



## 11<sup>th</sup> International Conference

### Photosynthesis and Hydrogen Energy Research for Sustainability

*in honor of*

*Robert Blankenship (USA), Gyozo Garab (Hungary), Michael Graetzel (Switzerland), Norman Huner (Canada), and Gunnar Oquist (Sweden)*

July 03–09 2023

İstanbul, Türkiye

# ABSTRACTS AND PROGRAMME



**International Conference “Photosynthesis and Hydrogen Energy Research for Sustainability-2023:** in honor of Robert Blankenship (USA), Gyozo Garab (Hungary), Michael Graetzel (Switzerland), Norman Huner (Canada), and Gunnar Oquist (Sweden)

**Editors:** Hüseyin Günhan Özcan, Gürkan Soykan, Nezihe Yıldırım, Selen Yoldaş, Girayhan Yılmaz, Aras İyitanır, Suleyman Allakhverdiev

The volume contains abstracts of the lectures and poster presentations at 11th International Meeting «Photosynthesis and Hydrogen Energy Research for Sustainability – 2023», in honor of Robert (Bob) Blankenship (USA), Gyozo Garab (Hungary), Michael Graetzel (Switzerland), Norman (Norm) Huner (Canada), and Gunnar Oquist (Sweden), which was held on July 3 – 9, 2023, in Istanbul, at Bahcesehir University.

Experimental and theoretical works covering a wide range of photosynthetic and biohydrogen topics, from the primary processes of electron transfer and energy bioconversion to the physiological aspects of photosynthesis and applied aspects of hydrogen production are discussed at the conference. Considerable attention is paid to discussion of structural organization of photosynthetic reaction centers, structure and function of photosystems, artificial photosynthesis, mechanisms of hydrogen production etc. The book will be of interest to researchers and students involved in the study of photosynthesis and bio-hydrogen production



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## Committee's Note

We cordially invite you to participate in this outstanding occasion, the 11th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability-2023: in honor of Robert Blankenship, Gyozo Garab, Michael Graetzel, Norman Huner, and Gunnar Oquist," which will be held at the prestigious Future Campus of T.C. Bahcesehir University in Istanbul, Turkey. Organized under the Faculty of Engineering and Natural Sciences, this Conference serves as a prestigious platform for scholars, researchers, and industry professionals from all corners of the world to convene.

The Conference embraces a profound significance by stimulating vibrant debates on previous, current, and future research endeavors concerning photosynthesis, hydrogen, encompassing a broad spectrum of molecular to global perspectives. Furthermore, it delves into the diverse realm of biohydrogen production, exploring studying processes and applied aspects, while providing a unique opportunity to engage with eminent researchers dedicated to furthering photosynthesis and biohydrogen studies.

With a focus on information exchange and professional growth, this Conference offers an engaging venue for students, postdoctoral fellows, and scientists from diverse nations. It promotes intellectual horizons extension by nurturing meaningful connections, and fostering collaboration through joint projects and informal partnerships. The Conference program encompasses a wide range of captivating topics, divided into two main subjects: photosynthesis and biohydrogen.

The photosynthetic section provides a thorough examination of fundamental processes, revealing the complexities of the structure, function, and biogenesis of the photosynthetic apparatus, as well as the enlightening elements of photosystem I and II. Additionally, it delves into the intriguing realm of water oxidation mechanisms, artificial photosynthesis, the regulation of photosynthesis under environmental stress, applied aspects of photosynthesis, and emerging techniques with enormous potential.

The biohydrogen section is equally enthralling, focusing on the outstanding advancements in biological hydrogen production, exploring the complexities of hydrogenases and the fascinating domain of artificial photosynthesis for hydrogen energy. The conference, further discusses hydrogen purification and storage, the dynamics of the hydrogen economy, and the significance of hydrogen energy education. Moreover, developing methodologies dedicated to studying hydrogen energy will be highlighted, illuminating new avenues for future research.

The conference thrives on its interdisciplinary orientation, embracing a harmonious blend of topics and compelling lectures. Throughout the event, participants will have the opportunity to interact with over 110 captivating presentations, including lectures and posters.

As we anticipate this momentous gathering, we eagerly look forward to a week filled with inspiring presentations, enthralling discussions, and invaluable insights into the fields of science and research. Together, let us embark on a truly engaging journey, defining the future of photosynthesis and hydrogen energy research for sustainability.

Suleyman Allakhverdiev  
Yücel Batu Salman  
Girayhan Yılmaz



**11<sup>th</sup> International Conference of Photosynthesis and Hydrogen Energy Research for Sustainability Conference Program**

**3-9 July 2023**

03.07.2023	BAU FUTURE CAMPUS	
13:00	OPENING CEREMONY	<b>SULEYMAN ALLAKHVERDIEV</b> (RUSSIA) – (COORDINATOR) <b>JULIAN J. EATON-RYE</b> (NEW ZELAND) (ONLINE) – (CHAIRMAN) <b>GÜRKAN SOYKAN</b> (TÜRKİYE) – (HEAD OF THE ENER. SYS. DEP.) <b>TATSUYA TOMO</b> (JAPAN) – (SECRETARY) <b>GOVINDJEE</b> (USA) (ONLINE) – (ADVISOR)
	PLENARY LECTURES	CHAIRS: <b>GYŐZŐ GARAB</b> (HUNGARY) <b>SULEYMAN ALLAKHVERDIEV</b> (RUSSIA/TURKEY)
13:30	GUNNAR ÖQUIST (SWEDEN)	Breakthrough Research and the Nobel Prize
14:00	ROBERT BLANKENSHIP (USA)	More than 50 years of Photosynthesis: My Scientific Journey (ONLINE)
14:40	İBRAHİM DİNÇER (CANADA/TURKEY)	Introduction of Hydrogen 1.0: A New Age and Discussion of Recent Developments in Green Hydrogen Production
15:40 16:00	<b>COFFEE/THE BREAK</b>	
	PLENARY LECTURES	CHAIRS: <b>İBRAHİM DİNÇER</b> (CANADA/TURKEY) <b>TATSUYA TOMO</b> (JAPAN)
16:00	GYŐZŐ GARAB (HUNGARY)	Challenges of stability and flexibility of photosynthetic machineries
16:40	MICHAEL GRAETZEL (SWITZERLAND)	Solar hydrogen generation and artificial photosynthesis (ONLINE)
17:20	NORM HUNER (CANADA)	Photosynthetic Adaptation to Stress Induces Multicellularity in the Antarctic

	Psychrophile, <i>Chlamydomonas priscuii</i> (ONLINE)
<b>18:00</b>	<b>SPECIAL EVENT</b>
<b>19:00</b> <b>21:00</b>	<b>WELCOME PARTY</b>

<b>04.07.2023</b>	<b>BAU FUTURE CAMPUS</b>	
	<b>PLENARY LECTURES</b>	<b>CHAIRS: KENTARO IFUKU</b> (JAPAN) <b>LORENZO FERRONI</b> (ITALY)
<b>09:00</b>	THOMAS D. SHARKEY (USA)	Cytosolic carbon metabolism pathways that support the Calvin-Benson-Bassham cycle
<b>09:40</b>	MAHDI NAJAFPOUR (IRAN)	Water-oxidation Reaction in the Presence of Manganese Compounds: How do Manganese Compounds Oxidize Water in Artificial Photosynthetic Systems?
<b>10:20</b>	MARC NOWACZYK (GERMANY)	Structural insights into photosystem II assembly
<b>11:00</b> <b>11:30</b>	<b>COFFEE/THE BREAK</b>	
	<b>PLENARY LECTURES</b>	<b>CHAIRS: THOMAS D. SHARKEY</b> (USA) <b>KEISUKE KAWAKAMI</b> (JAPAN)
<b>11:30</b>	YUTAKA SHIBATA (JAPAN)	Cryo-optical microscopy study on regulation mechanism of photosynthetic light harvesting
<b>12:10</b>	JAN KERN (USA)	Sequence of events during the water oxidation reaction in Photosystem II visualized by time resolved X-ray studies
<b>12:50</b>	IFTACH YACOBY (ISRAEL)	Ambient H <sub>2</sub> production from green algae, achievements, and challenges

<b>13:30 14:30</b>	<b>LUNCH</b>	
	<b>INVITED LECTURES</b>	<b>CHAIRS: BARRY BRUCE (USA) MARC NOWACZYK (GERMANY) MAHDI NAJAFPOUR (IRAN)</b>
<b>14:30</b>	STEFANO SANTABARBARA (ITALY)	Photoinduced triplet state in the chlorophyll-d based photosystem I of <i>Acaryochloris marina</i>
<b>15:00</b>	RAJAGOPAL SUBRAMANYAM (INDIA)	High light-induced changes in whole-cell proteomic profile and its correlation with the organization of thylakoid supercomplex in cyclic electron transport mutants of <i>Chlamydomonas reinhardtii</i>
<b>15:30</b>	CRISTOPHER GISRIEL (USA)	Structure-function relationships of far-red light-absorbing allophycocyanins
<b>16:00</b>	ANNA FRANK (GERMANY)	Rational Design of Biophotoelectrodes for <i>In Vitro</i> Biocatalysis
<b>16:15 16:30</b>	<b>COFFEE/THE BREAK</b>	
<b>16:30</b>	<b>POSTERS</b>	<b>CHAIRS: RAJAGOPAL SUBRAMANYAM (INDIA) STEFANO SANTABARBARA (ITALY) KOSTAS STAMATAKIS (GREECE) MARIA BORISOVA-MUBARAKSHINA (RUSSIA)</b>

05.07.2023	BAU FUTURE CAMPUS	
	<b>INVITED LECTURES</b>	<b>CHAIRS: YUTAKA SHIBATA (JAPAN)</b> <b>ANJANA JAJOO (INDIA)</b> <b>BEKJAN KOSSALBAYEV (KAZAKHSTAN/JAPAN)</b>
<b>09:00</b>	LORENZO FERRONI (ITALY)	Chloroplast and photosynthetic acclimation in a lycophyte, <i>Selaginella Martensii</i> : a window on the photosynthesis of ancient vascular plants?
<b>09:30</b>	TATSUYA TOMO (JAPAN)	Red-chlorophyll in photosystem I
<b>10:00</b>	YOSHIFUMI UENO (JAPAN)	Molecular mechanisms of state transition in a glaucophyte <i>Cyanophora paradoxa</i>
<b>10:30</b>	ARVI FREIBERG (ESTONIA)	Exiton prominence in photosynthetic color-tuning and light-harvesting
<b>11:00</b> <b>11:30</b>	<b>COFFEE/THE BREAK</b>	
	<b>INVITED LECTURES</b>	<b>CHAIRS: AGEPATI S. RAGHAVENDRA (INDIA)</b> <b>JAN KERN (USA/GERMANY)</b> <b>CHRISTOPHER GISRIEL (USA)</b>
<b>11:30</b>	KEISUKE KAWAKAMI (JAPAN)	Structure of the far-red light utilizing photosystem I of <i>Acaryochloris marina</i>
<b>12:00</b>	FRANCESCO FRANZIA (ITALY)	Use of bacterial photosynthetic vesicles to evaluate the effect of ionic liquids on the permeability of biological membranes

<b>12:30</b>	KENTARO IFUKU (JAPAN)	The extrinsic subunits of photosystem II optimizing the oxygen-evolving reaction
<b>13:00</b>	KOSTAS STAMATAKIS (GREECE)	Enhanced hydrogen production of <i>Synechococcus</i> sp. PCC 7942 cells
<b>13:30 14:30</b>	<b>LUNCH</b>	
	<b>INVITED LECTURES</b>	<b>CHAIRS: FRANCESCO FRANCIA (ITALY) MAREK ŽIVČÁK (SLOVAKIA) KOSTAS STAMATAKIS (GREECE)</b>
<b>14:30</b>	BARRY BRUCE (USA)	Evolutionary changes in the oligomeric states of photosystem I Three- Two- Four- One
<b>15:00</b>	MARIA BORISOVA-MUBARAKSHINA (RUSSIA)	Examining the Impact of Stress Conditions on the Functionality of the Plastoquinone Pool in Higher Plant Chloroplasts
<b>15:30</b>	SULEYMAN ALLAKHVERDIEV (RUSSIA)	Artificial Photosynthesis, an Energy Technology of the Future
<b>16:30 17:00</b>	<b>COFFEE/THE BREAK</b>	
<b>17:00</b>	<b>POSTERS</b>	<b>CHAIRS: RAJAGOPAL SUBRAMANYAM (INDIA) STEFANO SANTABARBARA (ITALY) KOSTAS STAMATAKIS (GREECE) MARIA BORISOVA-MUBARAKSHINA (RUSSIA)</b>

06.07.2023	BAU FUTURE CAMPUS	
	<b>INVITED LECTURES</b>	<b>CHAIRS: IFTACH YACOBY (ISRAEL)</b> <b>RAJAGOPAL SUBRAMANYAM (INDIA)</b>
<b>09:00</b>	AGEPATI S. RAGHAVENDRA (INDIA)	Redox signals between cellular organelles to modulate photorespiratory enzymes in pea leaves
<b>09:30</b>	MAREK ŽIVČÁK (SLOVAKIA)	Monitoring activity and regulation of electron transport at PSI to uncover specific stress responses in crop plants
<b>10:00</b>	ONDREJ DLOUHY (CZECH REPUBLIC)	Role of non-lamellar lipid phases in the structure and function of plant thylakoid membranes
<b>10:30</b> <b>11:00</b>	<b>COFFEE/THE BREAK</b>	
<b>11:00</b>	MILAN SZABO (HUNGARY)	Alternative electron transport pathways in microalgae, as reflected by flash-induced Chl- <i>a</i> fluorescence relaxation
<b>11:30</b>	VACLAV KARLICKY (CZECH REPUBLIC)	Acclimation of photosynthetic apparatus to different light intensities in norway spruce
<b>12:00</b> <b>13:00</b>	<b>LUNCH</b>	

<b>13:00</b>	<b>POSTERS</b>	<b>CHAIRS: RAJAGOPAL SUBRAMANYAM (INDIA) STEFANO SANTABARBARA (ITALY) KOSTAS STAMATAKIS (GREECE) MARIA BORISOVA- MUBARAKSHINA (RUSSIA)</b>
<b>13:00 14:00</b>	<b>Lunch</b>	
<b>14:00</b>	IRADA HUSEYNOVA & ISMAIL ZULFUGAROV (AZERBAIJAN)	Interaction between the thylakoid protein phosphorylation and the energy-dependent quenching of chlorophyll fluorescence in plants
<b>14:30</b>	VASILY V. PTUSHENKO (RUSSIA)	The low temperature-acclimated green microalgae <i>Lobosphaera incisa</i> reveals significant nonphotochemical quenching, minor changes in VAZ pool size, prolonged expression of PsbS-encoding gene and decreased ROS production
<b>15:00</b>	BOLATKHAN ZAYADAN (KAZAKHSTAN)	Potential cultures of cyanobacteria as feedstock for biohydrogen production
<b>15:30 16:00</b>	<b>COFFEE/THE BREAK</b>	
<b>16:00</b>	NATALIA N. RUDENKO (RUSSIA)	The participation of thylakoid carbonic anhydrases in photosynthesis of higher plants
<b>16:30</b>	<b>POSTERS</b>	<b>CHAIRS: RAJAGOPAL SUBRAMANYAM (INDIA) STEFANO SANTABARBARA (ITALY) KOSTAS STAMATAKIS (GREECE) MARIA BORISOVA- MUBARAKSHINA (RUSSIA)</b>

