistance genes *Lr26* and *Lr35* in wheat cultivars in Azerbaijan

Farida B. Guliyeva*, Samira M. Rustamova, Irada M. Huseynova, Jalal A. Aliyev

Institute of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan
* Fax: +994-12-510-2433, e-mail: quliyeva-farida@mail.ru

Leaf rust is the most widely distributed fungal disease of wheat which can cause yield losses throughout the world. One of the most effective and environmentally beneficial measures to combat wheat rust disease is creation of cultivars insensitive to the infection. In order to produce resistant cultivars, it is necessary to identify wheat rust resistance genes against the fungal pathogen *Puccinia recondite* f. sp. *tritici*. The determination of resistance genes occurring in the registered cultivars in gene pool of the Institute of Crop Husbandry is important for the compilation of information about wheat genetic resources in Azerbaijan. Sixty-one wheat cultivars have been tested by means of molecular markers confirming presence of *Lr26* and *Lr35* resistance genes. Polymerse chain reaction was performed for the markers of IB267L/IB267R (GCAAGTAAGCAGCTTGATTTAGC/AATGGATGTCCCGGTGAGTGG′)and Lr35F/Lr35R (AGAGAGAGTAGAAGAGCTGC/AGAGAGAGAGCATCCACC) which amplified 267- and 900-bp bands, respectively. Based on the results, the resistance gene *Lr26* was only present in the 89% of wheat cultivars. This result shows the presence of *Lr26* gene in the short arm of the first chromosome in studied wheat genotypes. When using Lr35F/Lr35R marker specific fragments, linked adult plant resistance genes *Lr35* must have been synthesized in 900-bp genomic region. However, fragments were not visualized in this area of the obtained electrophoresis profiles. Thus the existence of *Lr35* gene in 2B chromosomes of studied genotypes was not proved with using this marker. The obtained results provide us with the evidence of need for testing the genotypes and use of resistant cultivars in wheat breeding programs as an effective way of controlling leaf rust.

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Poster S7.24

Generation of free radicals and antioxidative defense system in wheat plants subjected to long-term soil drought

Irada M. Huseynova*, Durna R. Aliyeva, Jalal A. Aliyev

Institute of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan
* Fax: +994 12 510 2433, e-mail: huseynova-i@botany-az.org

Drought stress leads to the overproduction of reactive oxygen species (ROS) in different compartments of plant cells. Plants possess antioxidant defense systems which prevent oxidative damage by scavenging of ROS. The objective of this study was to determine the generation of ROS and the responses of antioxidant enzyme activities as well as their isoenzyme patterns at different developmental stages of six contrasting durum (*Triticum durum* Desf.) and bread (*Triticum aestivum* L.) wheat varieties grown in field under drought. Drought increased the level of superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in leaves of wheat plants compared to the control. Drought leads to increase in antioxidant enzymes activities and changes in their isoforms in wheat plants. One isoform of catalase (CAT), seven isoforms of ascorbate peroxidase (APX) and seven isoforms of glutathione reductase (GTR) were shown by native gel electrophoresis in wheat leaves in response to drought. CAT activity increased in all varieties, especially in drought tolerant varieties under water deficiency. Four isoforms of APO were detected in the flowering phase at the beginning of the drought. The long-term drought caused an increase in the intensity of a high molecular isoform and led to appearance of three additional isoforms of this enzyme. The maximum APX activity in durum wheat was observed at the end of the flowering phase, while in bread wheat varieties at the end of the earing phase. The maximum APX activity was found in drought tolerant genotypes. The APX activity of durum wheat varieties did not differ significantly in milk ripeness and wax ripeness phases. Electrophoresis spectrum of GTR contained only three isoforms in all studied genotypes at the beginning of the drought. Maximum increases in the enzyme activities and isoform amounts were observed at the end of ontogenesis. The stress caused by soil drought increased GTR heterogeneity due to formation of four new forms with low and medium mobility.

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Poster S7.25 *(in absentia)*

Thermal acclimation of photosynthesis in cucumber leaves is enhanced by a daily short-term temperature drop

Elena Ikkonen*, Tatjana Shibaeva and Aleksander Titov

Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russia
* Fax: +7(8142)769810, e-mail: likkonen@gmail.com

The aim of this work was to study the effect of a daily short-term temperature drop on the ability of photosynthesis acclimation to temperature shifts and a role of leaf age in plant response. Cucumber (*Cucumus sativus* L.) plants were grown under constant temperature of 23°C (control) or treated daily by 2 h temperature decrease from 23 to 10°C at the end of the night (DROP). Plants were treated by DROP during 6 days starting from the time when the second leaf was young (emerging) ($DROP_{young}$), or mature (about four-fifth the area of the fully expanded leaf) ($DROP_{mature}$) or during 12 days, i.e. the whole period of leaf expansion ($DROP_{y+m}$). The chlorophyll *a* fluorescence (F_v/F_m) and temperature response of the net CO_2 assimilation rate (A) and light respiration (R_{light}) were measured on the fully expanded leaves.

There were no significant differences in the F_v/F_m values indicating no damage to the structure and function of chloroplasts. The temperature responses of A and R_{light} differed between treatments. The values of A and R_{light} of $DROP_{mature}$ leaves did not differ from control leaves at all measurement temperatures. $DROP_{young}$ and $DROP_{y+m}$ leaves did not differ from control and $DROP_{mature}$ leaves in A measured at optimal (23°C) temperature, but exhibited significantly higher rates of A measured at sub-optimum (12°C) temperature. At super-optimum (38°C) measurement temperature the A rate of $DROP_{y+m}$ leaves was the highest at high light (PPFD of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the lowest at low light (PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The optimum temperature for A_{150} shifted slightly lower for $DROP_{mature}$ and significantly lowers for $DROP_{young}$ and $DROP_{y+m}$ compared to control leaves. $DROP_{young}$ and $DROP_{y+m}$ leaves showed the R_{light} rates that were lower than those of control and $DROP_{mature}$ leaves at 23°C and higher at 38°C.

Thus, we suggest that the daily short-term temperature drop may enhance acclimation capacity of photosynthesis and respiration of cucumber leaves to temperature shifts when the immature leaves are affected.

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Poster S7.26

Role of CBF-mediated alternative electron pathways in balancing chloroplast redox signaling and cold acclimation of photosynthesis

Alexander G. Ivanov[1], David P. Sprott[2], Mona Sedeek[2], Marc Rosembert[1], Leonid Kurepin[1], Jas Singh[2], Norman P.A. Huner[1], Leonid V. Savitch[2,*]

1 – Department of Biology, University of Western Ontario, 1151 Richmond Street N., London, ON N6A 5B7, Canada
2 – Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Ave., Ottawa, ON K1A 0C6, Canada
* Fax: +1-613-759-1701, e-mail: savitchl@agr.gc.ca

Compared to control wild-type (WT) plants of *Brassica napus*, the *BnCBF17*-over-expressor (*BnCBF17*-OE) grown at 20°C mimicked cold-acclimated WT canola with respect to increased photosynthetic electron transport, light saturated rates of photosynthesis and compact dwarf phenotype. This was accompanied by significant enhancement of the expression of photosynthesis related genes as well as increased enzyme activities of Rubisco, SPS, FBPase, NADP-NDH and NAD-NDH (Savitch et al. 2005 Plant Cell Physiol. 46: 1525–1539) thus indicating significant alteration in the components and functional performance of the photosynthetic apparatus of *BnCBF17*-OE plants. Detailed analysis of energy partitioning identified that the proportion of linear electron transport not utilized in carbon assimilation and not directed to photorespiration was six-fold higher in *BnCBF17*-OE compared to WT canola plants grown in a control conditions. This was associated with the enhanced expression level and overall protein abundance of the plastid terminal oxidase (PTOX) in control grown *BnCBF17*-OE plants when compared to the WT plants grown in either control conditions or even acclimated to low temperatures. This suggests that observed discrepancies in energy partitioning might be associated with increased PTOX-mediated alternative electron flow to oxygen in *BnCBF17*-OE plants. Indeed, immunoblot analysis demonstrated that the reaction center polypeptides of PS I (PsaA) as well as PS I-related light harvesting proteins (Lhca1-4) were all significantly up-regulated, while the components of PS II were less affected. This well corresponded with a 60% higher level of the far-red light induced steady state oxidation of P700 ($P700^{+}$) observed in *BnCBF17*-OE compared to WT. The possible involvement of *BnCBF17* in inducing PTOX-dependent alternative electron transport pathways and their role in photosynthetic stress tolerance during cold stress/acclimation of *Brassica napus* is discussed.