07.07.2023	BAU FUTURE CAMPUS	
	<b>INVITED LECTURES</b>	<b>CHAIRS: ONDREJ DLOUHY (CZECH REPUBLIC) MILAN SZABO (HUNGARY) ARVI FREIBERG (ESTONIA)</b>
<b>09:00</b>	ANJANA JAJOO (INDIA)	Arbuscular Mycorrhizal fungi (AMF) protects photosynthetic apparatus of wheat under drought stress
<b>09:30</b>	BEKJAN KOSSALBAYEV (KAZAKHSTAN)	Study and optimization of hydrogen production of cyanobacterial species isolated from different ecosystems of the Kazakhstan
<b>10:00</b>	DMÍTRY DUNIKOV (RUSSIA)	Heat and mass transfer in a metal hydride reactor for hydrogen storage and purification
<b>10:30 11:00</b>	<b>COFFEE/THE BREAK</b>	
<b>11:00</b>	MOHAMMAD YUSUF ZAMAL (INDIA)	Mitigation effect of high light on the photosynthetic apparatus of Rhodobacter alkalitolerans when grown in an alkaline environment
<b>11:20</b>	GERGELY NAGY (HUNGARY)	The periodic organization and structural plasticity of thylakoid membranes as revealed by small-angle neutron scattering. (ONLINE)
<b>12:00 13:00</b>	<b>LUNCH</b>	
<b>13:00</b>	LECTURES BY YOUNG RESEARCHERS 6 PERSON X 10 MÍN	<b>CHAIRS: RAJAGOPAL SUBRAMANYAM (INDIA) STEFANO SANTABARBARA (ITALY) KOSTAS STAMATAKIS (GREECE) MARIA BORISOVA-MUBARAKSHINA (RUSSIA)</b>



<b>14:00</b>	<b>CERTIFICATE DISTRIBUTION CEREMONY</b>
<b>14:30</b>	<b>CLOSING CEREMONY</b>

<b>08.07.2023</b>	<b>ISTANBUL EUROPEAN SIDE</b>
<b>12:00</b>	<b>ALL DAY-TOUR</b>
<b>17:00</b>	<b>BANQUET - GOOD BYE PARTY</b>

<b>09.07.2023</b>	<b>BAU FUTURE CAMPUS, ISTANBUL</b>
<b>10:00</b>	<b>WORKSHOP AT BAU FUTURE CAMPUS</b>
<b>12:00 13:00</b>	<b>LUNCH</b>
<b>13:00</b>	<b>FREE TIME</b>

## **BREAKTHROUGH RESEARCH AND THE NOBEL PRIZE**

Gunnar ÖQUIST

Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, S-90187 Umeå,  
Sweden

*E-mail: [gunnar.oquist@umu.se](mailto:gunnar.oquist@umu.se)*

Breakthrough research is distinguished by a higher potential for shifting or invalidating paradigms thus opening up for new opportunities for science, technology and society. Analyses show that national science policies over time have favoured incremental or consolidating research rather than transformative or disruptive research with a higher potential to result in scientific breakthroughs. What kind of science policy is required to increase the probability of scientific breakthroughs? Is it more research organized through strategic, more or less targeted top-down organized programs, or is it the development of environments where individuals are trusted with the freedom to pioneer in the pursuit of their own ideas? We need both types of research since none of them can in the long run be successful without the support of the other. We need incremental research to focus on solving problems within the conceptual framework of our time. But to nurture excellence in research of the highest transformative potential, we need to invest more in research that has a higher probability to open up for new discoveries and solutions. For that we need more scientists that are trusted with the individual, long-term freedom to explore and take risks. We need more of the kind of pioneering research that question accepted views and perspectives, research that addresses difficult problems with a greater potential to open up for new opportunities. In my talk, I will discuss how to foster more of breakthrough research that may qualify for a Nobel Prize. This is what we need to give our societies a better chance to successfully deal with present and future global challenges to mankind. In fact, the pressing need for excellence of the highest distinction has never been greater in the history of mankind. I will finish by describing the process behind identifying breakthrough research findings worthy of a Nobel Prize.

## MORE THAN 50 YEARS OF PHOTOSYNTHESIS: MY SCIENTIFIC JOURNEY

Robert E. BLANKENSHIP

Lucille P. Markey Distinguished Professor of Arts and Sciences, Emeritus  
Departments of Biology and Chemistry, Washington University in St. Louis, St. Louis, MO 63130,  
USA  
*REBlankenship@gmail.com*

I was born and grew up in southeast Nebraska, USA. After undergraduate studies in chemistry at Nebraska Wesleyan University in Lincoln, my scientific journey began in 1970 as a chemistry graduate student at the University of California, Berkeley. I chose Kenneth Sauer as my adviser, and my PhD thesis work centered on the role of Mn in photosynthetic oxygen evolution. Most of my thesis work was done using EPR as a technique and utilized higher plant chloroplasts as a system. After a brief but exciting interlude at the American University of Beirut in 1975, I became a postdoctoral fellow with William Parson at the University of Washington in Seattle. My work there used rapid kinetic optical spectroscopy and the system studied was isolated reaction centers from purple photosynthetic bacteria. In 1979 I took an Assistant Professor position at Amherst College in Massachusetts. While there I became introduced to the filamentous green bacterium *Chloroflexus aurantiacus* and the newly discovered organism *Heliobacterium chlorum*, through collaborations with R. Clinton Fuller. During this time, I developed an interest in the origin and early evolution of photosynthesis, which has continued throughout my career. In 1985, I moved to Arizona State University, where I stayed for 21 years. During that time, my research was focused on chlorosome antenna complexes from green bacteria and reaction centers from heliobacteria and also primary photochemistry in Photosystem I of cyanobacteria. Much of this work utilized ultrafast optical spectroscopy. In 2006 I moved to Washington University in St. Louis, where I stayed until my retirement in 2019. In St. Louis, I worked on both antenna complexes and reaction centers from a wide range of organisms. A significant portion of my work there involved using advanced mass spectroscopic techniques in collaboration with Michael Gross. During my career, I have mentored 30 PhD students, 13 MS students, 34 postdoctoral fellows and hundreds of undergraduate researchers. I wish to thank all my former students, collaborators and other associates for all their great work and friendship over more than 50 years studying photosynthesis.

## **INTRODUCTION OF HYDROGEN 1.0: A NEW AGE AND DISCUSSION OF RECENT DEVELOPMENTS IN GREEN HYDROGEN PRODUCTION**



**Name: Ibrahim Dincer**

**Organisation: Ontario Tech. University, Oshawa, Ontario**

**Title: Professor**

President, Hydrogen Technologies Association

Editor-in-Chief, Energy Storage

Editor-in-Chief, International Journal of Exergy

Editor-in-Chief, International Journal of Global Warming

Editor-in-Chief, International Journal of Research, Innovation and Commercialisation

Special Issues Coordinating Editor, International Journal of Hydrogen Energy

### **Biography:**

Ibrahim Dincer is a full professor of Mechanical Engineering at Ontario Tech. University. Renowned for his pioneering works in the area of sustainable energy technologies he has authored/co-authored many books and book chapters, along with many refereed journal and conference papers. Dr. Dincer has chaired many national and international conferences, symposia, workshops and technical meetings. Dr. Dincer has delivered many keynotes and invited lectures. Dr. Dincer is an active member of various international scientific organizations and societies, and serves as editor-in-chief, associate editor, regional editor, and editorial board member on various prestigious international journals. Dr. Dincer currently serves as President for Hydrogen Technologies Association in Turkey and Chair for Energy Working Group in Turkish Academy of Sciences. Dr. Dincer is a recipient of several research, teaching and service awards, including the Premier's research excellence award in Ontario, Canada. During the past nine years he has been recognized by Thomson Reuters as one of the Most Influential Scientific Minds in Engineering and one of the most highly cited researchers. During the past 25 years Dr. Dincer's research and activities have been diverse and primarily focussed on sustainable energy solutions, sustainable communities and cities, district energy systems, green buildings, renewable energy technologies, energy storage technologies, hydrogen energy technologies, and waste to energy technologies. His group has developed various novel technologies for commercialization. He is known for his engineering education related talks as a committed educator.

**Abstract:**

This plenary talk will highlight the importance of a new age with hydrogen (so-called: Hydrogen 1.0) and evaluate the challenges and opportunities of this new era after hydrocarbon age. The talk will also discuss recent developments in the areas of green hydrogen production and related technologies along with sectoral needs.

**CHALLENGES OF STABILITY AND FLEXIBILITY OF PHOTOSYNTHETIC MACHINERIES**

Győző GARAB<sup>1,2</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

<sup>2</sup>Department of Biophysics, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

*E-mail: garab.gyozo@brc.hu*

During my half a century in photosynthesis research, I had the privilege to participate in numerous prestigious collaborations – with excellent scientists and enthusiastic, talented young researchers, in intellectual friendly atmosphere – on topics ranging from the primary processes and model systems to stress physiology and ecology. This was a great experience and a gift from Life. With the treasured support from my research partners, I could also afford the luxury of having a few ‘pet’ topics, a bit of our own, keeping us busy for many years. In the most challenging projects we tried to find the answers for the duality of robustness and plasticity of the photosynthetic machineries at different levels of complexity.

We have established that LHCII and PSII assemble into structurally flexible macrodomains with long-range chiral order; their roles in membrane energization and regulatory processes remain to be clarified. The same holds true for the biological thermo-optic effect, light-induced reversible reorganizations driven by dissipation of excess excitation energy. The quasi-helical organization of the granum-stroma thylakoid membranes (TMs) is evolutionary the most successful membrane system, which exhibit remarkable plasticity with largely unknown mechanisms. Part of TMs’ structural plasticity might be ‘borrowed’ from the marked polymorphism of their bulk lipids, which can only be interpreted by extending the fluid-mosaic bilayer membrane model; this, in turn, poses questions about some fine but potentially important details of the energization of TMs. The primary functions of PSII can be understood based on the (static) atomic resolution structure; nonetheless the reaction center matrix exhibits remarkable structural / functional dynamics, the exact nature, mechanism and significance of which are not well understood.

After all these years and efforts, I have to realize that John Green is perfectly right about science: “as you learn, you don’t really get answers; you just get better questions”.

# MESOSCOPIC PHOTOSYSTEMS FOR THE GENERATION OF ELECTRICITY AND FUELS FROM SUNLIGHT

Michael GRAETZEL

Laboratory of Photonics and Interfaces, Ecole Polytechnique Federale de Lausanne, Lausanne, 1015, Switzerland

*E-mail: michael.graetzel@epfl.ch*

Learning from the concepts used by green plants photosynthesis, we have developed molecular photosystems affording efficient solar light harvesting and conversion to electricity and fuels. Solar cells using dyes, semiconductor quantum dots or perovskite pigments as light harvesters have emerged as credible contenders to conventional silicon cells photovoltaic devices. Dye sensitized solar cells (DSCs) were the first to use a three-dimensional mesoscopic junction for solar electricity production. The power conversion efficiency for DSC's is currently 15.1 % in direct sunlight and 35 % in ambient light. DSCs are simple and inexpensive to manufacture and they possess unique practical advantages including flexibility and transparency. These features along with excellent long-term stability have fostered first commercial applications large scale industrial production. Dye sensitized solar cell have engendered the advent of perovskite solar whose rapid efficiency rise from 3 % to over 25 % has stunned the photovoltaic community. Due to their exceptional performance, they are presently being intensively investigated as one of the most promising future PV technology. We have applied these fundamentally new concepts to realize highly efficient generation of hydrogen and reduction of carbon dioxide to ethylene and ethanol by sunlight using water as electron source mimicking fuel generation by natural photosynthesis.

## Biography



**Michael Graetzel** is a Professor in School of Chemical Science & Engineering at the Ecole Polytechnique Fédérale de Lausanne (EPFL). He received his PhD from the Technical University in Berlin in 1971. After a postdoctoral training at the University of Notre Dame Indiana, USA, he joined EPFL since 1977. He published over 1700 peer-reviewed scientific papers regarding the conversion of solar energy to electricity and chemical fuels and storage of electricity in batteries. He pioneered research on energy and electron transfer reactions in mesoscopic systems and their use to generate electricity and fuels from sunlight. He is credited with moving the solar cell field beyond the principle of light absorption via diodes to the molecular level exploiting the sensitization of 3-dimensional networks of wide band gap semiconductor oxides nanoparticles by dyes, pigments or semiconductor nanocrystals for light energy harvesting. His dye-sensitized solar cells engendered the advent of perovskite solar cells the most exciting break-through in the recent history of photovoltaics. Current research focuses on dye sensitized and perovskite solar cells as well as the photoelectrochemical generation of hydrogen and reduction of carbon dioxide semiconductor. He received numerous honors and awards including the BBVA Frontiers of Knowledge Award in Basic Science, Millennium

Technology Grand Prize, the Marcel Benoist Prize, the King Faisal International Science Prize, the Albert Einstein World Award of Science, the Balzan Prize as well as honorary doctor's degree from 11 European and Asian Universities. He is an elected member of the European Academy of Science, the German Academy of Science (Leopoldina) and the Swiss Academy of Technical Sciences as well as several other learned societies. A recent bibliometric ranking by Stanford University places Graetzel in the first position on top of a list of 100'000 world-wide leading scientists across all fields.

## **PHOTOSYNTHETIC ADAPTATION TO STRESS INDUCES MULTICELLULARITY IN THE ANTARCTIC PSYCHROPHILE, *CHLAMYDOMONAS PRISCUII***

Norman P.A. HÜNER

Department of Biology, Western University, London ON, Canada N6A 5B7

The psychrophile, *Chlamydomonas priscuii*, is endemic to Lake Bonney, Antarctica and in culture exists as motile, single cells as well as immobile, multicellular palmelloids. Palmelloids are developmental structures associated with the cell cycle and may consist of up to as many as 36 individual single cells encapsulated within an outer limiting membrane derived from the mother cell. Comparative biochemical, physiological, microscopic and spectroscopic analyses of purified single cells and palmelloids indicate that the conversion of single cells to multicellular palmelloids alters the composition and organization of the photosynthetic apparatus. This phenotypic change enhances photoprotection of the photosynthetic apparatus from temperature and salt stress by minimizing potential cellular energy imbalances and safely dissipating excessive excitation energy by nonphotochemical quenching mechanisms. Since *Chlamydomonas priscuii* does not aggregate in response to either temperature or salt stress, the mechanism of palmelloid formation is distinct from the random aggregation phenomenon observed in the model mesophile, *Chlamydomonas reinhardtii*, in response to environmental stress. It is suggested that an advantage of palmelloid formation in green algae is decreased susceptibility to stress-induced photoinhibition. Thus, in addition to decreased susceptibility to predation, enhanced photoprotection from photoinhibition associated with palmelloid formation may be a complementary, selective, evolutionary advantage for the induction of multicellularity in green algae.