Poster S7.27

Investigation of reutilization of photosynthetic products in wheat genotypes with contrasting morpho-physiological parameters under water stress

Atabay Jahangirov[1,*], Gamid Hamidov[1], Ali Jahangirov[1], Djavanshir Talai[2], Irada Huseynova[3], Jalal Aliyev[2,3]

1 – Gobustan Regional Experimental Station, Research Institute of Crop Husbandry, Gobustan, Azerbaijan
2 – Department of Plant Physiology and Biotechnology, Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan, Baku AZ1098, Azerbaijan
3 – Department of Fundamental Problems of Biological Productivity, Institute of Botany, Azerbaijan National Academy of Sciences, Baku AZ1073, Azerbaijan
* e-mail: a.cahangir@hotmail.com

Along with the products of photosynthesis, synthesized after conception, remobilization and reutilization of substances formed before the reproduction phase and accumulated in the stem as reserve substances also play an important role in the formation of wheat grains. The presented work is devoted to the study of the reutilization process in 21 genotypes of winter bread wheat (*Titicum aestivum* L.) with contrasting morpho-physiological parameters grown under arid rainfed conditions. Experiments were carried out in three variants, including plants supplied with water, grown under natural rainfalls and subjected to stress after earing (plots were covered with polyethylene film, thereby preventing the ingress of rain). The mean values of the reutilization in studied genotypes were 9.3%, 13% and 18.3% in aforementioned variants, respectively. It is obvious that reutilization has the most important role in the water-stressed plants. Moreover, under water stress, reutilization correlated with the plant height at r=−0.51*, with the length of the second internode from the top of the stem at r=−0.56**. Correlation between productivity and plant growth was r=0.49* under water stress, while under natural conditions no correlation was revealed. In this variant reutilization correlated with the plant height at r=−0.45*, with the length of the second internode from the top of the stem at r=−0.52*. No correlation was observed between reutilization and other morpho-physiological parameters in well watered wheat plants.

Poster S7.28 *(in absentia)*

Effects of planting and harvest dates on quantity and quality of sugar beet seed in Iran

Hassan Khanzade[1], Rasoul Fakhari[2], Ahmad Tobeh[3]

1 – Member of Scientific Board, Agricultural and Natural Resources Research Centre of Moghan, Parsabad Iran
2 – Weed science M.Sc. Student, University of Mohaghegh Ardabili, Ardabil, Iran
3 – Department of Agronomy and Crop breeding, University of Mohaghegh Ardabili, Ardabil, Iran
* e-mail: h_khanzadeh@yahoo.com

The experiment was conducted in Agricultural Research Station of Ardebil based on split plot experiment with completely randomized blocks in 4 replications in order to evaluate the effects of different planting dates (PDs) and harvesting dates (HDs) of qualitative and quantitative characteristics of sugar beet seed. PDs as the main factor were 10 March, 25 March, 9 April and 24 April in main plots and four HDs included 15, 30, 45 and 60 days after flowering as sub-main factor. After harvesting and transporting the seeds to the laboratory, classification and tests relating to qualitative characteristics of seeds were done under standard conditions and resulting data was analyzed and means were compared according to LSR Duncan test. It is found that PD significantly affected seed effective filling period (EFP) and rate. HD had significant effect (α=1%) on seed yield/plant, seed percentage in all sizes and classifications, germination rate and EFP and maximum seed weight. According to the variance analysis tables, over size seeds (>4.5 mm in diameter), EFP and eventually, maximum seed weight were significantly affected by planting × harvesting interaction. The highest seed yield was obtained in third and second harvest, respectively. Likewise, the highest seed yield/plant in all PDs was obtained in third harvest. The third harvest from third and fourth PD was more effective on favorite characteristics than other treatments. From first to third HD, the percentage of seeds with the diameter of 3.5–4.5 and >4.5 mm increased. The longest effecting filling period occurred in third PD × fourth HD that accompanied by the lowest seed filling rate. The highest seed weight was obtained in first PD × fourth HD that was not appropriate for seed production because of the increase in shattering amount in this treatment.

Poster S7.29

The time course of non-photochemical quenching and fluorescence recovery in *Synechocystis sp.* PCC6803

E. G. Maksimov[1,*], K. E. Klementiev[1], G. V. Tsoraev[1], I. V. Elanskaya[2], and V. Z. Paschenko[1]

1 – Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
2 – Department of Genetics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
* e-mail: emaksimoff@yandex.ru

Under high photon flux density of solar radiation an extensive damaging of photosynthetic apparatus can happen. To prevent this photo destruction cyanobacteria have developed a special mechanism of non-photochemical quenching (NPQ) of excitation in phycobilisome. In *Synechocystis* NPQ is triggered by the orange carotenoid protein (OCP), which conformation is sensitive to blue-green illumination allowing it to bind to the phycobilisome and reduce the flow of energy to the photosystems. Consequent decupling of OCP and recovery of phycobilisome fluorescence *in vivo* is controlled by the so-called fluorescence recovery protein (FRP). The mechanism of NPQ and its regulation role is still remains unclear. In this work the role of the phycobilisome core components, ApcD and ApcF, in NPQ and consequent phycobilisome fluorescence recovery in the cyanobacterium *Synechocystis sp.* PCC6803 has been investigated. The genes encoding these proteins have been disrupted in the genome of *Synechocystis sp.* PCC6803 PS I and PS II deficient mutant *ΔPSI/PSII* by inserting antibiotic resistance genes into their coding regions. Using a single photon counting technique we have registered fluorescence decay spectra with picosecond time resolution during NPQ activation and consequent fluorescence recovery. In order to estimate activation energy of those processes studies were performed in a wide range of temperatures – from 5 to 45°C. It was found that fluorescence quenching and recovery were strongly temperature dependent for all mutants exhibiting characteristic nonlinear time-courses. It was shown that fluorescence recovery of ApcD and ApcF deficient mutants is characterized by significant (10 kcal/mol) energy barrier comparing to *ΔPSI/PSII* mutant. This phenomenon indicates that ApcD and ApcF products may be required for proper interaction of FRP and OCP coupled to phycobilisome core.

Poster S7.30

Mechanical stress in the plant cell: relation to photosynthesis

Anna V. Komarova*, Alexander A. Bulychev and Tatyana N. Bibikova

Lomonosov Moscow State University, Moscow, Russia
* Tel.: +7(495)9393503, e-mail: AnVKomarova@gmail.com

Perception of environmental mechanical stimuli is crucial for survival of all living organisms, especially plants, because of their sessile life habit and inability to actively avoid stress factors. Mechanical signals arising from invasion of pathogens or feeding of herbivorous insects can be experimentally mimicked with a glass micropipette impalement of the cell wall (CW). The pH measurements in the microscopic zone of CW incision in internodal cells of *Chara corallina* revealed rapid localized apoplast alkalinization by 2–3 pH units, which may rigidify the cell wall structure, thus protecting the cell from further injury. The origin of rapid pH changes was partly clarified by studying mechanically induced pH responses in cells treated with channel blockers and metabolic inhibitors. Our results indicate that this localized increase in apoplastic pH is an early event in mechanoperception and depends on osmotic pressure, cytoskeleton, and intracellular calcium.

The mechano-induced pH response was light-dependent, although its disappearance in darkness proceeded very slowly. However, the transfer of the cell to light after long incubation in darkness resulted in a rapid recovery of mechano-induced pH changes. These results imply that the pH response may have a complex origin and comprise stages with different light requirements. The supposed role of photosynthetic electron transport in the incision-induced pH response is confirmed by inhibition of this response by DCMU that blocks intersystem electron flow after the primary quinone acceptor, thus causing complete oxidation of the plastoquinone pool. The results suggest that the plasma-membrane H^+/OH^- channels activated by photosynthesis and by CW microperforation share a number of common properties, even though the activation of these channels proceeds through the remarkably different pathways.

Poster S7.31

Slr2019, lipidA transporter homolog, is essential for acidic tolerance in *Synechocystis sp.* PCC6803

Ayumi Matsuhashi[1,*], Hiroko Tahara[1], Junji Uchiyama[2], Satoru Ogawa[3] and Hisataka Ohta[1,2]

1 – Graduate School of mathematics and science education Tokyo University of science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo, Japan
2 – Research Center for RNA Science, RIST, Tokyo University of Science
3 – Laboratory of electron microscopy school of medicine Mie University
* tel: +81(03)5228-8374, e-mail: c114703@ed.tus.ac.jp

We have previously reported that cell envelope biosynthesis is important for acid stress tolerance in *Synechocystis sp.* PCC6803 (Synechocystis 6803). But it has not been clarified whether other genes involved in extracellular matrix biosynthesis, are essential for acid stress response.

In this study, we showed *slr2019* mutant strain (*slr2019*), which has defective LPS, was more sensitive to acid stress than the wild type. Furthermore, we performed qRT-PCR analysis using *slr2019* primers and western blotting using Slr2019 antibody. There was no difference in the expression of *slr2019* and the amount of Slr2019 protein after acid stress. We are going to try to reveal localization of Slr2019.

To examine influence on various stress tolerance, *slr2019* was spotted on BG-11 plate under various stress conditions. In high salt and low temperature condition, the *slr2019* grew significantly slower than wild type. These results indicated that Slr2019 is necessary so that *Synechocystis* 6803 survives in various stress conditions. This suggests that LPS involved in various stress tolerance in *Synechocystis* 6803.

Poster S7.32

Evidence on superoxide anion radical formation on the electron donor side of Photosystem II: EPR spin trapping study

Ankush Prasad, Aditya Kumar, Pavel Pospíšil*

Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic
* Tel: +420(58)5634174, e-mail: pavel.pospisil@upol.cz

Light-induced production of superoxide anion radical ($O_2^{\bullet-}$) is known in Photosystem II (PS II) of higher plants where evidences have been provided that $O_2^{\bullet-}$ is formed by reduction of molecular oxygen on the electron acceptor side of PS II. In the current study, $O_2^{\bullet-}$ coupled with carbon-centered ($R^{\bullet}$) radical has been shown to be formed in the donor side of PS II. Light-induced $O_2^{\bullet-}$ and $R^{\bullet}$ production in Tris-treated PS II membranes was studied by electron paramagnetic resonance (EPR) spin-trapping spectroscopy, as monitored by EMPO-OOH and EMPO-R adduct EPR spectra. Based on the effects observed by the exogenous addition of catalase and superoxide dismutase, it is hypothesized that $O_2^{\bullet-}$ formation correlates with $R^{\bullet}$ formation on the donor side of PS II. In addition to this, the involvement of Type I photosensitization reaction has also been explored to describe the mechanism on the formation of $R^{\bullet}$.

Poster S7.33

Homo-FRET for the investigation of the oligomeric state of proteins to observe the D1 repair cycle in PS II

Franz-Josef Schmitt[1,*], M. Broser[1], C. Junghans[1], V. D. Kreslavski[2,3], A. Zorina[3], D. A. Los[3], S. I. Allakhverdiev[2,3], T. Friedrich[1]

1 – Institute of Physical Chemistry, TU Berlin, Berlin, Germany
2 – Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
3 – Institute of Plant Physiology, RAS, Moscow, Russia
* e-mail: schmitt@physik.tu-berlin.de

The combination of various microscopic techniques allows for addressing complex problems in biophysics. We present a multi-parameter setup based on a Nikon TI Eclipse microscope equipped with a new detection system for fluorescence lifetime microscopy (FLIM) in form of a high throughput multi-anode (MA) microchannel plate detector driven in the single photon counting mode (up to 2 million photons/sec). The setup combines fluorescence lifetime imaging microscopy (FLIM) and simultaneous anisotropy microscopy by splitting the MA detector optically into two independent areas. All microscopic techniques (confocal, wide field, total internal reflection) can be done with spectral, temporal and polarization resolution.

Förster Resonance Energy Transfer (FRET) between identical fluorophores without the discrimination between donor and acceptor (Homo-FRET) allows the determination of the aggregation state of identical chromophors.

Microscopic measurements of time resolved Homo-FRET were used to determine the aggregation state of a model system of fluorescence proteins (FKBP-GFP constructs) that can dimerize or form larger aggregates (up to pentamers) by adding a specific membrane-permeable agent.

The technique has great application potential for the observation of the repair cycle of the D1 protein in the photosystem II (PS II). Monomerisation of the PS II during the D1 repair in *Synechocystis sp.* PCC 6803 will be measured by the fluorescence anisotropy emitted from genetically modified PS II cores labeled with yellow fluorescent proteins (YFP). For that purpose we expressed the genetically modified PsbM and PsbT proteins tagged to YFP. During monomerisation the anisotropy of the YFP emission rises due to the interruption of Homo-FRET that occurs in the dimer.

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Poster S7.34

Effect of water stress on photosynthetic and physiological parameters in spring barley

Pavol Slamka*, Katarína Olšovská, Marek Živčák and Marián Brestič

Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
* Fax: +421-6415494, e-mail: pavol.slamka@uniag.sk

Effect of N-nutrition (0 g; 1 g; 2 g N per pot, respectively) at two levels of water regime (optimal water regime vs. water stress) during three growth stages (tillering, shooting and earing) were investigated in three-year pot vegetation experiment with spring barley (variety of Kompakt) under atmosferic conditions on brownsoil. Before and after stress period in respective growth stages the plants were grown under optimal moisture conditions. Each treatment was four times repeated. Chosen photosynthetic, physiological, yield and qualitative parameters of spring barley were examined in the experiment. Achieved physiological results showed that application of water stress (drought) decreased nitratreductase activity (NRA), relative water content in leaves (RWC), stomatal conductivity (g_S) and net assimilation rate (P_n) and increased intercellular concentration of CO_2 (C_i) at the same time. N-fertilization had positive and statistically significant effect on NRA and C_i but on the contrary negative effect was confirmed in P_n, RWC and g_S, respectively. On the average of three years net assimilation rate of leaves of main barley stalk (P_n) achieved significantly higher values in all investigated growth stages on optimally moistured treatments than in stressed conditions. Application of N fertilization influenced P_n negatively decreasing P_n relatively slightly under optimum water conditions, but very intensively (several times) under drought stress conditions in comparison to unfertilized control, dropping up to the interval of 1–5 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. By the evaluation of three years results barley achieved the highest yield of grain (24.6 g per pot) when it was grown under optimal moisture condition and fertilzed with 1 g N per pot. Double dose of N had negative effect on yield (14.26 g per pot). Plants exposed to drought stress in growth stage of tillering were the most tolerant against water stress and provided high grain yield. When stress was induced during growth stage of shooting the spring barley grain yield showed the highest sensitivity to stress. Induction of water stress (dryness) similarly like N fertilization decreased thousand kernel weight of barley. Also content of starch in grain was reduced by both water stress and N-nutrition. Water stress significantly increased content of crude protein in grain in comparison with optimal water regime. N-application increased content of crude protein in barley grain under both levels of water regime.

Poster S7.35

SltB2 and SltC2 are involved in acid stress tolerance in *Synechocystis sp.* PCC6803

Hiroko Tahara[1,*], Ayumi Matsuhashi[1], Junji Uchiyama[2], Satoru Ogawa[3], Kouji Matsumoto[4] and Hisataka Ohta[1,2]

1 – Department of biology, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku, Tokyo, Japan
2 – Research center for RNA science, RIST, Tokyo University of Science
3 – Laboratory of Electron Microscopy, School of Medicine, Mie University, Tsu
4 – Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University
* Fax: +81(3)5228-8374, e-mail: c112704@ed.tus.ac.jp

To identify ABC transporters involved in acid tolerance, deletion mutants of ABC transporter genes with unknown substrates were screened for acid stress sensitivities in low pH medium. It was found that cells expressing the deletion mutant of *sltA1* (*Synechocystis* lipid transporter) were more sensitive to acid stress than the wild-type cells. In particular, SltA1 was demonstrated to be homologous to Tgd1 from *A. thaliana*. Interestingly, we found that *Synechocystis* have two sets' ABC transporters which have homology to Tgd. However, the counterparts of each gene remain unknown.

In this study, we found that SltA1 plays a role in cell membrane stabilization during environmental changes with other ABC transporter components. To elucidate whether these proteins interact with SltA1, we performed that these genes disrupted and performed analyses of phenotypes under acid stress condition. The *sltA2* deletion mutant didn't displayed acid stress sensitivities. However the *sltB2* (substrate binding subunit) and *sltC2* (ATP-binding subunit) deletion mutants didn't display it. These results might suggest that this ABC transporter consists of the SltA1, SltB2 and SltC2 proteins.

Poster S7.36

Non-photochemical quenching of chlorophyll fluorescence revealed from picosecond time-resolved fluorimetry

E. G. Maksimov[1,*], K. E. Klementiev[1], G. V. Tsoraev[1], M. D. Mamedov[2], and V. Z. Paschenko[1]

1 – Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, 119992, Moscow, Russia
2 – Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia
* e-mail: emaksimoff@yandex.ru

As high-intensity solar radiation can lead to extensive damaging of photosynthetic apparatus, higher plants and algae have developed a mechanism of non-photochemical quenching (NPQ) of chlorophyll (Chl) fluorescence. Xanthophyll cycle was found to play a key role in the excess energy dissipation within light-harvesting antenna proteins by NPQ. Under high photon flux densities in the dark adapted sample Chl fluorescence intensity undergoes significant changes due to complex relations between rate constant of photochemical and non-photochemical quenching resulting a nonlinear time-course, which is known as an induction curve.