## **CYTOSOLIC CARBON METABOLISM PATHWAYS THAT SUPPORT THE CALVIN-BENSON-BASSHAM CYCLE**

Thomas D. SHARKEY

MSU-DOE Plant Research Laboratory, Plant Resilience Institute, and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, United States  
*Corresponding author email: tsharkey@msu.edu*

David Walker and Alice Herold, in a 1977 paper, asked “can the chloroplast support photosynthesis unaided?” Their conclusion was that cytosolic sucrose synthesis was essential in most cases to enable moderate to high rates of photosynthesis in leaves. As a result of the oxygenation reaction of rubisco the photorespiratory metabolic pathway that involves mitochondria and peroxisomes is required. There are other metabolic pathways that are closely aligned with the Calvin-Benson-Bassham (CBB) cycle, notably fatty acid synthesis, isoprenoid synthesis, and phenylpropanoid metabolism. Two other cytosolic pathways that support the Calvin-Benson-Bassham (CBB) cycle will be described. One of these is the oxidative pentose phosphate pathway that begins with glucose 6-phosphate in the cytosol and forms a shunt bypassing many of the CBB cycle reactions, returning five of the original six carbons to the CBB cycle. This metabolism can explain the observed lack of complete labeling of the CBB cycle when carbon isotopes are fed to leaves. It also may be the primary source of non-photorespiratory CO<sub>2</sub> emission during photosynthesis, known in photosynthesis models as  $R_d$ , or more appropriately,  $R_L$ , for respiration in the light. A glucose-6-phosphate shunt inside the chloroplast can be induced by high temperature stress. Another pathway can support the CBB cycle when plastid triose phosphate isomerase, aldolase, or fructose 1,6-bisphosphatase are inhibited. This cytosolic bypass involves glyceraldehyde 3-phosphate export from the chloroplast and glucose 6-phosphate return. This pathway normally does not occur because the glucose phosphate transporter needed is usually not expressed in leaves. However, the transporter can be induced, allowing plants to survive while lacking what should be essential enzymes. Two metabolic pathways that do not occur during photosynthesis are glycolysis and the tricarboxylic acid, or Krebs, cycle. The simultaneous operation of glycolysis and the gluconeogenic reactions of the CBB cycle would make a futile cycle. As early as 1952, Calvin published that essentially no labeled carbon was found in citrate when algae were labeled in the light, but that label was found in citrate as soon as the light was turned off. The metabolic pathways and the evidence to support them will be presented in this talk.



**WATER-OXIDATION REACTION IN THE PRESENCE OF MANGANESE COMPOUNDS:  
HOW DO MANGANESE COMPOUNDS OXIDIZE WATER IN ARTIFICIAL  
PHOTOSYNTHETIC SYSTEMS?**

Mohammad Mahdi Najafpour\*

Department of Chemistry, Institute for Advanced Studies in Basic Sciences , Zanjan, Iran

\*E-mail: [mmnajafpour@iasbs.ac.ir](mailto:mmnajafpour@iasbs.ac.ir)

Water-oxidation reaction (WOR) is a bottleneck and sluggish reaction for water splitting [1]. Mn oxides are promising for WOR because the compounds are stable, low-cost, and environmentally friendly. Interestingly, plants, algae, and cyanobacteria use a  $\text{CaMn}_4\text{O}_5$  cluster for WOR [2,3]. This CaMn cluster is housed in Photosystem II, a membrane-protein complex that functions as a light-driven water oxidase in oxygenic photosynthesis, and efficiently oxidizes water at low overpotential. Thus, Mn compounds are interesting to be used as WOR catalysts in artificial photosynthetic systems [4,5]. Recently, modern characterization techniques and different spectroscopic methods have shown a better understanding of WOR mechanism in the presence of Mn compounds. Herein, our findings on the conversion of Mn compounds to true catalysts during WOR are presented. I show that many Mn compounds before WOR or during WOR could convert to Mn-oxide based catalysts that are true catalysts for WOR. Some biomimetic models for biological  $\text{CaMn}_4\text{O}_5$  are also decomposed during WOR. For many Mn compounds, Mn(II) or (III) leaching into the electrolyte and then deposition of the leached Mn ions result in  $\text{MnO}_x$  formation.

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**STRUCTURAL INSIGHTS INTO PHOTOSYSTEM II ASSEMBLY**

Marc M. Nowaczyk<sup>1,2,\*</sup>

<sup>1</sup>Molecular Mechanisms of Photosynthesis, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Germany

<sup>2</sup>Department of Biochemistry, Institute of Biosciences, University of Rostock, Albert-Einstein-Str. 3, 18059 Rostock, Germany

\*Corresponding author: [Marc.Nowaczyk@uni-rostock.de](mailto:Marc.Nowaczyk@uni-rostock.de)

Photosystem II (PSII) assembly is a stepwise process that involves the transient formation of intermediate PSII complexes with varying protein compositions. The assembly process is facilitated by

assembly factors, like the lipoprotein Psb27 [1], which form intermediate complexes with a certain subset of PSII subunits. Using cryo-electron microscopy, we solved the structure of a partially functional PSII assembly intermediate from the thermophilic cyanobacterium *Thermosynechococcus vestitus* BP-1 (formerly known as *T. elongatus* BP-1) at 2.94 Å resolution [2]. It contains three assembly factors (Psb27, Psb28, Psb34) and provides detailed insights into their molecular function. The structure further demonstrates how the PSII active site is prepared for the incorporation of the Mn<sub>4</sub>CaO<sub>5</sub> cluster, which performs the unique water splitting reaction. Recently, we used twin-strep-tagged Psb27 for the isolation and subsequent cryo-EM analysis of PSII assembly intermediates, which resulted in structural models of two distinct Psb27-containing PSII complexes with ~2.9 Å and ~3.0 Å resolution, respectively. Both structures revealed the presence of the small membrane protein PsbJ, which was absent from all previous structures of PSII intermediates. In presence of PsbJ, the acceptor side becomes fully matured and binds Q<sub>B</sub>, while the donor side still contains only one single ion at the position of the Mn<sub>4</sub>CaO<sub>5</sub> cluster of mature PSII. One of the two Psb27-PSII complexes additionally binds the extrinsic subunit PsbV and the assembly factor Psb32, which indicates a role in late PSII assembly prior to photoactivation. The data further imply that the extrinsic subunit PsbO, which is important for PSII activity, does not form a stable complex with the intermediate, which might be related to the immature Mn<sub>4</sub>CaO<sub>5</sub> cluster and the resulting structural differences at the PsbO binding site.

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T1.9

## CRYO-OPTICAL MICROSCOPY STUDY ON REGULATION MECHANISM OF PHOTOSYNTHETIC LIGHT HARVESTING

Yutaka SHIBATA

Department of Chemistry, Graduate School of Science, Tohoku University, Japan

*E-mail:* [shibata@m.tohoku.ac.jp](mailto:shibata@m.tohoku.ac.jp)

We have developed and continuously improved the cryogenic laser-scanning confocal microscope systems. The systems have been applied to the study of intracellular rearrangement of LHCs during the state transitions (ST) and the fluctuation of energy-transfer pathways within single PSI's.

Recent advancement of the cryo-electron microscopy has increased the structural understanding of the ST. However, it is still elusive whether the LHCI detached from PSII in state2 all bind to PSI or partially remain isolated from both of the photosystems (PSs). The developed system provides the high lateral resolution (0.4 μm) and the ability to detect cryogenic fluorescence spectrum at each pixel, enabling resolutions of the intracellular PSI-rich and PSII-rich domains. The information about the PS segregation is effective to evaluate the LHC rearrangement during the ST [Fujita et al. *J.Photo.Photo.B*]. Our improved system achieved the simultaneous detection of the fluorescence spectrum and lifetime at each pixel. Owing to this advancement, we captured key evidence for the free LHCs isolated from both of the PSs in ST2. The observation revealed that the free LHCs were in the highly quenched state and accumulated in the PSI-rich domains. We also developed a microscope system which acquires both the excitation and emission spectra at each pixel. Observation of *Chlamydomonas* cells by this excitation

spectral microscope at room temperature enabled the real-time visualization of the rearrangement of LHCs upon the ST. We surprisingly found that the ST is less active in the region around the pyrenoid, which is a subcellular compartment specialized for the CO<sub>2</sub> fixation.

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## SEQUENCE OF EVENTS DURING THE WATER OXIDATION REACTION IN PHOTOSYSTEM II VISUALIZED BY TIME RESOLVED X-RAY STUDIES

J. Kern<sup>1</sup>, A. BHOWMICK<sup>1</sup>, P. SIMON<sup>1</sup>, H. MAKITA<sup>1</sup>, I. BOGACZ<sup>1</sup>, R. HUSSEIN<sup>2</sup>, M. IBRAHIM<sup>2</sup>,  
M. ZHANG<sup>1</sup>, S. KEABLE<sup>1</sup>, T. FRANSSON<sup>3</sup>, M. CHEAH<sup>4</sup>, A. BREWSTER<sup>1</sup>, N.K. SAUTER<sup>1</sup>, R.  
ALONSO-MORI<sup>5</sup>, J. MESSINGER<sup>4</sup>, U. BERGMANN<sup>5</sup>, H. DOBBEK<sup>2</sup>, A. ZOUNI<sup>2</sup>, J. YANO<sup>1</sup>, V.  
YACHANDRA<sup>1</sup>

1. Lawrence Berkeley National Laboratory, MBIB Division, Berkeley, CA 94720, USA.
2. Humboldt University Berlin, Department of Biology, 10099 Berlin, Germany
3. Department of Theoretical Chemistry and Biology, KTH Royal Institute of Technology, Stockholm, Sweden
4. Uppsala University, Department of Chemistry, SE 75120 Uppsala, Sweden
5. Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA, USA
6. Department of Physics, University of Wisconsin–Madison, Madison, WI, USA.

Obtaining information about changes in the electronic and geometric structure of the active site of a metalloenzyme during catalytic turnover is essential to obtain a detailed understanding of its reaction mechanism. Utilizing femtosecond X-ray pulses from XFELs it is possible to record “undamaged” snapshots of metalloenzymes at room temperature. Given adequate reaction triggering options these can be collated to a “movie” that shows the sequence of events at the catalytic site necessary for the reaction to take place. We used this approach to record data from crystals of Photosystem II, at different time points during the light driven water oxidation reaction. We obtained structures at around 2 Å resolution for the four stable states (S0 to S3) [1] as well as for time points in the S2-S3 transition [2,3] and the S3-S0 transition [4]. We also obtained X-ray emission (XES) data collected in parallel to the diffraction data that confirm the intactness of the sample and effective turnover during in-situ illumination. The XES data provide kinetic information about the Mn redox changes that can be correlated with structural observations at different time points in the reaction cycle. We will present details from these studies regarding the steps involved in the incorporation of an additional oxygen atom into the Mn<sub>4</sub>CaO<sub>5</sub> cluster during the S2-S3 transition as well as the resetting of the cluster in the S3-S0 transition, including indications for the presence of an observable reaction intermediate at around 500 to 1200 μs into the S3-S0 transitions. We will also discuss the importance of the network of channels connecting the Mn cluster with the lumen and their potential role in proton and water transport during catalysis, based on our structural observations.

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We acknowledge funding by NIH NIGMS and DOE BES.

## **CURRENT ACHIEVEMENTS AND CHALLENGES OF PHOTOBIOLOGICAL HYDROGEN PRODUCTION**

Prof. Itach Yacoby, Ph.D.

School of Plant Sciences and Food Security  
The George S. Wise Faculty of Life Sciences, Tel Aviv University  
Tel Aviv 69978, Israel  
[Itachy@tauex.tau.ac.il](mailto:Itachy@tauex.tau.ac.il)

Photosynthetic energy is primarily utilized for the conversion of CO<sub>2</sub> into organic matter; however, it also offers potential for driving other crucial processes, such as hydrogen (H<sub>2</sub>) production via the enzyme Hydrogenase through electron flow. Unfortunately, in natural systems, H<sub>2</sub> production is transient, lasting only about 2 minutes, due to electron loss to competing processes, primarily the Calvin cycle, and later on the accumulation of inhibitory levels of oxygen. These limitations, however, are overcome in a *Chlamydomonas* mutant carrying a mutation in the Proton-Gradient-Regulation-Protein-5 (pgr5) gene. This mutant exhibits enhanced respiration and a slower Calvin cycle, enabling sustained H<sub>2</sub> production for an extended period of 12 days under ambient mixotrophic conditions. Notably, this scalable and sustainable production can be achieved using culture volumes as 1 liter and above.

However, we have encountered an additional barrier in our research. In anoxic cultures of green microalgae, we discovered that the main limitation to achieving rapid hydrogen production lies in an anoxic switch occurring in photosystem II. Upon a 20 seconds of light exposure, a significant three-fold reduction in photosynthetic electron flow is observed, which does not recover to its original rate. Our findings demonstrate a notable alteration in the activity of photosystem II, resulting in a three-fold decrease in electron output. This discovery challenges the prevailing notion that changes in electron transfer rate within the Cytochrome b<sub>6</sub>f complex determine the rate-limiting control of photosynthesis. Instead, this down-regulation process, potentially involving the photoreduction of O<sub>2</sub> at the acceptor site of PSII and an alternative conformation of its acceptor site residue arrangement, ultimately leads to a substantial reduction in H<sub>2</sub> production. Overcoming this switch represents the primary target for obtaining a viable agro-economic photosynthetic H<sub>2</sub> production.

**PHOTOINDUCED TRIPLET STATE IN THE CHLOROPHYLL-D BASED PHOTOSYSTEM  
I OF ACARYOCHLORIS MARINA**

Stefano SANTABARBARA<sup>1\*</sup>, Alessandro AGOSTINI<sup>2</sup>, Anastasia A. PETROVA<sup>1,3</sup>, Marco  
BORTOLUS<sup>2</sup>, Anna Paola CASAZZA<sup>1</sup>, Donatella CARBONERA<sup>2</sup>

<sup>1</sup>IBBA, CNR, Via Bassini 15a, 20133 Milano, Italy;

<sup>2</sup>DSC, Università di Padova, Via Marzolo 1, 35131 Padova, Italy;

<sup>3</sup>A.N. Belozersky IPCB, MSU, 119992 Leninskiye Gory 1, Moscow, Russia.

*Corresponding author: E-mail: stefano.santabarbara@cnr.it*

In *Acaryochloris marina* the long-wavelength absorbing Chlorophyll (Chl)-*d* substitutes almost completely the otherwise ubiquitous Chl-*a*. The involvement of Chl-*d* in photochemistry and electron transfer (ET) in *A. marina* Photosystem I (PSI) is well established, since the electron donor P740 is significantly red-shifted with respect to P700 of Chl-*a* PSI. Structural models of *A. marina* PSI identified Pheophytin (Pheo)-*a* as an ET cofactor, which may compensate for the 0.1 eV loss in photon energy, being more oxidizing by the same extent with respect to Chl-*d* or -*a* in organic solvents. The role of residual Chl-*a* is still debated. Photo-induced Chl triplets are informative markers of photosynthetic RCs, where they are typically populated by charge recombination giving rise to a distinctive electron spin polarization (esp). These states were studied in thylakoids (TM) and isolated PSI of *A. marina* by Optically Detected Magnetic Resonance (ODMR) and time-resolved EPR (TR-EPR). Both in TM and PSI four <sup>3</sup>Chl-*d* populations were detected by ODMR. Two were assigned to antenna and two to RC triplets based on their response to illumination in mild reducing conditions. Microwave induced triplet-*minus*-singlet spectra at 1.8K have a main bleaching at 740 nm and a rich spectral structure, dominated by Chl-*d* signatures, thus excluding the participation of Chl-*a* in this RC. The TR-EPR detected esp indicates that these triplets are not populated as expected by the recombination mechanism but by intersystem crossing (ISC). An explanation for the unusual population is that the recombination from singlet state precursor to the low energy state of the RC (RC\*) outcompetes its spin dephasing rate to the triplet state, implying that the energy gap between RC\* and either primary or secondary radical pair, that involves Pheo-*a*, shall be much smaller than in Chl-*a* PSI. Low energy gaps for both radical pairs population were obtained from ultrafast transient absorption data in Chl-*d* PSI (~60 meV). Yet, even smaller gaps or the presence of significant distributions of energy levels shall be postulated to make the proposed <sup>3</sup>RC population mechanism active, at least in a subpopulation of PSI RCs.