In this work we present an approach allowing the estimation of Chl fluorescence life-time during different stages of induction curve. Using time correlated single photon counting technique we studied NPQ of chloroplasts Chl fluorescence from Spinach. Picosecond laser was operated in 50 MHz regime, fluorescence signal was recorded as cycles (f(t,T) mode of Becker & Hickl SPC) with a duration of signal accumulation up to one second for each cycle. It was shown that during adaptation to actinic light Chl fluorescence life-time gradually reduces up to 30%. This stage corresponds to NPQ observed via conventional OJIP fluorimeter (Handy-PEA, Hansatech). Treatment of chloroplasts by nigericin almost completely removed NPQ resulting high values of Chl fluorescence life-times. This fact confirms that transmembrane proton gradient plays essential role in the regulation of xanthophyll cycle enzymes and NPQ influence on Chl fluorescence. Thus time-resolved measurements allow us to estimate relations between the rate constants of photochemical and non-photochemical quenching because of the fluorescence life-time is directly connected with fluorescence quantum yield.

Poster S7.37

Genomic analysis of parallel-evolved cyanobacterium *Synechocystis sp.* PCC6803 under acid stress

Junji Uchiyama[1,*], Yu Kanesaki[2], Naoya Iwata[3], Ryousuke Asakura[3], Kento Funamizu[3], Rizumu Tasaki[3], Mina Agatsuma[3], Hiroko Tahara[3], Ayumi Matsuhashi[3], Hirofumi Yoshikawa[4] and Hisataka Ohta[1,3]

1 – Research center for RNA science, Research Institute for Science and Technology, Tokyo University of Science, Katsushika-ku, Tokyo, Japan
2 – Genome Research Center, Nodai Research Institute, Tokyo University of Agriculture, Setagaya-ku, Tokyo, Japan
3 – Department of biology, Faculty of Science, Tokyo University of Science, Shinjyuku, Tokyo, Japan
4 – Department of Bioscience, Tokyo University of Agriculture, Setagaya-ku, Tokyo, Japan
* Fax: +81(3)52288374, e-mail: junjiutiyama@yahoo.co.jp

Experimental evolution is a powerful tool to clarify phenotypic and genotypic changes responsible for adaptive evolution. By long-term cultivation of a microorganism under an environmental stress conditions, we can expect to obtain stress-tolerant strains after cycles of mutation and selection. To search for a gene involved in acid tolerance, we isolated the acid acclimated *Synechocystis sp.* PCC6803. They are widely known to *Synechocystis sp.* PCC6803 are rarely found in habitants with pH values below 5.75.

In this study, parent cells were cultured in BG-11 at pH 6.0. We gradually lowered pH of the medium from pH 6.0 to pH 5.5 for 3 months. Our acclimation cells could grow in acid stress condition at pH 5.5, which parent cells could not grow at.

We analyzed DNA sequence using next generation sequencer. Using comparative genomic analysis of acid acclimatized strains and parent strain, we identified 11 SNPs in acid acclimatized strain. These SNPs might be involved in acid stress tolerance.

Poster S7.38

Efficient PS II repair in the cyanobacterium *Synechocystis* PCC 6803 requires the cry-DASH cryptochrome

István Z. Vass[1,*], P. B. Kós[1], J. Knoppová[2], J. Komenda[3], Imre Vass[1]

1 – Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, P. O. Box 521, H-6701 Szeged, Hungary
2 – Faculty of Science, University of South Bohemia, Branisovska 31, Ceske Budejovice, Czech Republic
3 – Institute of Microbiology, Academy of Sciences, Opatovicky Mlyn, 379 81 Trebon, Czech Republic
* Temesvari krt. 62, 6726 Szeged, Hungary; Fax: 0036-62-433-188, e-mail: vass.istvan_zoltan@brc.mta.hu

The decade long study of cry-DASH cryptochromes resulted in no conclusive function to which they could be associated. This indicates a protein with diverse roles. Since we showed that the homologue of the *Synechocystis* cry-DASH (Syn-CRY), a DNA photolyase, is involved in PS II repair, we examined whether Syn-CRY participates in the same process to some degree? We found that the absence of Syn-CRY renders the *Synechocystis* cells increasingly sensitive to not only UV-B but also high intensity PAR. However, unlike its homologue, Syn-CRY is not involved in UV-B damaged DNA repair, nor does it affect the transcription of genes, indispensable for PS II repair. Nevertheless, its lack does interfere with the accumulation of D1 protein, the structural backbone of PS II, as well as other, mostly cytoplasmic proteins, such as PilA1 and bicarbonate transporter SbtA. We concluded that Syn-CRY is required for efficient restoration of Photosystem II activity following UV-B and PAR induced photodamage. Its effect, unlike that of DNA photolyase, is not exerted through DNA repair, but most likely effects either D1 translation by inhibiting CO_2 transport and fixation, or PS II assembly through the putative involvement of PilA1 in Chl binding.

Poster S7.39

Regulation of electron and proton transport in different wheat genotypes in conditions of high temperature

Marek Živčák[*], Marián Brestič, Zuzana Balátová, Katarína Olšovská

Slovak University of Agriculture, Nitra, Slovak Republic
* Fax: +421-37-641-54-94, e-mail: marek.zivcak@uniag.sk

Effect of high temperature were studied in genotypes of wheat of different origin. Plants grown in growth chamber under moderate temperature conditions were exposed to air temperature 42°C for 12 hours in light and then the effect of high temperature was assessed by comparison of photosynthetic parameters measured before and after high light treatment. As expected, the CO_2 assimilation measured at 42°C was substantially lower compared to non-stress conditions, which was not caused by stomatal closure; the genotypes differed in decrease of photosynthetic rate. The analysis of fast chlorophyll fluorescence kinetics uncovered significant genotypic differences in the level of PS II donor side impairment shown by the increase of variable fluorescence in 0.3 ms (K-step), number of active reaction centers as well as cooperation between photosystems indicated by IP amplitude. These results were confirmed also by simultaneous measurements of chlorophyll fluorescence and P700 by saturation pulse method, which indicated a shift in equilibrium between photosystems indicated by the PS II and PS I redox poises. In some genotypes, the insufficient regulation of electron transport in high temperature conditions led to overreduction of PS I acceptor side, which may be associated with harmful effects due to oxidative stress. Our results indicate that wheat genotypes differ in regulation of electron and proton transport and related processes, which might be crucial to prevent damage of photosynthetic structures in high temperature conditions.

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Section 8: Applied aspects of Photosynthesis: $BioH_2$ and Bioelectricity

Lecture S8.1

Direct solar conversion using thermophilic cyanobacteria

Barry D. Bruce

University of Tennessee-Knoxville, Energy Science and Engineering Program, Bredesen Center for Interdisciplinary Research and Education
* e-mail: bbruce@utk.edu

World petroleum production rates are predicted to reach its peak before mid-century and the higher cost of recovering recalcitrant deposits is expected to drive up the price of petroleum-derived fuels. Although fossil fuels rely on photosynthesis-driven biomass accumulation from millions of years ago, there is hope that directly harnessing photosynthesis can shorten the cycle time for creating fuels from solar energy. Ligno-cellulosic biomass-derived fuels are potentially a clean, renewable and sustainable source of fuel but several challenges exist. One of the major challenges is the high-energy cost and low yield of cellulosic conversion. However, there exists a more direct photosynthesis-driven biomass production, using algae and cyanobacteria, that are now becoming actively pursued. In the proposed research, we are exploring a novel organism (*Chroococcidiopsis*) that is capable of producing large quantities of complex carbohydrates in an extracellular structure known as a sheath. During their growth cycle of cell division and expansion, the sheath is enzymatically converted into simple sugars that continually accumulate in their media. Their robust growth, extremely thick carbohydrate-rich sheath, and their process of auto-conversion, combine for a very efficient production of simple sugars that can be used to support bioethanol or other advanced fuels production via yeast fermentation.

Lecture S8.2

Photoelectron conversion using combination of bio-components with artificial molecules

Hiroshi Nishihara*

Department of Chemistry, School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
* Fax: +81(3)58418063, e-mail: nisihara@chem.s.u-tokyo.ac.jp

Highly efficient photoelectron conversion is an important target of research on molecular devices, for which is necessary to control electron conduction in a molecular circuit precisely by combining appropriate molecular-based components. Photosystem I (PS I) and photosystem II (PS II) are excellent candidates of the components for molecular devices, since these bio-components undergo most efficient and optimized photoelectric conversion performance seen in nature with a quantum yield of nearly 100%. To utilize these bio-components effectively in a molecular circuit, it is a smart method to use reconstitution of a redox component in a redox cascade in PS I or PS II by an artificial molecular wire which is connected to electrode or transistor gate for developing a photosensor or to Pt nanoparticles for realizing photo-energy to chemical energy conversion.

We have been studying bio-conjugated systems in which PS I or PS II is connected to Au or Pt nanoparticles for fabrication of bio-photo sensor [1–5] or dihydrogen production, respectively.

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Lecture S8.3

Photo fermentative hydrogen production in combination with lactate and methane fermentation to maximize the bioenergy recovery from food waste

Mi-Sun Kim*, Dong-Hoon Kim, Mo-Kwon Lee

Biomass and Waste Energy Laboratory, Korea Institute of Energy Research,
152 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea
* Fax: +82-42-860-3739, e-mail: bmmskim@kier.re.kr

This study develops a three-stage (lactate- + photo-H_2 + CH_4) fermentation system that substantively improves the efficiency of bioenergy production with an emphasis on achieving high H_2 yield. Previous studies have sought to convert organic wastes into H_2 and CH_4 via two-stage fermentation systems. However, with a two-stage dark (H_2 + CH_4) fermentation system, energy conversion to H_2 was low, while a two-stage (dark- + photo-) H_2 fermentation system showed a limited total bioenergy recovery. The three-stage fermentation system demonstrates overall improvement over previous research by converting 41% and 37% of the energy content in food waste into H_2 and CH_4, respectively. It also achieves an H_2 yield based on hexose input of 8.35 mol H_2/mol hexose added, the highest value ever reported to date, using actual organic waste as a feedstock. The novelty of this process begins by first fermenting food waste to lactate, rather than acetate and butyrate, since lactate is considered a more favorable substrate for photo-fermentative H_2 production. Within one day, food waste was successfully fermented to lactate by indigenous lactic acid bacteria, showing a lactate production yield of 1.65 mol lactate/mol hexose added. Lactate fermentation effluent (LFE) was then centrifuged, and the supernatant was used for H_2 production by photo-fermentation, while the residue was used for CH_4 production by anaerobic digestion. By adding a trace metal solution at the proper level (5 mg Fe/L and 15 mg Mo/L), the supernatant of LFE was successfully converted to H_2 with 71% H_2 conversion efficiency. The CH_4 production was optimized by applying response surface methodology. The optimized conditions were found to be inoculum/substrate (I/S) ratio of 1.70 and substrate level of 8.80 g VS/L, under which yielded 88% CH_4 conversion efficiency. In terms of electrical energy, the developed novel three-stage fermentation system could yield 1,146 MJ/ton-food waste, which is 1.4 times higher value than that of previous two-stage dark (H_2 + CH_4) fermentation system.

Lecture S8.4

Photobiological hydrogen production by *Anabaena* PCC 7120 mutants with increased heterocyst frequency

Hajime Masukawa[1,*], Hidehiro Sakurai[1], Kazuhito Inoue[2]

1 – Research Institute for Photobiological Hydrogen Production
2 – Department of Biological Sciences, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa, Japan
* Fax: +81-463-58-9684, e-mail: wtk-0488gg@kanagawa-u.ac.jp

We are proposing large-scale photobiological hydrogen production by mariculture raised cyanobacteria where the microbes convert solar energy into hydrogen with water as the source of electrons to reduce protons. The hydrogen gas is produced as the inevitable by-product of nitrogen fixation by nitrogenase, an oxygen-labile enzyme. In the absence of N_2, the enzyme directs all electrons to hydrogen production.

Heterocyst-forming cyanobacteria reconcile two incompatible processes, oxygenic photosynthesis and O_2-sensitive nitrogenase reaction, by spatially separating them. In response to combined-nitrogen deprivation, approximately one cell of 10–20 vegetative cells develops into differentiated cells called heterocysts, the site of nitrogen fixation, that provide a microaerobic environment, allowing the O_2-labile nitrogenase to function in aerobic environments. Among several genes involved in differentiation and patterning of heterocysts, *hetR* is considered the master regulator of heterocyst differentiation. Since it can be expected that higher frequency of heterocysts would increase the rates of H_2 production, we have constructed several HetR mutants of *Anabaena* PCC 7120 by random mutagenesis and shown that some mutants formed heterocysts at frequencies significantly higher than those for the parental strain. The effects of increased heterocysts on photobiological H_2 production will be presented.

Lecture S8.5

Modeling the HydSL-hydrogenase from *Thiocapsa roseopersicina*

Azat Abdullatypov*, Nikolay Zorin, Anatoly Tsygankov

Institute of Basic Biological Problems RAS, Pushchino, Moscow Region, Russia
* e-mail: azatik888@yandex.ru

The HydSL-hydrogenase from purple sulphur bacterium *Thiocapsa roseopersicina* BBS is considered as a potential catalyst for hydrogen biosensors, hydrogen fuel cells and light-driven H_2-producing systems (when coupled to Photosystem I) due to its high thermal stability and quite low sensitivity to oxygen, carbon monoxide and hydrogen sulphide.

However, the reasons of such attractive features of this enzyme remain unclear because of poor knowledge of its three-dimensional structure. The experimental 3D-structure of this enzyme with atomic resolution hasn't been obtained yet, so the methods of homology modeling are quite actual for further studies.

The first homology model of this enzyme was made by Szilagyi et. al. [Szilagyi et al., 2002]. In this work, a proposal for stabilizing role of inter-subunit ionic pairs was put forward. The C-end fragment of the small subunit of this enzyme was not built in this work. The assessment of this model by a novel statistical potential implemented into MODELLER package showed low confidence level for this model, so we decided to build a new model of this enzyme using new program packages (MODELLER9.10 for homology modeling and QUARK for *ab initio* modeling of the C-end fragment of the small subunit) and a template 3D-structure of HydSL-hydrogenase from *Chromatium vinosum*, which is highly homologous to the enzyme of our study (84% and 87% for large and small subunits, respectively). The results of modeling showed very high quality of the obtained models. A full-size model of the small subunit of the enzyme was built for the first time. The C-end fragment of the small subunit, which is considered as a potential membrane anchor interacting with cytochrome-like protein Isp1, was shown to have certain degree of freedom, rotating independently of the main protein globule. The stabilizing role of ionic pairs was also shown.

Mapping of intramolecular tunnels was carried out in MOLE2.0 program package in order to detect the determinants of oxygen resistance. However, the results showed that these tunnels are very sensitive to alterations of the structure caused by energy minimization in solution.

In order to define the role of electrostatic interactions in binding the *in vitro* redox partner of hydrogen, i.e. methyl viologene, we carried out experiments showing a strong inhibitory effect of positively charged polypeptides. To detect the viologene and peptide binding sites, we performed docking of these substances to the hydrogenase model in Autodock Vina. The results of docking showed two possible sites for binding methyl viologene, where electron transfer could occur. The peptides K_{20} and $K(KLK)_6K$ also bound to these sites.

The results of the work could be useful in planning the mechanisms of immobilizing and modifying the HydSL hydrogenase for its further application in hydrogen fuel cells, hydrogen biosensors and light-driven hydrogen-producing systems.

Poster S8.6

Simultaneous bio-hydrogen and microbial oil production by *Rhodobacter sp.* KKU-PS1

Thitirut Assawamongkholsiri[1,2], Alissara Reungsang[1,2,3,*]

1 – Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
2 – Fermentation Research Center for Value Added of Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand
3 – Research Group for Development of Microbial Hydrogen Production Process from Biomass, Khon Kaen University, Khon Kaen 40002, Thailand
* Fax: +66-43-362-121, e-mail: alissara@kku.ac.th

Effects of four main media compositions including malic acid, glutamate, Fe and vitamin on hydrogen and microbial oil productions from malic acid by *Rhodobacter sp.* KKU-PS1 were investigated using response surface methodology with central composite design. Malic acid, glutamate and Fe concentrations showed the greatest individual effect on a maximum hydrogen production rate (R_m) ($P \leq 0.001$) while their effects on oil contents ($P \geq 0.05$) were not found. Concentrations of glutamate and Fe revealed the interactive effect ($P \leq 0.05$) on R_m and oil contents. The optimum conditions for simultaneously maximizing R_m and oil contents were malic acid concentration of 4.7 g/L, glutamate concentration of 159 mg/L, vitamin solution of 21.7 mL/L and Fe concentration of 330 mg/L at a controlled initial pH, incubation temperature and illumination intensity of 7.0, 25.6°C and 7500 lux, respectively. Under the optimum conditions, R_m and oil contents were 4.1 mL H_2/L·h and 31.9% (w/w), respectively. Confirmation experiments were conducted under the optimum conditions in which an R_m of 4.1 mL H_2/L·h and oil contents of 29.4% (w/w) were achieved. The lipid composition were analyzed by GC-MS. It was found that C18:1 (57.0% of total fatty acid (TFA)) was the main free fatty acid followed by C18:0 (25.9% of TFA), C16:0 (9.3% of TFA), C19:0 (3.7% of TFA), C16:1 (2.7% of TFA), C14:0 (0.2% of TFA), C18:2 (0.2% of TFA) and other 1.0% of TFA.