**HIGH LIGHT-INDUCED CHANGES IN WHOLE-CELL PROTEOME AND  
ORGANIZATION OF THYLAKOID SUPERCOMPLEX IN CYCLIC ELECTRON  
TRANSPORT MUTANTS OF CHLAMYDOMONAS REINHARDTII**

Ranay Mohan Yadav, Sureshbabu Marriboina, Yusaf Zamal Mohammad, Jayendra Pandey and  
Rajagopal Subramanyam

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli,  
Hyderabad- 500046, India

Rajagopal Subramanyam ([srgsl@uohyd.ac.in](mailto:srgsl@uohyd.ac.in)) Phone: +91-40-23134572

*Chlamydomonas (C.) reinhardtii* is the most extensively cultivated algae worldwide, and light constitutes the leading environmental cause limiting its growth and productivity. We have correlated the biophysical, biochemical, and potential proteomic data to understand targeted sites of proteins and photosynthetic organization grown in high light from cyclic electron transport mutants. From proteomic data, the 320 proteins were significantly affected in high light compared to the control. In which mainly the up and down-regulated proteins originated in photosynthesis, glycolysis, protein synthesis, and proteins involved in cytoskeleton assembly. In high light stress ~250 induced differentially abundant proteins (DAPs), in which 118 DAPs determined metabolites revealed the tricarboxylic acid cycle, especially the malate to oxaloacetate conversion step. Also, cilia and flagella-associated proteins were strongly stimulated under high light. Additionally, we observed that the Cyt *b6* expression is increased ~280 times more in the *pgr5* mutant to regulate pmf and pH across the thylakoid membrane. The increased Cyt *b6* function in *pgr5* could be due to the compromised function of (cp) ATP synthase subunits for energy generation and photoprotection under high light. Moreover, our proteome and western-blot data shows the PSBS protein isoforms (PSBS1 and PSB2) expressed more than LHCSR in *pgr5* compared to WT and *pgr11* under high light, which also agrees with protein data. In addition, we immunoblotted the PSII, PSI, and associated light-harvesting complexes proteins to compare with the total cell proteome. The obtained proteins representing PSII core PsbA (D1) and PsbD (D2) accumulated more in *pgr11* and *pgr5* than in the WT under high light. In high light, CP43 and CP47 showed a reduced amount in *pgr5* due to changes in chlorophyll and carotenoid content around the PSII protein, which coordinates with the cofactors for efficient energy transfer from the light-harvesting antenna to the photosystem core. BN-PAGE and circular dichroism studies indicate that high light alters macromolecular assembly and prevalent thylakoid destacking in *pgr11* and *pgr5* allows due to change in the proteome of thylakoids. Based on this study, we emphasize that this is an excellent aid to the scientific community in understanding the role of CET mutants in high light.

Tx.x

## STRUCTURE-FUNCTION RELATIONSHIPS OF FAR-RED LIGHT-ABSORBING ALLOPHYCOCYANINS

Christopher J. GISRIEL,<sup>1\*</sup> Eduard ELIAS,<sup>2</sup> Gaozhong SHEN,<sup>3</sup> Nathan T. SOULIER,<sup>3,†</sup> David A. FLESHER,<sup>4</sup> M. R. GUNNER,<sup>5</sup> Gary W. BRUDVIG,<sup>1,4\*</sup> Roberta CROCE,<sup>6\*</sup> Donald A. BRYANT,<sup>3\*</sup>

<sup>1</sup>Department of Chemistry, Yale University, New Haven, CT 06520, USA.

<sup>2</sup>Department of Physics and Astronomy and Institute for Lasers, Life and Biophotonics, Faculty of Sciences, VU University Amsterdam, 1081 HV Amsterdam, Netherlands

<sup>3</sup>Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.

<sup>4</sup>Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.

<sup>5</sup>Department of Physics, City College of New York, New York, NY 10031, USA.

<sup>6</sup>Biophysics of Photosynthesis, Department of Physics and Astronomy, Faculty of Science, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, Netherlands.

Cyanobacteria and red algae contain phycobiliproteins that absorb light and transfer energy to the photosystems involved in oxygenic photosynthesis. Some cyanobacteria express a paralog of the phycobiliprotein, allophycocyanin, that strongly absorbs far-red light (FRL). Using cryo-electron microscopy and time-resolved absorption spectroscopy, we reveal the structure-function relationship of this FRL-absorbing allophycocyanin complex that is expressed during acclimation to low light. FRL-allophycocyanin assembles as helical nanotubes rather than typical toroids due to alterations of the domain geometry within each subunit, and likely associates with photosystem I rather than photosystem II. Spectroscopic characterization suggests that FRL-AP nanotubes are somewhat inefficient antenna but the enhanced ability to harvest FRL when visible light is severely attenuated represents a beneficial tradeoff. The results expand the known diversity of light-harvesting proteins in nature and exemplify how biological plasticity is achieved by balancing resource accessibility with efficiency.

T1.2

**CHLOROPLAST AND PHOTOSYNTHETIC ACCLIMATION IN A LYCOPHYTE,  
*Selaginella martensii*: A WINDOW ON THE PHOTOSYNTHESIS OF ANCIENT VASCULAR  
PLANTS?**

Lorenzo FERRONI

Department of Environmental and Prevention Sciences, University of Ferrara, Italy  
E-mail: [lorenzo.ferroni@unife.it](mailto:lorenzo.ferroni@unife.it)

Vascular plants are a monophyletic group, whose evolutionary history begins with the early divergence dividing two sister clades: lycophytes and euphyllophytes. After the great success of the former up to the Carboniferous period, they became a small group, currently accounting for ca. 1% of extant vascular plant species. With ca. 750 species, *Selaginella* is the dominant genus; despite a cosmopolitan distribution, most species are found in the humid shady understorey of equatorial rainforests. As members of an ancient vascular plant lineage, *Selaginella* species conceivably exhibit some features recalling ancestral photosynthetic traits, e.g., a low carbon fixation capacity, likely related to the high CO<sub>2</sub> pressure in the Carboniferous. However, other traits are better interpreted as results of the chloroplast evolution occurred in parallel with, and independent of, that of euphyllophytes and especially angiosperms. The study of the photosynthetic apparatus in *Selaginella martensii* offers interesting information about how this plant combines the phenotypic features related to shade adaptation with a noticeable ability to successfully manage high light intensities. On one hand, the monoplastidy of the leaf epidermal cells and the overall organisation of the thylakoid system appear particularly suited to emphasize light absorption in a shade environment; on the other hand, *S. martensii* can induce NPQ rapidly and to a high level upon exposure to intense light, more than one can expect for a shade-adapted species. In context of the shade-type thylakoid system, the plant's ability not only to survive, but also to long-term acclimate to high irradiance has been related to processes such as the energy spillover from PSII to PSI, the energetic uncoupling of LHClI from PSII, and the electron flux to sinks alternative to photosynthesis and photorespiration.

T1

## **EXITON PROMINENCE IN PHOTOSYNTHETIC COLOR-TUNING AND LIGHT-HARVESTING**

Arvi FREIBERG\*

Institute of Physics, University of Tartu, Estonia

*\*Corresponding author: E-mail: [arvi.freiberg@ut.ee](mailto:arvi.freiberg@ut.ee)*

Photosynthesis is a vital process that converts sunlight into energy for the Earth's ecosystems. Color adaptation is crucial for different photosynthetic organisms to thrive in their ecological niches. Although the presence of collective excitons in light-harvesting complexes is well known, the role of delocalized excited states in color tuning and excitation energy transfer remains unclear. This study evaluates the characteristics of photosynthetic excitons in sulfur and non-sulfur purple bacteria using advanced optical spectroscopic techniques at reduced temperatures. The exciton effects in these bacteriochlorophyll containing species are generally much stronger than in plant systems that rely on chlorophylls. Their exciton bandwidth varies based on multiple factors such as chromoprotein structure, surroundings of the pigments, carotenoid content, hydrogen bonding, and metal ion inclusion. The study nevertheless establishes a linear relationship between the exciton bandwidth and Qy singlet exciton absorption peak, which covers almost 160 nm. These findings provide important insights into bacterial color-tuning and light-harvesting, which can inspire sustainable energy strategies and devices.

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## **ARTIFICIAL PHOTOSYNTHESIS, AN ENERGY TECHNOLOGY OF THE FUTURE**

Suleyman I. ALLAKHVERDIEV

Controlled Photobiosynthesis Laboratory, K.A. Timiryazev Institute of Plant Physiology, RAS, Moscow,  
Russia

Photosynthesis and Hydrogen Energy Laboratory, Faculty of Engineering and Natural Sciences,  
Bahcesehir University, Istanbul, Turkey

*E-mail: [suleyman.allakhverdiev@gmail.com](mailto:suleyman.allakhverdiev@gmail.com)*

Natural photosynthesis is a proven for thousands of years example of alternative energy efficiency, producing organic compounds and O<sub>2</sub>, and under certain conditions H<sub>2</sub>, from water and CO<sub>2</sub> at the expense of solar energy. Burning H<sub>2</sub> provides the maximum energy among other fuels and water, an environmentally



friendly product. Production of H<sub>2</sub> by artificial photosynthesis systems is a promising and high priority. Solar energy can be converted into electricity (in solar cells) or used in systems generating H<sub>2</sub> from water. We are investigating the creation and operation of solar energy converters based on phototroph components to produce environmentally friendly energy. We created an original setup to analyze the operation of solar cells based on photosynthetic systems in a wide range of temperatures and light intensities. We have obtained original data on the "work" of solar cells capable of generating photocurrents, based on different components of the photosynthetic apparatus, including thylakoids and photosystem II membranes immobilized on the surface of titanium dioxide under different conditions. Particular attention is paid to the search for efficient catalysts for water oxidation, since such catalysts are key components of solar cells producing molecular hydrogen from water in the light. We found that the most effective catalyst for water oxidation under artificial photosynthesis conditions is a manganese-containing complex. To produce photohydrogen, we modified photosystem I (PSI) in which the secondary electron acceptor, vitamin K, was replaced with platinized naphthoquinone (PtNP), which increases the efficiency of electron transfer to the electrode. With this modification of PSI, it was possible to create an artificial system capable of efficiently generating molecular hydrogen at the expense of light energy.

*The results were obtained under the state assignment of the Ministry of Science and Higher Education of the Russian Federation (122050400128-1) and supported by the Russian Science Foundation (Grants No. 19-14-00118; No. 22-44-08001).*

T1.8

## **RATIONAL DESIGN OF BIOPHOTOELECTRODES FOR IN VITRO CATALYSIS**

Anna FRANK<sup>1</sup>, Marc M. NOWACZYK<sup>1</sup>

<sup>1</sup> Chair for Biochemistry, Institute of Biological Sciences, University of Rostock, Rostock, Germany

\*Corresponding author : [anna.frank@uni-rostock.de](mailto:anna.frank@uni-rostock.de)

The use of photosynthetic protein complexes for the fabrication of solar energy conversion devices is a promising strategy due to their natural abundance and high quantum efficiency. Particularly, one of the main photosynthesis-driving enzymes, photosystem I (PSI), is a stable protein complex able to convert visible light into high energy electrons – making it an attractive candidate for the fabrication of biohybrid devices. A challenge in such devices is to overcome short circuiting processes between the light-generated electrons and the electrode. One approach is oriented immobilization of PSI complexes in so-

called Langmuir monolayers. Charge recombination occurring at the gaps between disc-shaped PSI trimers could be successfully minimized by additionally employing smaller PSI monomers, filling the gaps and resulting in increased surface coverage and overall performance<sup>1</sup>. Depending on the species, cyanobacterial PSI is mostly present in trimeric form, with neglectable amounts of monomers. However, a novel preparation method for the isolation of functional PSI monomers from *T. vestitus* with high yield enabled not only the fabrication of above-mentioned mixed monolayers but also structural analysis of PSI monomers<sup>2</sup>. To promote efficient wiring between PSI complexes and the electrode surface, rationally designed redox polymers can be used as an effective tool. They enhance electron transfer and film stability and furthermore enable the deposition of additional enzymes such as oxidoreductases to the electrode, in order to make use of the high energy electrons generated by PSI, as could be shown for a hydrogenase<sup>3</sup> as well as for a PSI-hydrogenase fusion complex<sup>4</sup>, resulting in light-driven H<sub>2</sub> production.

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T1.1.3

### **STRUCTURE OF THE FAR-RED LIGHT UTILIZING PHOTOSYSTEM I OF ACARYOCHLORIS MARINA**

Keisuke KAWAKAMI<sup>1</sup>, Tasuku HAMAGUCHI<sup>2</sup>, Kyoko SHINZAWA-ITOH<sup>3</sup>, Natsuko INOUE-KASHINO<sup>3</sup>, Shigeru ITOH<sup>4</sup>, Kentaro IFUKU<sup>5</sup>, Eiki YAMASHITA<sup>6</sup>, Kou MAEDA<sup>3</sup>, Koji YONEKURA<sup>1,2</sup>, Yasuhiro KASHINO<sup>3</sup>

<sup>1</sup>Biostructural Mechanism Laboratory, RIKEN SPring-8 Center, Japan; <sup>2</sup>Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Japan; <sup>3</sup>Graduate School of Life Science, University of Hyogo, Japan; <sup>4</sup>Department of Physics, Graduate School of Science, Nagoya University, Japan; <sup>5</sup>Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Japan; <sup>6</sup>Laboratory of Supramolecular Crystallography, Institute for Protein Research, Osaka University, Japan.

Photosynthetic organisms such as plants and algae, absorb solar energy and convert it into chemical energy by utilizing numerous pigments in the photosynthetic membrane protein complexes (photosystems I and II; PSI and PSII)<sup>1-4</sup>. *Acaryochloris marina* (*A. marina*), one of the cyanobacteria, uses chlorophyll (Chl) d to absorb far-red light to carry out photochemical reactions<sup>5</sup>, whereas many oxygen-evolving photosynthetic organisms utilize Chl a for carrying out photochemical reactions. Recently, we analyzed the structure of *A. marina* PSI at 2.6 Å resolution using cryo-electron microscopy (cryo-EM)<sup>6</sup>. Interestingly, the primary electron acceptor (A0) in the *A. marina* PSI was pheophytin (Pheo) a but not Chl a. The structure revealed a unique type I reaction center that uses Chl d and Pheo a as electron carriers and its pigment arrangement. Herein, we show the architecture of *A. marina* PSI and discuss how the *A. marina* PSI utilizes low-energy far-red light to drive photochemical reactions.