Poster S8.7

Applied photosynthesis: Putting PS I to work

Barry D. Bruce

University of Tennessee-Knoxville, Energy Science and Engineering Program, Bredesen Center for Interdisciplinary Research and Education
* e-mail: bbruce@utk.edu

Oxygenic photosynthesis is driven via sequential action of Photosystem II (PS II) and (PS I) reaction centers via the Z-scheme. Both of these pigment–membrane protein complexes are found in cyanobacteria, algae, and plants. Unlike PS II, PS I is remarkably stable and does not undergo limiting photo-damage. This stability, as well as other fundamental structural differences, makes PS I the most attractive reaction centers for applied photosynthetic applications. These applied applications exploit the efficient light harvesting and high quantum yield of PS I where the isolated PS I particles are redeployed providing electrons directly as a photocurrent or, via a coupled catalyst to yield H_2. I will review the recent advances in molecular genetics, synthetic biology, and nanotechnology have merged to allow PS I to be integrated into a myriad of biohybrid devices. In our lab we have made several devices with PS I immobilized onto various electrode substrates and using various electron donors. We are still making significant advances in the efficiency of these innovative yet highly variable designs. I will provide an update on the recent advances in PS I mediated PV devices with a focus on identifying the similarities and differences in electrode surfaces, immobilization/orientation strategies, and artificial redox mediators. The potential drawbacks and attractive features of some of these schemes are also discussed with their feasibility on a large-scale. As an environmentally benign and renewable resource, PS I may provide a new sustainable source of bioenergy.

Poster S8.8

Sustained hydrogen photoproduction by phosphorous-deprived marine green microalgae *Chlorella* C65.

Khorcheska A. Batyrova*, Anastasia I. Gavrisheva, Anatoly A. Tsygankov

Institute of Basic Biological Problems RAS, Pushchino, Moscow region 142290 Russia
* Tel: +7(496)773-27-91, Fax: +7(496)73-305-32, e-mail: horcheska@gmail.com

Different strains of photoautotrophic microalgae have the remarkable ability to reduce protons into H_2 using light energy. Consequently, renewable H_2 photoproduction can be achieved in these microorganisms using energy provided by solar irradiation and electrons derived from water. Previously it has been shown, that green microalgae *Chlamydomonas reinhardtii* deprived of sulfur are capable of prolonged H_2 photoproduction [1]. To the best of our knowledge sustained H_2 photoproduction by marine microalgae under sulfur deprivation was not shown. We hypothesized that marine microalgae grow in the sea water with high sulfur content. Artificial sea water also cannot produce S-deprived conditions due to high sulfur content in salts like NaCl which is added to the medium at high concentration. Besides sulfur deprivation, sustained H_2 photoproduction in *C. reinhardtii* cultures can be achieved under phosphorus-deprived conditions [2]. The physiological response of algal cultures to phosphorus deprivation demonstrated significant similarities with the response of algae to sulfur depletion. Similar to sulfur deprivation, the phosphorus deprivation limits O_2 evolving activity in algal cells and causes other metabolic changes that are favorable for H_2 photoproduction.

It the present study phosphorous deprivation was optimized for generation of hydrogen production in marine green microalgae *Chlorella* C65. The use of marine microalgae is also remarkable for the fact that we can utilize unlimited resources of sea water, whereas the resources of fresh water on earth are limited and in some regions, fresh water is predicted to become scarcer by 2020.

Two types of mediums were used to create high salinity conditions: Tris-Acetate-Phosphate (TAP) medium with addition of 30 g/L of pure NaCl (TAP/NaCl), and TAP medium where, instead of distilled water, marine water from the Black Sea was used (TAP/SW). Similar to the previous study of H_2 production under phosphorous deprived condition [2] in order to achieve H_2 photoproduction in P-deprived marine green algae C65, the dilution approach [3] was applied. Tris-Acetate-minus-Phosphate with NaCl (TA-P/NaCl) and TA-P based on sea water (TA-P/SW) media were inoculated with different quantities of cells (1–50%) withdrawn from a phosphorous-replete culture and washed once in ether TA-P/NaCl or TA-P/SW medium. Cultures diluted to about 0.5–1 μg Chl/mL in the beginning of P-deprivation were able to establish anaerobiosis. The H_2 accumulation was up to 40 ml H_2 gas per 1L of the culture and depended on the volume of vials. Impact of CO_2, light intensity and other factors to H_2 photoproduction by *Chlorella* C65 were investigated.

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Poster S8.9

Human photosynthesis?

Arturo Solís Herrera

Human Photosynthesis Study Center. López Velarde 108, Centro, Aguascalientes, Ags., México
* Tel: +524499160048, +524499150042, e-mail: comagua2000@yahoo.com

The dissociation of the water molecule by the chlorophyll in plant leaves began to loom by Jan Baptista van Helmont about 1643. It has been widely accepted although, to date, is not understood.

The first reaction in photosynthesis is the dissociation of the water molecule, producing diatomic hydrogen and oxygen. It is a process by which light energy is converted into chemical energy. However, the real value is the product of molecular hydrogen, because it is the quintessential carrier of energy in the entire universe, and on the other hand, oxygen is toxic at any concentration.

The reaction in plants can be written as follows: $2H_2O \rightarrow 2H_2 + O_2$

The plant expels oxygen to the atmosphere and hoards molecular hydrogen. Chlorophyll uses the ends of the visible light: purple and red, to carry out the dissociation, since it is a very expensive energetically reaction.

The laboratory is required to heat the water to two thousand degrees Celsius to separate water into its components.

To date it was not known that our body could conduct a similar chemical reaction, however, during an observational, descriptive study of changes in the morphology of the vessels of the optic nerve that occur in the three leading causes of blindness in the world, eventually identified the melanin had unsuspected intrinsic ability not only to dissociate the water molecule, but also to re-form it.

Melanin reaction can be written as follows: $2H_2O \leftrightarrow 2H_2 + O_2 + 4e^-$

For each two molecules of water re-formed 4 high-energy electrons are generated.

Melanin, being the darkest substance known, since it is able to absorb the entire electromagnetic spectrum, that is, from gamma to radio wave beams thus is many times more efficient than the chlorophyll.

And unlike chlorophyll, which requires being inside a cell to perform its function, melanin is capable of dissociating and re-form the molecule of water into and out of the cell.

Concluding: melanin is nothing less than human chlorophyll.

Poster S8.10

Construction of a photochemical system using PS II and a molecular wire equipped with a platinum nanoparticle

Shu Ikehira[1,*], Mariko Miyachi[1], Kyoko Okuzono[1], Yoshinori Yamanoi[1], Tatsuya Tomo[2] and Hiroshi Nishihara[1]

1 – University of Tokyo, Tokyo, Japan
2 – Tokyo University of Science, Tokyo, Japan
* Fax: +81(3)58414346, e-mail: ikehira@chem.s.u-tokyo.ac.jp

Photosynthesis in nature is a photochemical reaction which converts light energy to chemical energy. The quantum yield of photosynthesis is nearly 100%, and thus its combination with artificial systems will give a useful device using solar energy. Especially, the light absorption components, Photosystems I and II (PS I and PS II), are attractive materials for such devices. We previously reconstituted PS I by exchanging vitamin K1 in the redox cascade of PS I for an artificial molecular wire terminated with a gold nanoparticle. We developed a new bio-photosensor in the combination of the reconstituted PS I and FET.

In this research, we focused on PS II, isolated from *Thermosynechococcus elongatus*, which can extract electrons from water without sacrificial reagents. A platinum nanoparticle (PtNP) is introduced into PS II as a hydrogen evolution catalyst by the modified method based on PS I reconstitution. In the reconstituted PS II, a photo-excited electron is expected to transfer to a PtNP through a molecular wire to produce dihydrogen accompanied by oxygen evolution with electron extraction from water.

The molecular wire was designed for a structural analogue of plastoquinone, synthesized in 3 steps and connected to a PtNP surface through ligand substitution reaction. Amphipathicity of a PtNP provides easy modification of a molecular wire and reconstitution with PS II. The size of PtNPs was found to be 4.1±1.4 nm by the observation of TEM. The reconstitution of PS II will be presented.

Poster S8.11

Hydrogen photoproduction by mixed culture of *Rhodobacter sphaeroides* and *Clostridium butyricum*

Tatyana Laurinavichene[*], Evgeny Shastik and Anatoly Tsygankov

Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia
* Fax: +7(496)7731134, e-mail: laurinaoke@mail.ru

The application of mixed culture *Clostridium* + *Rhodobacter* seems to be promising for hydrogen production. To make the most of this system we examined the influence of carbon and nitrogen sources at different *Rb. sphaeroides*/*Cl. butyricum* ratio as compared to individual species. Hydrogen production by mixed culture in glucose + glutamate medium did not exceed that of *Rb. sphaeroides*, being 6 times that of *Cl. butyricum*. When using glucose + ammonium medium, the 1.4-times stimulation of hydrogen production by mixed culture over that of *Rb. sphaeroides* was shown. In the starch + glutamate medium *Rb. sphaeroides* did not produce hydrogen, whereas the mixed culture produced about 2.6-times more hydrogen as compared to *Cl. butyricum*.

Interrelations between two species during hydrogen production are of particular interest. In other studies the special methods for quantifying bacterial cells during the process have been suggested. However the current activity need not be in close agreement with cells number. We put forward the functional test to estimate the contribution of each species of the mixed culture to the hydrogen production at any phase of the process. The data suggest that the relative contribution of *Rb. sphaeroides* varied over a wide range depending on experimental conditions. In any case, the hydrogen production mediated by *Cl. butyricum* ceased within 3 days. The competition for glucose between two species during the hydrogen production was unlikely since the addition of glucose did not change their relative contribution to the hydrogen production.

This work was supported by RFBR (14-04-00246).

Poster S8.12 *(in absentia)*

Effects of ascorbate on Photosystem II during sulphur-deprivation of *Chlamydomonas reinhardtii*

Valéria Nagy[1], André Vidal-Meireles[1], Roland Tengölics[2], Gábor Rákhely[2], Győző Garab[1], László Kovács[1], Szilvia Z. Tóth[1,3]

1 – Biological Research Centre of the Hungarian Academy of Sciences, Szeged
2 – Department of Biotechnology, University of Szeged, Hungary
3 –Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany
* Fax: +36-62/433-434, e-mail: nagy.valeria@brc.mta.hu

In *Chlamydomonas reinhardtii* photosynthesis results in the release of H_2 that may be exploited as renewable energy. The hydrogenase enzyme is located at the acceptor side of photosystem I (PS I); it is highly efficient but very sensitive to O_2. Recently, we demonstrated that upon the addition of 10 mM ascorbate (Asc), linear electron transport from photosystem II (PS II) to the hydrogenase was significantly accelerated, leading to an approximately three-fold increase in H_2 production under sulphur-deprived conditions. This effect was ascribed partly to the PS II alternative electron donor function of Asc.

In the present study, we investigated several strains and found that externally supplied Asc promoted the establishment of anaerobiosis. Thermoluminescence measurements showed that oxygen-evolving complexes (OEC) became inactivated within a few hours after the addition of Asc, whereas Chl *a* fluorescence measurements suggested that PS II reaction centers remained functional for a prolonged period of time, enabling PS II to accept electrons from Asc; these findings were confirmed by Western blot analysis.

We also found that Asc had no major effect on respiration and the activity of hydrogenase. Overall, Asc was advantageous in a strain with modest H_2 production (i.e. our wild type strain, S-01), which has a relatively low respiration rate and low hydrogenase activity; in this strain Asc enhances H_2 production by promoting anaerobiosis and acting as a PS II electron donor. However, Asc had a negative effect on the H_2 production in CC-124, because in this strain respiration is highly active and can cope with relatively high levels of O_2; once OEC activity is reduced by Asc, H_2 production drops to the level of moderate H_2 producing strains.

Poster S8.13

Study the effect of copper on primary photosynthetic processes in the green microalga *Scenedesmus quadricauda* by using M-PEA-2 instrument

Daria A. Todorenko, T. K. Antal*, D. N. Matorin

Lomonosov Moscow State University, Faculty of Biology, Department of Biophysics, Moscow, Russia
* e-mail taras_an@mail.ru

Analysis of time resolved induction curves of chlorophyll fluorescence (OJIP transient) recorded by PEA (Plant Efficiency Analyzer) is a widely used approach to study photosynthetic reactions. A newly designed instrument M(Multi)-PEA-2 measures simultaneously light-induced kinetics of prompt and delayed fluorescence, as well as light absorbance at 820 nm (redox transitions of P700 in PS I) that allows to gain a comprehensive picture of primary photosynthetic events.

In the present paper we carried out investigations of copper sulfate effect on photosynthetic electron flow in the green alga *S. quadricauda* by using M-PEA-2. Changes in the fluorescence yield and light absorbance at 820 nm were induced by a 60 s pulse of red light of photon flux density 1000 μmol photon$\cdot m^{-2}\cdot s^{-1}$.

In our experiments a gradual decrease in F_v/F_m ratio was observed upon increase in copper sulfate concentration from 10 to 50 μM, indicating decrease in PS II photochemistry. Incubation of algal cells with copper significantly influenced the shape of the OJIP curve. Analysis of the OJIP curve by JIP-test showed disturbance of electron flow at the acceptor side of PS II that together with the reduced PS II photochemistry significantly slows down the rate of photochemical reduction of the plastoquinone pool. Moreover, we observed the increase in the rate of the initial fluorescence rise in the OJIP curve that may reflect destruction of the oxygen evolving complex in PS II. The amplitudes of the fast and slow peak in the kinetics of the delayed light emission were significantly reduced in the presence of copper, indicating low level of the membrane energization. The increase in the light absorbance signal at 820 nm which characterize the rate of P700 oxidation by PS I acceptor side and ferredoxin was slowed down in the cells treated by copper, suggesting impairment of electron flow via PS I and activation of the cyclic electron flow around PS I.

Section 9: Emerging Techniques for Studying Photosynthesis

Lecture S9.1

Photosynthetic performance indexes based on fast Chl *a* fluorescence induction data: advantages and limitations

Alexandrina Stirbet*

204 Anne Burras Ln, Newport News, VA 23606, USA
* e-mail: sstirbet@gmail.com

Performance indexes (PI) are algorithms that capture information contained in various parameters in one number. There is no one formula to design a PI, but they can be powerful tools when correct parameters are chosen and combined in a manner that supports the intended purpose of the index. In photosynthetic studies, fast Chl *a* fluorescence induction data are often used to characterize photosynthetic samples. A fluorescence parameter assumed to predict photosynthetic performances is the F_v/F_m ratio (where $F_v=F_m-F_0$ is the maximum variable fluorescence, F_0 the initial fluorescence measured on dark adapted sample, and F_m the maximum fluorescence), which is a proxy of the maximum quantum yield of Photosystem II photochemistry. Further, several photosynthetic performance indexes were defined by Strasser at al. (2000) [In: Yunus M, Pathre U, Mohanthy P (eds): Probing Photosynthesis, Mechanism, Regulation and Adaptation, Taylor & Francis, London, U.K, pp. 445–483], which incorporate 3 to 5 different fluorescence parameters. Here we discuss the advantages and limitations of these indexes, and compare the information on photosynthetic performance obtained with them with that given by the F_v/F_m ratio.

Poster S9.2

A red/blue light emitting diode intermittent light system for investigating fluctuation tolerance actions of respiratory chain proteins

Abdelghafar M. Abu-Elsaoud[1,2,*] and Allan G. Rasmusson[2]

1 – Dept. of Botany, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt
2 – Dept. of Biology, Lund University, Lund 22362, Sweden
* Fax. +46-462224113, e-mail: Abdoacacia@gmail.com

Light emitting diodes (LED) allow efficient high photosynthetically active light with a faster response and a more controlled environment. In the current study pulsed light modulation using light dimming system composed of red and blue light emitting diodes was designed and constructed. The pulsed light chambers were supported by an active ventilation system for better performance of LED lamps, and temperature and air quality control within the chamber. Pulsing was obtained using special electronic relay timers for controlling pulsing time; asymmetrical type was used to precisely adjust light/dark time ranges. Two types of pulsing light chambers were designed and constructed, one for soil-grown plants and the other for agar plate sterile cultures. Two *Arabidopsis thaliana* lines suppressed for alternative pathways in mitochondria were compared to *A. thaliana* Col-0 using current red/blue LED light system. *A. thaliana* genotypes were cultivated under various pulsed red/blue LED light (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) timings of 12 h day length. Different growth parameters were assessed including biomass, fresh and dry weights, root length, rosette diameter and leaf area. Maximum biomass was recorded in half-strength continuous red/blue LED light (70 $\mu mol \cdot m^{-2} \cdot s^{-1}$) followed by 5 minutes pulsed red/blue LED light. A comparative study were performed and assessed for successfully using both system in studying plant growth and photosynthesis under red/blue LED light. Combination of red and blue light emitting diodes supported by asymmetrical relay timers can be considered as an efficient tool for *Arabidopsis thaliana* growth and development.