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T1.8

## USE OF BACTERIAL PHOTOSYNTHETIC VESICLES TO EVALUATE THE EFFECT OF IONIC LIQUIDS ON THE PERMEABILITY OF BIOLOGICAL MEMBRANES

Francesco FRANCIA<sup>1\*</sup>, Tancredi BIN<sup>1</sup>, Anna Maria GHELLI<sup>1</sup> and Giovanni VENTUROLI<sup>1</sup>

<sup>1</sup>University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy

\*Corresponding author: Via Irnerio n.42, Bologna, Italy; Tel. +39 51 2091293;  
Fax +39 51 242576; Email : francesco.francia@unibo.it

Ionic liquids (ILs) are salts composed by a combination of organic or inorganic cations and ions. Their low melting point is due to structural elements that shield the ion-charged center and prevent the formation of crystals, causing them to become liquid below 100°C. This property, together with an extremely low vapor pressure, low flammability and high thermal stability, makes them suitable for replacing canonical organic solvents, with a reduction of industrial activities impact on the environment. Despite in the last decades the eco-compatibility of ILs has been extensively verified through toxicological tests performed on macro and microorganisms, a detailed understanding of the interaction of these compounds with biological membranes are far from being exhaustive. In this context, we've

chosen to evaluate the effect of some ILs on native membranes using chromatophores, photosynthetic vesicles that can be isolated from *Rhodobacter capsulatus*, a representative member of the purple non-sulfur bacteria. Here, carotenoids associated with the light-harvesting complex II act as endogenous spectral probes of the membrane electrical potential ( $\Delta\Psi$ ). In fact, when a membrane electric field is generated by photoexcitation of the photosynthetic reaction center (RC), the visible spectrum of the carotenoids undergoes an electrochromic red shift that responds linearly to the  $\Delta\Psi$  amplitude. By measuring the time evolution of the carotenoid band shift induced by a single RC photoexcitation, information on the  $\Delta\Psi$  dissipation due to ionic currents across the membrane can be obtained. Using a statistical model to analyze the non-homogeneous decay of the carotenoid signals, we attempted to interpret at a mechanistic level the marked increase of ionic permeability caused by specific ILs.

T1

## THE EXTRINSIC SUBUNITS OF PHOTOSYSTEM II OPTIMIZING THE OXYGEN-EVOLVING REACTION

Ko IMAIZUMI and Kentaro IFUKU\*

Graduate School of Agriculture, Kyoto University

\*Corresponding author: E-mail: [ifuku.kentaro.2m@kyoto-u.ac.jp](mailto:ifuku.kentaro.2m@kyoto-u.ac.jp)

Photosystem II (PSII) catalyzing photosynthetic water oxidation is composed of more than 20 subunits, including membrane-intrinsic and -extrinsic proteins. PSII extrinsic proteins protect the catalytic  $\text{Mn}_4\text{CaO}_5$  cluster from the outside bulk solution and stabilize the binding of inorganic cofactors, such as  $\text{Ca}^{2+}$  and  $\text{Cl}^-$ , in the oxygen-evolving center (OEC) of PSII. Among the extrinsic proteins of PSII, PsbO exists in all oxygenic organisms. PsbP and PsbQ are specific to green plants including land plants and green algae, while PsbU, PsbV, CyanoQ, and CyanoP has been found in cyanobacteria.

Here, we have examined the functional role of a specific loop region, Loop 4, of the PsbP subunit inserted close to the Cl-2 binding site in OEC. Reconstitution experiments suggest that mutations in Loop 4 have large effects on the oxygen-evolving activity of PSII. Notably, a specific mutation, D139N, was found to enhance the oxygen-evolving activity *in vitro*. Light-induced Fourier transform infrared (FTIR) difference spectroscopy and theoretical calculations suggest that the D139N mutation increases the  $\text{Cl}^-$  retention ability of PsbP inducing a unique structural change near the Cl-2 binding site of OEC. Structural comparison of the green plant PSII structure with cyanobacterial, red algal, and diatom PSII structures suggests that PsbP-Loop 4 seems to replace the C-terminal region of PsbU in spatial and functional manners. This also indicates the functional significance of Cl-2 in the water-oxidizing reaction.

T2.3

### **ENHANCED HYDROGEN PRODUCTION OF SYNECHOCOCCUS SP PCC 7942 CELLS.**

Broussos P-I<sup>1</sup>, Romanos GE<sup>2</sup>, Stamatakis K<sup>1\*</sup>

<sup>1</sup>Institute of Biosciences and Applications, NCSR Demokritos, Aghia Paraskevi, 15310 Attikis, Greece

<sup>2</sup> Institute of Nanoscience and Nanotechnology, NCSR Demokritos, Aghia Paraskevi, 15310 Attikis, Greece

*\*Corresponding author: E-mail: [kstam@bio.demokritos.gr](mailto:kstam@bio.demokritos.gr)*

The cyanobacterium *Synechococcus elongatus* PCC7942 (S7942) accumulates sucrose under salt stress. We developed a new method, using a genetically engineered strain of S7942 (1) that produces sucrose as a viable alternative to Hydrogen (H<sub>2</sub>) formation via anaerobic dark fermentation (2). We studied it with the aim of increasing H<sub>2</sub> production. Cells are also able to accumulate glycogen together with sucrose during salt stress and to degrade it in dark fermentation conditions.

Simultaneously, in cells forced to glycogen breakdown, under dark anaerobic and nitrogen-depleted conditions, the procedure results to a significantly augmented H<sub>2</sub> evolution up to 3200-fold higher than in nitrogen replete cells. The sustainability of the procedure, was tested by the viability of the cyanobacterial biomass following its dark fermentation. Cells were able to proliferate upon their dark fermentation, in the double BG-11 either in enriched nitrogen, BG-11 medium. The present study demonstrates a significant H<sub>2</sub> production and indicates the glucose derived from the endogenous glycogen as the main carbon substrate fermented.

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T1.9

### **MOLECULAR MECHANISMS OF STATE TRANSITION IN A GLAUCOPHYTE CYANOPHORA PARADOXA**

Yoshifumi UENO<sup>1,\*</sup>, Seiji AKIMOTO<sup>2</sup>

<sup>1</sup>Institute of Arts and Science, Tokyo University of Science, Tokyo, Japan

<sup>2</sup>Graduate School of Science, Kobe University, Hyogo, Japan

*\*Corresponding author: E-mail: [yueno@rs.tus.ac.jp](mailto:yueno@rs.tus.ac.jp)*

A balance of excitation between two photosystems (PSI and PSII) is necessary for efficient oxygenic photosynthesis. In nature, oxygenic photosynthetic organisms control the excitation balance by using a variety of regulatory mechanisms. State transition is one of the mechanisms, as proposed in red algae and green algae in 1969 [1, 2]. This mechanism has been investigated for a long time in cyanobacteria, red algae, and green algae. Glaucophytes are one of the three groups of primary symbiotic algae, together with red algae and green algae. Compared with cyanobacteria and other primary symbiotic algae, the

regulatory mechanism is poorly understood in glaucophytes. In this study, we aimed to reveal the molecular mechanisms of state transition in glaucophytes. To this end, we prepared glaucophyte *Cyanophora paradoxa* cells illuminated with PSI-exciting light (blue, 470 nm) or PSII-exciting light (red, 655 nm) for 5 minutes, and measured their steady-state fluorescence spectra with absolute intensities and time-resolved fluorescence spectra at 77 K. Based on the results, we will discuss the molecular mechanisms of state transition in the glaucophyte *C. paradoxa*.

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T1.5

## RED-CHLOROPHYLL IN PHOTOSYSTEM I

Tatsuya TOMO

Graduate School of Science, Tokyo University of Science, Tokyo, Japan

Chlorophylls (Chls) play a role in light-harvesting, energy transfer and electron transfer in photosynthesis. Chl is classified into *a*, *b*, *c*, *d* and *f* molecular species.

The absorption maxima of Chl *d* and Chl *f* in organic solvents are located at 30 and 40 nm longer wavelengths than Chl *a*, respectively. On the other hand, that of Chl *b* and *c* is shifted towards the short wavelength side. The initial electron donor of photosystem I and II is often Chl *a*. Chl with absorption maxima at longer wavelengths than the initial electron donor is called low energy Chl or red-Chl and is present in photosystems I and II.

Photosystem I generally contains 7–10 molecules of Chl with absorption maxima at longer wavelengths than the electron donor. The 77 K fluorescence spectrum of photosystem I in most species has an emission maximum at 720–740 nm derived from red-Chl, but some cyanobacteria have fluorescence bands at longer wavelengths. The initial electron donor in photosystem I of *Halomicronema hongdechloris*, which contains Chl *f*, is Chl *a*. Thus, in its photosystem I, Chl *f* contributes to energy transfer as red Chl. The cyanobacterium *Arthrospira platensis*, previously named *Spirulina platensis*, has an additional fluorescence band around 760 nm in addition to 730 nm. It has been known that this cyanobacteria has no Chl other than Chl *a*. We have isolated and characterized monomeric, dimeric and trimeric complexes of photosystem I of this species; the intensity of the 760 nm fluorescence band was found to vary with aggregation. The reasons for red-Chl formation and its function will be reported at this conference

Tx.x

## **EVOLUTIONARY CHANGES IN THE OLIGOMERIC STATES OF PHOTOSYSTEM I: THREE- TWO- FOUR- ONE!**

Barry D. BRUCE

University of Tennessee, Knoxville

Photosystem I (PSI) is the largest photosynthetic and type I reaction centers. In cyanobacteria, this complex is organized as a symmetric trimer with a three-fold axis of symmetry. Recently, a new class of PSI, tetrameric in structure, has been identified in two cyanobacteria, *Chroococcidiopsis* TS-821, and *Nostoc* sp. PCC-7102. This tetramer is a dimer-of-dimers and may represent a transition between a more primitive trimeric system and the monomeric PSI found in plants and algae. We have investigated the distribution of this tetrameric form of PSI using genomic analysis, bioinformatics, and biochemical characterization. We have shown that it is widespread throughout all the heterocyst-forming filamentous cyanobacteria and their close relatives (HCR). Despite this oligomeric difference, pump-probe spectroscopy reveals that the ultrafast (<100 fs) charge transfer between the primary electron donor P700 and the primary chlorophyll acceptor is very similar to that of trimeric PSI complexes from *Synechocystis* sp PCC 6803. However, with the recently solved Cryo-EM structure for two different tetrameric forms of PSI (TS-821 and *Nostoc*), the interfaces are different between the two dimers, with both the number and placement of select chlorophylls altered. This is possibly reflected in the low-temperature fluorescence emission profile. We have now shown that in three cyanobacteria, the transition from a dimeric to the tetrameric form of PSI is induced by high light with an associated increase in accumulation of the keto-carotenoids, canthaxanthin, echinenone, and the glycosylated carotenoid, myxoxanthophyll. Keto-carotenoids have been shown to provide a much higher tolerance to UV and oxidative stresses. This high-light photo-adaptation may be an early evolutionary transition that preceded the origin of chloroplasts and was associated with the early transition from aquatic/marine habitats to a high-light more terrestrial environment. This basal role of tetrameric-forming cyanobacteria in plastid evolution is supported by the recent discovery of a tetrameric form of PSI in *Cyanophora paradoxa*, a glaucophyte in the Archaeplastida – a group of plastid-containing organisms that may share a unique common ancestor believed to be the first eukaryote to establish an endosymbiotic association with a cyanobacterium.

T1.9

## **EXAMINING THE IMPACT OF STRESS CONDITIONS ON THE FUNCTIONALITY OF THE PLASTOQUINONE POOL IN HIGHER PLANT CHLOROPLASTS**

Maria Borisova-MUBARAKSHINA<sup>1\*</sup>, Marina KOZULEVA<sup>1</sup>, Daria VETOSHKINA<sup>1</sup>, Daria VILYANEN, Ilya NAYDOV<sup>1</sup>, Aleksandr ASHIKHMIN<sup>1</sup>, Ekaterina PYKHOVA<sup>1,2</sup>, Tatiana KHOROSHAEVA<sup>1</sup>, Roman MARKIN, Boris IVANOV<sup>1</sup>

<sup>1</sup>Institute of Basic Biological Problems, RAS, Pushchino, Russia

<sup>2</sup>S.P. Korolev Samara National Research University, Samara, Russia

\*Corresponding author. Fax: +7(4967)33-05-32, E-mail: [mubarakshinamm@gmail.com](mailto:mubarakshinamm@gmail.com)

Plastoquinone (PQ), a lipophilic mobile electron carrier functioning between Photosystem II (PS II) and the cytochrome b6/f complex, is involved not only in linear electron transport but also in cyclic electron transport around photosystem I, the Mehler reaction, chlororespiration and in the antioxidant network. The redox state of the plastoquinone pool (PQ pool) influences light energy distribution between photosystems and regulation of plastid and nucleus gene expression under stress conditions. However, the mechanisms underlying these PQ pool functions are not fully deciphered. Under stress conditions, the Mehler reaction intensifies, resulting in increased production of superoxide anion radical ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) in chloroplasts. It is commonly accepted that the main pathway for  $H_2O_2$  production is the disproportionation of  $O_2^{\cdot-}$ , catalyzed by superoxide dismutase (SOD) in the chloroplast stroma. We have shown that an additional, SOD-independent pathway for  $H_2O_2$  production in the thylakoid membrane, occurs under stress conditions. This membrane-specific  $H_2O_2$  results from the reaction of  $O_2^{\cdot-}$  with the reduced plastoquinone molecule, plastoquinone. This reaction promotes the oxidation of the PQ pool and neutralization of  $O_2^{\cdot-}$  in the thylakoid membranes; the produced  $H_2O_2$  could act as a signaling agent, reflecting the redox state of the PQ pool for regulating gene expression. Under severe stress conditions, oxidized plastoquinone derivatives, known as plastoquinone C molecules, accumulate in thylakoid membranes. We have studied the effects of plastoquinone A and plastoquinone C on the activity of isolated PS II. Our findings indicate that plastoquinone C is less efficient as an electron acceptor from PS II than plastoquinone A. Possibly the suppression of the electron transfer rate at the PS II level due to the presence of oxidized derivatives of plastoquinone is one of the mechanisms contributing to inhibition of photosynthesis in higher plant cells. This work was supported by the Russian Science Foundation (Grant No. 23-14-00396).

T1.7

## **REDOX SIGNALS BETWEEN CELLULAR ORGANELLES TO MODULATE PHOTORESPIRATORY ENZYMES IN PEA (PISUM SATIVUM) LEAVES**

Agepati S. RAGHAVENDRA, Deepak SAINI, Ramesh BAPATLA, and Sunil BOBBA

Department of Plant Sciences, School of Life Sciences,  
University of Hyderabad, Hyderabad 500046, India  
email: [as\\_raghavendra@yahoo.com](mailto:as_raghavendra@yahoo.com), [asrsls@gmail.com](mailto:asrsls@gmail.com)

Photorespiration is essential to plant metabolism, coordinating with photosynthesis and dark respiration. It is unclear how the pathway located in different subcellular compartments of chloroplast, peroxisome, mitochondrion, and the cytoplasm responds to stress occurring exclusively in one of those. Metabolite movement can be one of the basis. In addition, reactive oxygen species (ROS) and reactive nitrogen species, particularly nitric oxide (NO), could also participate. We attempted to assess the basis of the inter-organelle interaction during the photorespiratory pathway. We induced oxidative stress by menadione (MD) in mitochondria and photo-oxidative stress by high-light (HL) in chloroplasts. Subsequently, the changes in selected photorespiratory enzymes, located in other subcellular compartments were examined. The presence of MD upregulated the enzyme activity, protein levels, and transcripts of four photorespiratory enzymes: Peroxisomal glycolate oxidase,



catalase chloroplastic glycerate kinase, and phosphoglycolate phosphatase in both normal and HL. The effect of MD was maximum in high light, indicating photo-oxidative stress was an influential factor in regulating photorespiration. The modulation by MD of photorespiratory enzymes was dampened when the scavengers of superoxide (Tiron) and H<sub>2</sub>O<sub>2</sub> (catalase) were present in the incubation medium. Thus, the changes were due to both superoxide and H<sub>2</sub>O<sub>2</sub>, two forms of ROS, in leaves. Further, ROS (both superoxide and H<sub>2</sub>O<sub>2</sub> produced in either chloroplasts (HL-stress) or mitochondria (oxidative stress by MD) could move across the cell, modulating enzymes in other organelles, including peroxisomes and chloroplasts. Since NO is also generated besides ROS during abiotic/biotic stress, we set out to assess the modulation of photorespiration by NO using a natural NO donor, S-nitrosoglutathione (GSNO). The increase in NO levels, nitrosothiols, and tyrosine-nitrated proteins confirmed the high NO levels induced by GSNO. Yet GSNO did not seriously affect photorespiratory enzymes. Unlike MD, we figured out that the ROS production and the antioxidant enzymes were only marginally affected by GSNO. The photorespiratory enzymes responded much more strongly to ROS rather than to NO. Further work is necessary to understand the interactions between NO and ROS. Our observations throw challenging open areas for further study. It would be interesting to examine the effect of HL and MD on N-metabolism enzymes, which are tightly coupled to photorespiration.

T.1

## **MONITORING OF PSI PHOTOCHEMISTRY TO EXAMINE GENOTYPE-SPECIFIC RESPONSES OF CROP PLANTS TO VARIOUS STRESS FACTORS**

Marek Živčák<sup>1\*</sup>, Erik Chovanček<sup>1,2</sup>, Andrej Filaček<sup>1</sup>, Marián Brestič<sup>1</sup>

<sup>1</sup>Institute of Plant and Env. Sciences, Slovak University of Agriculture, Nitra, Slovakia

<sup>2</sup> Molecular Plant Biology, Department of Life Technologies, University of Turku, Finland

*\*Corresponding author: e-mail: [marek.zivcak@uniag.sk](mailto:marek.zivcak@uniag.sk)*

Regulation of electron transport is crucial to protect components of the electron transport chain against photooxidative damage in plants exposed to fluctuating environmental conditions. The physiological significance of photosystem I photoinhibition (PSI) and the necessity of PSI protection has been previously demonstrated, but their prominence in different stress scenarios is still unclear. In a series of experiments with diverse wheat genotypes of different stress tolerance, we examined the regulation of electron transport, redox states of the PSI and PSII, and PSI inactivation in parallel with other photosynthetic parameters in conditions of abiotic stresses, especially drought, heat, and nitrogen deficiency. All stress factors led to changes in redox states of PSI donor and acceptor side, associated with alterations in transthylakoid proton gradient (ECS<sub>t</sub>), proton conductance (gH<sup>+</sup>), and non-photochemical quenching (NPQ). In the drought stress experiment, we observed very efficient regulation leading to the protection of PSI and no symptoms of PSI damage. On the other hand, high temperatures, especially heat waves, decreased PSI activity in sensitive but not in tolerant genotypes. The genotypes also differed in their recovery after heat stress relief; a poor recovery was associated with an overly reduced acceptor side of photosystem I and a high membrane potential in the chloroplast. A good recovery of photosynthetic capacity and photoprotective functions were clearly associated with enhanced ΔpH component of the proton motive force. Comparison of the redox states of PSII and PSI under nitrogen deficiency indicated mostly efficient regulation and protection of PSI, which was, however, provided by the genotype-specific mechanisms. In conclusion, our results confirmed the critical role of regulation of electron transport and keeping a low reduction level of PSI acceptor side.

In wheat germplasm, we identified significant genotypic variability in responses to evaluated stress conditions, including differences in photoprotective mechanisms employed by different genotypes or groups of genotypes. The research was supported by the projects VEGA 1-0664-22, VEGA-1-0425-23, and APVV-18-0465.

T1.2

### **ROLE OF NON-LAMELLAR LIPID PHASES IN THE STRUCTURE AND FUNCTION OF PLANT THYLAKOID MEMBRANES**

Ondřej DLOUHÝ<sup>1\*</sup>, Uroš JAVORNIK<sup>2</sup>, Kinga Ilona BÖDE<sup>1,3,4</sup>, Primož ŠKET<sup>2</sup>, Ottó ZSIROS<sup>3</sup>, Irena KURASOVÁ<sup>1</sup>, Václav KARLICKÝ<sup>1</sup>, Janez PLAVEC<sup>2</sup>, Bettina UGHY<sup>3</sup>, Vladimír ŠPUNDA<sup>3</sup>, Győző GARAB<sup>1,3</sup>

<sup>1</sup>Department of Physics, Faculty of Science, University of Ostrava, Ostrava, Czechia

<sup>2</sup>Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia

<sup>3</sup>Institute of Plant Biology, Biological Research Centre, ELKH, Szeged, Hungary

<sup>4</sup>Doctoral School of Biology, University of Szeged, Szeged, Hungary

\*Corresponding author: E-mail: [ondrej.dlouhy@osu.cz](mailto:ondrej.dlouhy@osu.cz)

Energization of plant thylakoid membranes (TMs) depends on their organization into a bilayer. However, about half of TM lipids are non-lamellar, monogalactosyldiacylglycerol (MGDG) lipids, which in aqueous environments tend to form non-lamellar phases (NLPs). It is now also well established that isolated functional spinach TMs, in addition to the lamellar (L, or bilayer) phase, contain (at least) two isotropic (I) phases and an inverted hexagonal (H<sub>II</sub>) phase; and TMs of plants belonging to different divisions and orders exhibit similar polymorphisms. Quantitative analysis of our <sup>31</sup>P-NMR spectra on spinach TMs have shown that only about 40% of the bulk lipid molecules form L phase, and about 40% and 20% constitute an H<sub>II</sub> and I phases, respectively. The phase behavior of granum and stroma TMs were similar to intact TMs, whereas the contribution of plastoglobuli to the observed lipid polymorphism were ruled out. Specific effects of selected lipases and proteinases have revealed that the NLPs are to be found outside the protein-rich L phase of TMs containing the main protein (super)complexes of the photosynthetic machinery: I phases are proposed to originate from membrane fusion and junctions, and the association of lipid molecules with the photoprotective enzyme VDE and/or other lipocalin(-like) molecules; H<sub>II</sub> phase appears to be given rise by lipid molecules encapsulating stroma-side proteins or polypeptides. The largely reversible pH- and temperature-induced changes in the polymorphism of TMs strongly suggest the participation of NLPs in regulatory processes. The polymorphic phase behavior of TMs are interpreted within the frameworks of the Dynamic Exchange Model, which proposes that NLPs contribute to the structural plasticity of the highly organized vesicular membrane system and safe-guard the high protein-to-lipid ratio of TMs.

T1.9

## **FLASH-INDUCED CHLOROPHYLL FLUORESCENCE RELAXATION IN MICROALGAE**

Milán SZABÓ<sup>1,\*</sup>, Priyanka Pradeep PATIL<sup>1,2</sup>, Sabit MOHAMMAD ASLAM<sup>1,3</sup> and Imre VASS<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

<sup>2</sup>Institute of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

<sup>3</sup>Doctoral School of Biology, University of Szeged, Szeged, Hungary

\*Corresponding author: E-mail: [szabo.milan@brc.hu](mailto:szabo.milan@brc.hu)

Relaxation of fluorescence yield upon a light pulse has been frequently used to investigate the electron transfer processes in various photosynthetic organisms. While the fluorescence relaxation occurs in well discernible monotonous phases, this signature can manifest itself in a less-characterized waving pattern. In cyanobacteria, this special case of chlorophyll fluorescence was found to be related to the operation of alternative electron transfer processes, the re-reduction of plastoquinone (PQ) pool by the type I NAD(P)H dehydrogenase (NDH-1) (Deák et al. 2014, BBA-Bioenergetics 1837, 1522-1532). However, in eukaryotic algae the nature of the fluorescence wave and its relation to the electron transfer to PQ pool via alternative (cyclic) pathways remained to be better understood. Our aim was to i) investigate the various environmental conditions that induce the wave phenomenon, ii) to reveal the components of various electron transfer processes related to the wave phenomenon in a range of microalgae species that are used as model organisms in basic as well as in applied research. Our studies showed that the phenomenology of the wave signature exhibit remarkable differences in various species of microalgae, and different alternative electron sources appear to contribute to its manifestation in the different species. Therefore, the chlorophyll fluorescence wave can provide insights into the mechanisms that mediate the regulation of interacting electron flow routes in a range of algal species.

This work was supported by the National Research, Development and Innovation Office (NKFIH FK 128977).

T1.9

## **ACCLIMATION OF PHOTOSYNTHETIC APPARATUS TO DIFFERENT LIGHT INTENSITIES IN NORWAY SPRUCE**

Václav KARLICKÝ<sup>1,2,\*</sup>, Irena KURASOVÁ<sup>1</sup>, Michal ŠTROCH<sup>1</sup>, Parveen AKHTAR<sup>3</sup>, Kristýna VEČEŘOVÁ<sup>2</sup>, Otmar URBAN<sup>2</sup>, Bettina UGHY<sup>3</sup>, Győző GARAB<sup>1,3</sup>, Petar LAMBREV<sup>3</sup>, Vladimír ŠPUNDA<sup>1,2</sup>

<sup>1</sup>Department of Physics, Faculty of Science, University of Ostrava, Ostrava, Czechia

<sup>2</sup>Change Research Institute of the Czech Academy of Sciences, Brno, Czechia

<sup>3</sup>Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

\*Corresponding author: E-mail: [Vaclav.Karlicky@osu.cz](mailto:Vaclav.Karlicky@osu.cz)

The photosynthetic apparatus of Norway spruce exhibits several distinct characteristics that differ from those of typical land plants. Notably, the absence of lhcb3 and lhcb6 proteins as well as the presence of Lhcb8 (often referred to as Lhcb4.3) instead of Lhcb4 in photosystem II (PSII) light-harvesting complexes (LHCII) has been found in the gymnosperm genera *Picea* and *Pinus* (family Pinaceae). This variation results in different structure of PSII-LHCII supercomplex (Kouřil et al. 2016, New Phytologist) and its macro-organization in the thylakoid membrane (Karlický et al. 2016, Photosynthesis Research). Our recent research has demonstrated marked differences in the acclimation of the photosynthetic apparatus to different light intensities between spruce and the model angiosperm plant *Arabidopsis thaliana*. High light (HL) acclimation in spruce mainly involves the reduction of PSII and PSI core complexes per LHCII, the loss of PSII macro-organization, and the lock-in of high zeaxanthin levels and non-photochemical quenching in darkness (Štroch et al., 2022, Photosynthesis Research). Furthermore, we observed that strongly reduced macro-organization of PSII-LHCII supercomplexes in thylakoid membranes of HL-acclimated spruce was accompanied by an unchanged distribution of the excitation energy between the two photosystems after grana unstacking. We propose that the considerable increase in the content of the bilayer anionic lipid sulfoquinovosyl-diacylglycerol, at the expense of the non-bilayer lipid monogalactosyl-diacylglycerol, in HL-acclimated spruce thylakoid membranes contributes to the reduced flexibility of grana stacking/unstacking. Thus, the modulation of thylakoids lipid composition is involved in the specific HL-acclimation strategy of the spruce photosynthetic apparatus.

T1.11

## **ARBUSCULAR MYCORRHIZAL FUNGI (AMF) PROTECTS PHOTOSYNTHETIC APPARATUS OF WHEAT UNDER DROUGHT STRESS**

Sonal MATHUR<sup>1</sup> and [Anjana JAJOO](mailto:Anjana.JAJOO@unipune.ac.in)<sup>1,2</sup>

<sup>1</sup>School of Life Science, <sup>2</sup>School of Biotechnology,  
Devi Ahilya University, Indore (M.P.) 452017, INDIA  
Corresponding author: E mail: [ajheadsbt@gmail.com](mailto:ajheadsbt@gmail.com)

Drought stress (DS) is amongst one of the abiotic factors affecting plant growth by limiting productivity of crops by inhibiting photosynthesis. Damage due to DS and its protection by Arbuscular Mycorrhizal fungi (AMF) was studied on photosynthetic apparatus of wheat (*Triticum aestivum*) plants in pot experiments. AMF plants showed increased relative water content (RWC) both for leaf and soil indicating that AMF hyphae penetrated deep into the soil and provided moisture to the plants. In Chl a fluorescence induction curve (OJIP), a declined J-I and I-P phase was observed in DS plants. Efficacy of primary photochemistry declined in DS plants as result of DS, while AMF plants showed maximum

photochemistry. DS leads to declined quantum efficiency of PSI and PSII in DS plants while it was restored in AMF + DS plants. Electron transport (ETRI and ETRII) decreased while quantum yield of non-photochemical quenching Y(NPQ) increased as a result of drought stress. CEF around PSI increased in DS-stressed plants. Efficient PSI complexes decreased in DS plants while in case of AMF plants PSI complexes were able to perform PSI photochemistry significantly. Thus, it is concluded that drought stress-induced damage to the structure and function of PSII and PSI was alleviated by AMF colonization.

T2.3

### **INVESTIGATION OF OXYGEN, CARBON DIOXIDE, AND NITROGEN GASES INFLUENCE ON HYDROGEN PRODUCTION OF CYANOBACTERIA**

Bekzhan D. KOSSALBAYEV<sup>1,2</sup>, Asemgul K. SADVAKASOVA<sup>2</sup>, Bolatkhan K. ZAYADAN<sup>2</sup>,  
Meruert O. BAUENOVA<sup>2</sup>, Gulzhanay K. KAMSHYBAYEVA<sup>1,2</sup>, Suleyman I. ALLAKHVERDIEV

3

<sup>1</sup>Department of Chemical and Biochemical Engineering, Institute of Geology and Oil-Gas Business Institute Named after K. Turyssov, Satbayev University, Almaty 050043, Kazakhstan

<sup>2</sup> Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Al-Farabi 71, Almaty 050038, Kazakhstan

<sup>3</sup>K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Str. 35, Moscow 127276, Russia

*Department of Chemical and Biochemical Engineering, Institute of Geology and Oil-Gas Business Institute Named after K. Turyssov, Satbayev University, Satpaev street 22, Almaty 050043, Kazakhstan, e-mail: [kossalbayev.bekzhan@gmail.com](mailto:kossalbayev.bekzhan@gmail.com)*

The low amount of H<sub>2</sub> that can be released from cells biologically has led to the search for producers in various ecosystems and mutants by genetic methods. To study the ability to excrete H<sub>2</sub>, 11 different strains of cyanobacteria from different ecosystems were selected. The ability of cyanobacterial strains to release H<sub>2</sub> was studied on a gas chromatograph under anaerobic conditions. During screening, *Anabaena variabilis* BTA-1047 was found to produce more H<sub>2</sub> (5.3 μmol H<sub>2</sub> mg<sup>-1</sup> Chl a h<sup>-1</sup>) than other strains. In studies of the effect of various concentrations of O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> gases on the production of H<sub>2</sub> by strains, it was found that 2% O<sub>2</sub> and 2% CO<sub>2</sub> positively affect the production of hydrogen by cells, while in cells treated with 2% N<sub>2</sub>, the release of H<sub>2</sub> was relatively low. It has been established that an increase in the concentration of CO<sub>2</sub>, intensifying the process of photosynthesis, reduces the release of H<sub>2</sub>, since the O<sub>2</sub> released into the environment at the same time suppresses the formation of H<sub>2</sub>. The research results show that a micro-anaerobic environment saturated with a carbon source has a positive effect on the hydrogen distribution of nitrogen-fixing strains of cyanobacteria.