Poster S9.3

Effect of point mutations on stability of bacterial photoreaction center: molecular dynamics studies

Azat G. Gabdulkhakov[1,*], V. G. Kljashtorny[1], T. Y. Fufina[2], L. G. Vasilieva[2] and Vladimir A. Shuvalov[2]

1 – Institute of Protein Research, 142290 Pushchino, Institutskaya 4, Moscow region, Russia
2 – Institute of Basic Biological Problems, 142290 Pushchino, Institutskaya 2, Moscow region, Russia
* Fax: +7(4967)318284, e-mail: azat@vega.protres.ru

Photosynthesis is the process of light energy transformation into a biologically usable form of chemical energy. Light is first absorbed by light-harvesting antenna complexes and the energy is then passed to the photosynthetic reaction centers (RC). RC of purple bacterium *Rhodobacter sphaeroides* is an integral membrane pigment-protein complex which consists of three protein subunits and ten cofactors of electron transfer. Cofactors are represented by four bacteriochlorophylls (BChl), two bacteriopheophytines, two ubiquinones, a molecule of carotenoid and a non-heme iron atom. BChl structure includes a central Mg atom, which considerably affects spectral properties of tetrapyrroles. The BChls microenvironment can greatly affects their photophysical and redox properties and can be altered by site-directed mutagenesis. Comparison of the crystal structures of mutant and wild type RCs can provide new information on interactions between protein and cofactors of electron transfer.

The Molecular Dynamics (MD) simulation method was used to preliminarily evaluate the impact of mutations in the RCs. We calculated 50 ns MD trajectories for the wild-type RC and mutants of RC. The all-atom simulations were performed with GROMACS software using NPT assemble and explicit water environment. The membrane parts of RC's were embedded to the "detergent belt" consisting of a LDAO (lauryldimethylamine oxide). Taking into account the results obtained, several mutants had been chosen for further spectral analysis and crystallization.

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Poster S9.4

Some unusual applications of chlorophyll fluorescence

Hazem M. Kalaji[1,*], Magdalena D. Cetner[1], Izabela A. Samborska[1], Izabela Łukasik[1], Vasilij Goltsev[2]

1 – Department of Plant Physiology, Warsaw University of Life Sciences SGGW-Nowoursynowska 159, 02-776 Warsaw, Poland
2 – Department of Biophysics and Radiobiology, Faculty of Biology, St. Kl. Ohridski University of Sofia, 8 Dragan Tzankov Blvd., Sofia, 1164, Bulgaria
* e-mail: hazem@kalaji.pl

Chlorophyll fluorescence signals have been used mainly for detection and monitoring the effect of different stressors on the performance of photosynthesizing organisms. Moreover, much biological and physiological research has been conducted to understand the functions of different components of photosynthetic apparatus, especially Photosystems I and II. In this study, some other aspects of this issue were investigated, including some examples where chlorophyll fluorescence analysis has been applied as the perfect solution in practical and everyday life applications, such as boats construction, national ecological/anti-terrorist alarms, water desalinization, stadium construction, food and flower production, and fertilization dosage optimization.

Poster S9.5

Production of singlet oxygen in cultured *Symbiodinium* cells, the photosyenthetic partner of the coral *Pocillopora damicornis*

Ateeq ur-Rehman[1], Zsuzsanna Deák[1], Anthony Larkum[2], Peter J. Ralph[2] and Imre Vass[1]

1 – Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
2 – Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney, Australia
* e-mail address: ateeq@brc.hu

Coral bleaching involves the expulsion of *Symbiodinium* cells from its host and resulting the breakdown of the coral and algae symbiosis, provides an opportunity to understand the molecular mechanism of this symbiotic association. In this study, we report a precise detection of singlet oxygen ($^1O_2$) production in the *Symbiodinium* cells and in intact coral *Pocillopora damicornis*. Singlet oxygen ($^1O_2$) is one of the most important reactive oxygen species (ROS) in photosynthetic systems. It is produced via interaction of molecular O_2 with ^{3}Chls in the Photosystem II (PS II) reaction center, as well as in the light harvesting antenna. Recently we have developed a method for detection of $^1O_2$ by His-mediated O_2 uptake in intact cyanobacterial cells [1]. The method is based on chemical trapping of $^1O_2$ by histidine, which leads to O_2 uptake during illumination that can be detected and quantified by commercial oxygen electrodes. We have employed chemical trapping technique and observed oxygen uptake in intact *Symbiodinium* cells, during illumination in the presence of histidine. His-mediated O_2 uptake data demonstrates the production of $^1O_2$, and the results show that O_2 uptake was enhanced during the thermal and light stress conditions. Recently we showed that the inhibition of the Calvin-Benson cycle by glycolaldehyde and potassium cyanide during thermal stress in *Symbiodinium* cells promotes $^1O_2$ formation [2]. Our study reveals that heat and light stress induce photo-inactivation of PS II and enhance $^1O_2$ production, while histidine provides protection against PS II photo-inactivation and pigment bleaching. Therefore, the inactivation of PS II and enhanced production of $^1O_2$ by heat and light stresses promotes bleaching event in cultured *Symbiodinium* cells. Based on our results, $^1O_2$ induced inactivation of *Symbiodinium* cells may be involved in triggering the expulsion of *Symbiodinium* cells from the coral host, which leads to coral bleaching.

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Poster S9.6

Fluorescence lifetime analysis of infrared-fluorescent protein variants derived from a bacterial phytochrome, RpBphP2

Thomas Friedrich*, Franz-Josef Schmitt and Neslihan N. Tavraz

Technical University of Berlin, Institute of Chemistry Sekr. PC 14, Max-Volmer-Laboratory for Biophysical Chemistry, Straße des 17. Juni 135, D-10623 Berlin, Germany
* Fax: +49 30 31478600, e-mail: friedrich@chem.tu-berlin.de

Bacterial phytochromes (BphPs) are red/far-red absorbing, water-soluble photoreceptors belonging to the superfamily of phytochromes with members in plants, algae, fungi and (cyano-)bacteria. Phytochromes share a characteristic domain structure composed of an N-terminal photosensory core domain (PAS-GAF) and a C-terminal, phytochrome-specific PHY transduction domain, which is followed, in many cases, by a histidin kinase output module. The PAS-GAF domain covalently binds the open-chain tetrapyrrole cofactor bilin (biliverdin IXα, BV, in bacteria) in an autocatalytic reaction via a thioester bond to a conserved cysteine at the N-terminus of the PAS domain. BphPs undergo reversible photoconversion between two stable states, a red-absorbing Pr state (680–710 nm), and a far red-absorbing Pfr state (740–760 nm), which can be interconverted by light of appropriate wavelengths.

Recent years have seen rapid development of phytochrome derivatives as spectroscopic tools and photoswitches for high-resolution fluorescence microscopy as well as photoacoustic microscopy and tomography. Whereas the fluorescence quantum yield (FQ_y) of "natural" phytochromes is low (1–2%), recent studies used the *Rhodopseudomonas palustris* bacteriophytochromes RpBphP2 and RpBphP6 for FQ_y optimization resulting in novel spectroscopic tools termed iRFPs with FQ_y values up to 11%, whereas other studies showed the applicability of phytochrome derivatives for bimolecular fluorescence complementation, and the photoswitching properties have been exploited to establish novel tools of optical superresolution microscopy.

In this work, we attempted to elucidate the molecular determinants underlying the increased FQ_y in iRFP713 derived from RpBphP2. Resonance Raman spectroscopy in conjunction with QM/MM calculations has provided first clues showing that the FQ_y increase relies on the slowing down of competing processes such as photoisomerisation and internal conversion of the excited state. This is due to reducing the stabilization of the distorted chromophore configuration in the excited state and a perturbation of a local H-bond network in the vicinity of the BV chromophore that lowers the probability of a thermal deactivation channel. Moreover, the chromophore embedment into the protein

matrix was found to be more rigid, in effect stabilizing the Pr ground state of the molecule (Biochemistry 53(1):20-29, 2014). Furthermore, we measured time-resolved fluorescence spectra and decay-assiciated spectra of iRFP713 and mutants thereof, and its predecessor, RpBphP2, at various temperatures down to 10K. In effect, iRFPs and other phytochrome derivatives are emerging tools for novel microscopic techniques with interesting perspectives for the use in photosynthesis research.

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+7 (499) 788-78-56
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