T2.13

## **HEAT AND MASS TRANSFER IN A METAL HYDRIDE REACTOR FOR HYDROGEN STORAGE AND PURIFICATION**

Dmitry DUNIKOV<sup>1,2</sup>, Dmitry BLINOV<sup>1,2</sup>

<sup>1</sup> Joint Institute for High Temperatures of the Russian Academy of Sciences, Laboratory for Hydrogen Energy Technologies, 125412 Izhorskaya st. 13 bld. 2, Moscow, Russia

<sup>2</sup> National Research University "Moscow Power Engineering Institute", 111250 Krasnokazarmennaya 14, Moscow, Russia

*\*Corresponding author: E-mail: ddo@mail.ru*

Sustainable development of energy sector includes rapid increase of share of renewables, including modern use of biomass, and requires new energy carriers with low carbon footprint. Low-carbon hydrogen from renewable sources is one of the most promising alternatives to fossil fuels. However, unlike electrolytic hydrogen produced from solar and wind power, hydrogen produced from biological resourced has to be upgraded before use. Metal hydrides selectively react with hydrogen at near ambient conditions and thus their applications include not only hydrogen storage, but also hydrogen purification and compression. To scale up metal hydride devices from laboratory samples to industrial applications, researchers need to overcome technical barriers, which connected with difficulties of enhancement of heat and mass transfer in porous metal hydride beds.

We present results of experiments and mathematical modelling of heat and mass transfer in a vertical flow-through metal hydride reactor with 1 kg of LaNi<sub>5</sub>-type alloy. The reactor can be used for hydrogen extraction from mixtures with high content of impurities such as CO<sub>2</sub> and CH<sub>4</sub>. Experiments show, that low permeability of activated metal hydride bed ( $\sim 10^{-12}$  m<sup>2</sup> due to small particle size  $\sim 10$   $\mu$ m) results in high Darcy pressure drop over the bed, and thus absorption requires higher feed pressures to purify hydrogen. It is proposed to increase particle and pore size to lower the pressure drop, and experiments on unactivated metal hydride beds (particle size  $\sim 1$  mm) show much higher permeability ( $\sim 10^{-9}$  m<sup>2</sup>), while the absorption speed is close to the values for the activated bed. Thus, prevention of particles decrepitation in metal hydride beds can help to increase efficiency of metal hydride purification of hydrogen.

*This work was supported by the Russian Science Foundation (No: 22-19-00516).*

T1:2

## **INTERACTION BETWEEN THE THYLAKOID PROTEIN PHOSPHORYLATION AND THE ENERGY-DEPENDENT QUENCHING OF CHLOROPHYLL FLUORESCENCE IN PLANTS**

Aynura PASHAYEVA<sup>1,2</sup>, Guangxi WU<sup>2</sup>, Irada HUSEYNOVA<sup>1</sup>, Choon-Hwan LEE<sup>2</sup>, Ismayil S. ZULFUGAROV<sup>1</sup>

<sup>1</sup>Institute of Molecular Biology and Biotechnologies, Azerbaijan National Academy of Sciences, 11 Izzat Nabiyev Str., Baku AZ 1073, Azerbaijan

<sup>2</sup>Department of Integrated Biological Science, Department of Molecular Biology, Pusan National University, Busan 46241, Korea

Corresponding author: [i.zulfugarov@imbb.science.az](mailto:i.zulfugarov@imbb.science.az)

Plants have established a diversity of molecular regulatory mechanisms in response to changes in light quality and quantity. Thylakoid protein phosphorylation (PP) and non-photochemical quenching of chlorophyll fluorescence (NPQ) are two close mechanisms that protect vascular plants through complex acclimation processes. The interaction between thylakoid PP and the NPQ is involved in the dynamic control of energy flow and photoprotection in the photosynthetic apparatus. To clarify the role of thylakoid PP in qE, we have used a direct Western Blot assay after BN-PAGE that allowed us to detect all phosphoproteins by P-Thr antibody as well as by P-Lhcb1 and P-Lhcb2 antibodies in rice plants. We observed that the bands corresponding to the phosphorylated proteins were enriched in the PsbS-KO mutant after illumination. In WT plants, the qE relaxation became slower after 10 min HL treatment, which correlated with PP of the Lhcb1 and Lhcb2 proteins in the LHCII trimers under the same experimental conditions. Consequently, we proposed that light-induced PP of PSII core and Lhcb1/Lhcb2 proteins enhancing in rice PsbS-KO plants might be due to more reactive-oxygen-species production in this mutant. The detailed molecular mechanisms and signaling pathways that connect thylakoid PP to the regulation of NPQ are still being investigated. Understanding the interaction between thylakoid PP and NPQ is important for elucidating the mechanisms underlying photosynthetic regulation and photoprotection in plants. This knowledge can contribute to the development of strategies to improve crop performance and stress tolerance.

T1.3

### **MITIGATION EFFECT OF HIGH LIGHT ON THE PHOTOSYNTHETIC APPARATUS OF RHODOBACTER ALKALITOLERANS WHEN GROWN IN AN ALKALINE ENVIRONMENT**

Mohammad Yusuf ZAMAL and Rajagopal SUBRAMANYAM\*

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad, Telangana, 500046 India

\*Corresponding author: [srgsl@uohyd.ernet.in](mailto:srgsl@uohyd.ernet.in)

Photosynthesis in purple bacteria is performed by pigment-protein complexes, including the light-harvesting complexes known as LH1 and LH2. The photosystem also encompasses carotenoids to assist in well-functioning of photosynthesis. Most photosynthetic bacteria are exposed to various abiotic stresses, and here, the *Rhodobacter (R.) alkalitolerans* was extracted from alkali pH. We report the comparative study of the photosynthetic apparatus of *R. alkalitolerans* in various light intensities in relation to the high pH tolerance ability of this bacterium. We found that as the light intensity increased, the stability of photosystem complexes decreased in normal pH (npH pH 6.8±0.05) conditions, whereas in high pH (hpH pH 8.6±0.05) acclimation was observed to high light. Looking at the content of bacteriochlorophyll *a*, absorbance spectra, and circular dichroism data, it is obvious

that the integrity of photosystem complexes is less affected in hpH compared to that of npH conditions. LP-BN of photosystem complexes also shows that LH2 is more affected in npH than hpH, whereas RC-LH1 monomer or dimer has shown interplay between monomer and dimer in hpH although the dimer and monomer both increased in npH. The pattern of monomer-dimer conversion is further evidenced by the sucrose density gradient of  $\beta$ -DM solubilized intracytoplasmic membranes. Further, thin layer chromatographic separation of isolated membrane lipids shows that phosphatidylcholine (PC) levels have increased in hpH conditions which further confirms the integrity of photosystem complexes in hpH conditions. Moreover, qPCR data showed that the subunit -c of ATPase levels was overexpressed in hpH. Consequently, the P515 measurement shows that more ATP production is required in hpH, which dissipates the protons from the chromatophore lumen. This could be the reason the photosystem protein complex stabilized due to more lumen acidification. To maintain homeostasis in hpH, the antiporter NhaD expressed more than in the npH condition. Our results will give clues to develop the alkali-tolerant species of algae and higher plants.

T1.2

## EVIDENCE FOR THE ROLE OF ISOTROPIC LIPID PHASE IN THE FUSION OF PHOTOSYSTEM II MEMBRANES

Kinga Ilona BÖDE<sup>1,2,3\*</sup>, Ondřej DLOUHÝ<sup>3</sup>, Ottó ZSÍROS<sup>1</sup>, Uroš JAVORNIK<sup>4</sup>, Avratanu BISWAS<sup>1,2</sup>, Primož ŠKET<sup>4</sup>, Janez PLAVEC<sup>4,5,6</sup>, Petar H LAMBREV<sup>1</sup>, Bettina UGHY<sup>1</sup>, Vladimír ŠPUNDA<sup>3</sup>, Győző GARAB<sup>1,3</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

<sup>2</sup>Doctoral School of Biology, University of Szeged, Szeged, Hungary

<sup>3</sup>Department of Biophysics, University of Ostrava, Ostrava, Czech Republic

<sup>4</sup>National Institute of Chemistry, Ljubljana, Slovenia

<sup>5</sup>EN-FIST Center of Excellence, Ljubljana, Slovenia

<sup>6</sup>Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

\*Corresponding author: [bode.kinga@brc.hu](mailto:bode.kinga@brc.hu)

Plant thylakoid membranes (TMs), in addition to the bilayer (or lamellar, L) phase, contain at least two isotropic (I) lipid phases and an inverted hexagonal (HII) phase. The non-bilayer propensity of bulk TM lipids has been proposed to safe-guard the lipid homeostasis of TMs; further, an I phase has been shown to arise from VDE:lipid assemblies (VDE is a luminal photoprotective enzyme) [1]. Effects of proteases and lipases on the lipid polymorphism of TMs have revealed that the HII phase originates from lipids encapsulating stroma-side proteins and that the non-bilayer phases are to be found in domains outside the protein-rich regions of TM vesicles; an I phase is proposed to be involved in the fusion of membranes and thus in the self-assembly of the TM network [2]. Here, using Photosystem II (BBY) subchloroplast particles, we tested this hypothesis. We capitalize on the fact that wheat-germ lipase (WGL) selectively eliminates the 31P-NMR-spectroscopy detectable I phases while exerts no effect on the L and HII phases and does not perturb the structure and function of the photosynthetic machinery [2]. We show that (i) BBY particles, compared to intact TMs, display weaker L and I phases and no HII phase – in accordance with the diminished lipid content of these particles and the absence of stroma TMs; (ii) similar to intact TMs, WGL has no effect on the molecular organization and functional activity of BBY particles but (iii) eliminates their I phase; and (iv) parallel with the diminishment of the I phase, WGL disintegrates the large (>10  $\mu$ m diameter) sheets of the BBY membranes, which are composed of stacked membrane pairs



of granum thylakoids of ~500 nm diameter. These data provide evidence for the involvement of I phase in the lateral fusion of stacked Photosystem II membrane pairs.

[1] Garab G. et al. (2022) Progr Lipid Res; [2] Dlouhý O. et al. (2022) Cells.

T1.2

## **COMPARATIVE ANALYSIS OF MESOPHYLL AND BUNDLE SHEATH CHLOROPLASTS FROM MAIZE PLANTS SUBJECTED TO SALT STRESS**

Nahida ALIYEVA, Durna. ALIYEVA, Saftar SULEYMANOV\*, Irada HUSEYNOVA

Institute of Molecular Biology and Biotechnologies, Ministry of Science and Education of the Azerbaijan Republic, 11 Izzat Nabiyev Str., Baku AZ 1073, Azerbaijan, Fax: +99412 510 2433

\*Corresponding author: E-mail: [saftar.suleymanov@mail.ru](mailto:saftar.suleymanov@mail.ru)

The leaves of  $C_4$  plants contain two distinct types of photosynthetic cells, mesophyll (M) and bundle sheath (BS) which are differently organized both structurally and functionally. The BS chloroplasts in the NADP-ME subtype are agranal with structures like stromal lamellae of chloroplasts  $C_3$  plants. Plants belonging to the  $C_4$  photosynthesis pathway are distinguished by the high efficiency of carbon dioxide ( $CO_2$ ) assimilation and the high level of specialization of the photosynthesis apparatus. The purpose of the presented work was the comparative study of M and BS chloroplasts isolated from maize under salt stress. The Zagatala 420 maize cultivar, which belongs to NADP-ME subtypes of  $C_4$  metabolism, was used as the object of this study. Chloroplasts were isolated from plants subjected to salt stress of various concentrations (0%, 1%, 2%, and 3% NaCl). Salt stress significantly affects the activity of PSII in M chloroplasts. The PSI complex showed tolerance to the effect of salt and although the rate of electron transport was reduced compared to the control, no drastic difference was detected. The analysis of fluorescence spectra of chlorophyll in M and BS chloroplasts showed the presence of three maxima, characteristic of the light-harvesting complex (LHC) at 686 nm, a PSII complex at 695 nm, and a PSI complex at 735 nm. Under the effect of 1% NaCl in the spectrum of M chloroplasts, the fluorescence ratio of 735 nm / 686 nm decreased and 3% salt led to a change in the short wavelength part of the spectrum characteristic for the PSII complex. The maximum intensity at 695 nm related to the core complex of PSII was weak in BS chloroplasts. This can be attributed to the low amount of LHCII, as well as the disruption of its anatomical structure under stress.

**ELECTROCHEMICAL CONTROL OF ELECTRON TRANSPORT IN THYLAKOID MEMBRANES AS STUDIED FOR MICROALGA AND CYANOBACTERIUM WITH MODELING STATE TRANSITIONS 2-1 DURING LIGHT INDUCTION**

N. BELYAEVA\*, A. BULYCHEV, V. PASCHENKO, G. RIZNICHENKO, A. RUBIN

Department of Biophysics, Biology Faculty of the M.V. Lomonosov Moscow State University,  
119234, Moscow, Russia,

\*e-mail: [natalmurav@yandex.ru](mailto:natalmurav@yandex.ru)

The closed systems of chloroplast and cyanobacterial thylakoid membranes (TM) support light powered electron / proton transfer (ET / PT) via the trans-membrane complexes that are similar for two types of photosynthetic membranes whereas the topological infrastructures differ for these TM systems [1]. The thylakoid membrane model (TM model) was developed as a system of ordinary differential equations [2-3] being modified in the present work to consider the state 2– 1 transitions that participate in the balance of light harvesting between photosystems II and I (PSII and PSI). As a result, quantitative interpretations were obtained for signals detected upon light induction prolonged to a few minutes: the fluorescence induction (FI) curves of *Scenedesmus obliquus* exposed to light during 5 min, as well as FI and P700 redox transitions of *Synechocystis* PCC 6803 recorded in parallel for 100 s intervals. Fittings the TM model to induction data allowed us to calculate the time-dependent concentrations of components for the ET/PT via PSII and PSI reaction centers mediated by PQ/PQH<sub>2</sub> pool, cytochrome b<sub>6</sub>f complex and plastocyanin or cyt c<sub>6</sub>, while reducing equivalents Fdr and NADPH are generated with an initial electron extraction from water by PSII and terminal Fdr – NADP reductase action. So, in silico, linear ET operation is considered along with the cyclic one, while charge fluxes between lumen/stroma depend on passive charge (H<sup>+</sup>, counterions) redistribution (V<sub>leak</sub>) and active energy consumption in the reversible H<sup>+</sup>-ATPase (V<sub>ATP</sub>). Fitting the parameter sets on different kinetic patterns for *Scenedesmus*, *Synechocystis* indicated the two TM types for chloroplasts/ cyanobacteria distinctive in PSI:PSII stoichiometry as proposed earlier [1]. Also, values V<sub>leak</sub> and V<sub>ATP</sub> were found different for *Scenedesmus* and *Synechocystis* TM systems. Within the framework of our quantitative analysis, the OJIPSMT fast and slow kinetics in FI might be explained according to TM energization stages coordinated to proton motive force generation upon light induction. Modifications of V<sub>leak</sub>, V<sub>ATP</sub> parameters were tested as factors influencing the OJIPSMT dynamics. The presented thylakoid membrane (TM) model can be used for systematically assessing the photosynthesizing organisms that function under different light or stress conditions.

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3. Belyaeva N, Bulychev A, Pashchenko V, Klementyev K, Ermachenko P, Konyukhov I, Riznichenko G, Rubin A. Dynamics of processes in thylakoid membranes of algae in vivo, studied in photosystem II and thylakoid models. *Biophysics* 2022, 67:5, 708-725

T1.4

## **EARLY-STAGE FORMATION OF OXO BRIDGES IN TRANSITION METAL CATALYSTS**

Aliya TYCHENGULOVA<sup>1,2</sup>, Aqerke BAURZHAN<sup>1</sup>, Noemi di STEFANO<sup>2</sup>, Daniele NARZI<sup>2</sup>,  
Giuseppe MATTIOLI<sup>3</sup> and Leonardo GUIDONI<sup>2</sup>

<sup>1</sup> Laboratory of Engineering Profile, Satbayev University, Almaty, Kazakhstan

<sup>2</sup> Department of Physical and Chemical Science, Università dell'Aquila, LAquila, Italy

<sup>3</sup> CNR-Istituto di Struttura della Materia, Area della Ricerca di Roma 1, CP 10, Monterotondo Scalo,  
Italy

*\*Corresponding author: E-mail: a.tychengulova@gmail.com*

Photosynthetic water oxidation is an important source of energy for all life forms which occurs during the oxidation and deprotonation reactions of the oxygen-evolving complex (OEC) in photosystem II (PS II). The Mn<sub>4</sub>Ca cluster is also of great interest to the scientific community due to the mechanism of its self-assembly through the photooxidation of Mn ions, called photoactivation.

Despite recent progress in describing the mechanism of water splitting catalyzed by PSII, much less is known about the process of self-assembly of the Mn<sub>4</sub>Ca cluster under the light, starting from the dissolved Mn<sup>2+</sup>. In the current situation, a theoretical approach to research can help rationalize the experimental data, shedding light on open questions regarding apo-PSII and the initial steps of photoactivation. The main idea of the work is to study the intermediate states of water-oxidizing catalysts in the process of self-assembly by computational chemistry methods based on the density functional theory. The present work can help to shed light on the structures of intermediate states of transition metal catalysts, as well as the mechanism of the second Mn<sup>2+</sup> attachment and the formation of oxo-bridges. Moreover, the role of acetate counterions on the self-assembly mechanism and the structure of the Mn catalyst was studied.

T1.4

## **RATE-LIMITING STEPS IN THE DARK-TO-LIGHT TRANSITION OF PHOTOSYSTEM II: DEPENDENCE ON THE TEMPERATURE AND THE LIPIDIC ENVIRONMENT OF THE REACTION CENTER**

Melinda MAGYAR<sup>1,\*</sup>, Gábor SIPKA<sup>1</sup>, Parveen AKHTAR<sup>1</sup>, Guangye HAN<sup>2</sup>, Petar H. LAMBREV<sup>1</sup>,  
Jian-Ren SHEN<sup>2,3</sup>, Győző GARAB<sup>1,4</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

<sup>2</sup>Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing, China

<sup>3</sup>Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan

<sup>4</sup>Faculty of Science, University of Ostrava, Ostrava, Czech Republic

\*Corresponding author: E-mail: [magyar.melinda@brc.hu](mailto:magyar.melinda@brc.hu)

Photosystem II (PSII) is the redox-active pigment–protein complex embedded in the thylakoid membrane (TM) that catalyzes the reduction of plastoquinone and the oxidation of water. By measuring variable chlorophyll-*a* fluorescence transients of PSII, elicited by single-turnover saturating flashes (STSFs), we have identified rate-limiting steps in the dark-to-light transition of PSII. It has been confirmed that in diuron-treated samples the first STSF, which generates the closed state (PSII<sub>C</sub>), induces an  $F_1 (< F_m)$  fluorescence level and additional excitations are required to reach the maximum ( $F_m$ ) level; and we have shown that  $F_m$  can only be reached by applying sufficiently long  $\Delta\tau$  waiting times between STSFs. We also revealed the gradual formation of the light-adapted charge-separated state, PSII<sub>L</sub>, linked to the  $F_1$ -to- $F_m$  transitions and possessing an increased stabilization of charges. Recently, we studied the effects of different physicochemical environments of PSII on the half-rise time ( $\Delta\tau_{1/2}$ ) and probed its presence during later steps ( $F_2$ ,  $F_3$  etc.). We show that (i) TM lipids shorten the  $\Delta\tau_{1/2}$  of PSII core complexes (CCs) of *T. vulcanus* to that of TMs – revealing the role of lipid matrix in the rate limiting steps; (ii) PSII CCs of *T. vulcanus* and spinach TMs, while exhibiting markedly different  $\Delta\tau_{1/2}$  values, display very similar temperature dependences, with increased values at low temperatures; and (iii) the  $\Delta\tau_{1/2}$  values in PSII CC are essentially invariant on the number of the STSF-induced increments at all temperatures. These data indicate the same physical mechanism involved during the PSII<sub>C</sub>-PSII<sub>L</sub> transition.

T1.4

## THE IMPACT OF CU(II) COMPLEXES ON THE PHOTOCHEMICAL ACTIVITY OF PHOTOSYSTEM II

M.S. SHABANOVA<sup>1,\*</sup>, I.M. HUSEYNOVA<sup>1</sup>, M.S. KARACAN<sup>2</sup>, N. KARACAN<sup>2</sup>, S.K. ZHARMUKHAMEDOV<sup>3</sup>, S.I. ALLAKHVERDIEV<sup>1,3,4</sup>

<sup>1</sup>Bionanotechnology Laboratory, Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, AZ1073 Baku, Azerbaijan

<sup>2</sup>Department of Chemistry, Science Faculty, Gazi University, Teknikokullar, Ankara 06500, Turkey

<sup>3</sup>Institute of Basic Biological Problems, FRC PSCBR Russian Academy of Sciences, Pushchino 142290, Russia

<sup>4</sup>Controlled Photobiosynthesis Laboratory, K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Str. 35, Moscow 127276, Russia

Herbicides are still the most effective method of weed control. However, frequent use of the same compounds leads to contamination of water and soil, and chemicals can damage the environment. Thus, in order to reduce the harm caused to the environment, special attention should be paid to new effective compounds acting by various mechanisms. Photosynthesis is a complex process of converting solar energy into the energy of chemical bonds. As a vital process for all photosynthetic organisms, photosynthesis is an attractive target for the use of inhibitory compounds.

Copper plays an important role in a number of metabolic processes in plants, cyanobacteria and algae. Although Cu cations are necessary for plant growth, a high concentration of Cu(II) has the highest

toxicity among heavy metal cations. It has been shown that the components of photosystem II (PSII) are more sensitive to the inhibitory effect of Cu(II) than the components of photosystem I.

In this study, thylakoid membranes enriched with PSII isolated from spinach leaves, *Spinacia oleracea* L, were used to study the inhibitory effect of Cu(II). This experiment was based on the effect of the copper complex on photosynthetic oxygen evolution as well as changes in the PSII chlorophyll fluorescence yield ( $F_V$ ) associated with photoreduction of the plastoquinone  $Q_A$ .

By efficiently suppressing both of the vital PSII photoreactions, we demonstrate that [CuL2] Br<sub>2</sub> effectively inhibits PSII photochemical activity. Without affecting the F<sub>0</sub> level or delaying the photoinduced rise in FM, [CuL2] Br<sub>2</sub> just lowers the FM level at the expense of the F<sub>V</sub>. The inhibitory action of [CuL2] Br<sub>2</sub> is not called off by artificial electron donors. It's likely that primary target for inhibitory activity of [CuL2] Br<sub>2</sub> complex is likely to be the components of PSII reaction center.

T1.5

## MODELLING OF ENERGY TRANSFER FROM PHYCOBILISOMES TO PHOTOSYSTEM I IN THE CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803

Avratanu BISWAS<sup>1\*</sup>, Parveen AKHTAR<sup>1</sup>, Ahmad BHATTI<sup>2</sup>, Ivo van STOKKUM<sup>2</sup>, Petar H. LAMBREV<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

<sup>2</sup>Department of Physics and Astronomy and LaserLaB, Faculty of Science, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands

\*Author to contact: [avratanu.biswas@brc.hu](mailto:avratanu.biswas@brc.hu)

In cyanobacteria, the main light-harvesting function is carried out by the phycobilisomes (PBS) – large water-soluble protein complexes attached peripherally to the thylakoid membrane, containing pigment-binding phycobiliproteins such as phycocyanin and allophycocyanin [1]. The energy transfer routes and dynamics from the PBS to photosystem I (PSI) and photosystem II (PSII) are still a matter of debate, especially regarding the connectivity of PBS and PSI. [2]

Here we investigated the pathways and dynamics of energy transfer from PBS to the photosystems in *Synechocystis* sp. PCC 6803. To measure the energy transfer to PSI directly, we used a mutant strain devoid of PSII [2]. The excitation kinetics of PBS and PSI were followed by picosecond time-resolved fluorescence spectroscopy using a synchroscan streak camera setup. A detailed kinetic model was developed that fits the experimental data and provides microscopic rate constants of energy transfer between different PBS, PSI and PSII chromophore groups. The model is based on measurements of isolated complexes (PBS, PSI) as well as a previously developed model of energy transfer in the PBS [3]. We found that PBS are capable of directly transferring energy to PSI in the PSII-deficient mutant, in a time scale of about 20 ps at room temperature. A significant fraction of PBS (~36%) in the mutant are not connected to PSI. These results will be used to refine a previous model of energy transfer in the intact cells of cyanobacteria [4].

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T1.6

### **ANALYSIS OF PHOTOCHEMICAL AND QUALITATIVE CHARACTERISTICS OF MICROGREENS LEAVES IN RELATION TO CHANGES IN MONOCHROMATIC LIGHT CONDITIONS**

Lucia JASENOVSKÁ<sup>1\*</sup>, Marián BRESTIČ<sup>1</sup>, Mária BARBORIČOVÁ<sup>1</sup>, Andrej FILAČEK<sup>1</sup>, Marek ŽIVČÁK<sup>1</sup>

<sup>1</sup>Institute of Plant and Environmental Sciences, Slovak University of Agriculture, Nitra, Slovakia

\*Corresponding author: e-mail: [xjasenovska@uniag.sk](mailto:xjasenovska@uniag.sk)

Currently, there is an increase in the cultivation of crops in high-tech greenhouses and controlled agricultural environments using light-emitting diodes that allow the regulation of plant growth, morphology, and qualitative traits through the control of artificial light (intensity, photoperiod, and spectrum). Conditions of cultivation, such as light quality, can influence the functions of photosynthetic apparatus, as well as the content of valuable nutritional elements of microgreens, the functional food resources. Our experiments focused on non-invasive pre-screening of the microgreens based on responsiveness to light spectra based on photochemical and qualitative traits. The photosystem II (PSII) photochemistry parameters were analyzed using the HandyPea device. The present work summarizes the results of analyses of the fast chlorophyll fluorescence kinetics in a collection of microgreens (19 genotypes of 17 species) cultivated in three light environments: control represented by white LEDs with balanced spectral composition, monochromatic blue (470 nm) and red (660 nm) LED lights. In red light, compared to blue light, we observed an increase in PSII antenna size indicated by the ABS/RC parameter, a decrease in the efficiency of electron flow between photosystems (PSII and PSI), and an overall reduction of the pool of electron carriers (Sm). Based on the values of the photochemical performance index (PIabs), we classified the tested genotypes into four groups: non-responsive to spectra with little significant differences (wheatgrass, spinach, pea jumbo, and fava bean), highly responsive to applied blue spectra (arugula, mung beans, pea, lettuce, and rose radish), highly responsive to red spectra (amaranth, cabbage, corn, fenugreek, kohlrabi, lentils, lettuce, sunflower, and watercress) and equally responding both to blue and red spectrum (mustard, red radish). The role of the light spectral quality on PSII photochemistry, but also on nutritional value of microgreens products was demonstrated.

Supported by the projects VEGA 1-0664-22, VEGA-1-0425-23 and APVV-18-0465.

T1.7

### **EFFECTS TEMPERATURE AND LIGHT INTENSITY ON ACTIVITIES OF KEY ENZYMES IN CAM PHOTOSYNTHESIS IN THE PHALAENOPSIS LEAVES**

Shahniyar BAYRAMOV<sup>1,2\*</sup>, Win van IEPEREN<sup>1</sup>

<sup>1</sup>Group Horticulture and Product Physiology, Wageningen University, 6700 AA, Wageningen, The Netherlands

<sup>2</sup>Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, AZ1073, Baku, Azerbaijan

\*Corresponding author: E-mail: [shahniyarb@yahoo.com](mailto:shahniyarb@yahoo.com)

The research on biochemical basis of CAM photosynthesis in the ornamental plant Phalaenopsis has been performed. The dynamics of diel changes in CO<sub>2</sub> rate during various phases of CAM photosynthesis were studied along with the changes of titratable acidity in the plant leaves, activities and the regulation mechanisms of some CAM pathway enzymes. According to the titratable acidity and dynamics of the diel changes in CO<sub>2</sub> assimilation, the studied Phalaenopsis genotypes are obligate CAM plants. Diel changes in activities of the primary enzymes of CAM photosynthesis-PEPCase and NAD-MDH were determined in 4-hour intervals. The activities of both enzymes were found to be higher during the first phase of CAM photosynthesis. The activities of both enzymes were less in the early morning hours compared to the night hours. Different PEPCase activities were detected depending on the leaf position. A positive correlation was observed between the ages of leaves and titratable acidity, and also between leaf ages and changes in PEPCase activity. Contrary to PEPCase, the highest activity of NAD-MDH was detected in old leaves. More inhibitory effect of malate on PEPCase activity was detected during daylight hours. The concentration of malate required for 50% inhibition (K<sub>i</sub>) of the day and night forms of PEPC from Phalaenopsis leaves were 0.5 and 5.0 mM, respectively. At low concentrations of malate, 5 mM glucose-6-phosphate more effectively prevents malate inhibition. Contrary to the light period, glucose-6-phosphate does not significantly affect enzyme activity during the dark period. Thus, glucose-6-phosphate has more effect on the dephosphorylation form of the enzyme than on the phosphorylation form.

T1.8

### **Mn-DEPLETED AND Mn-RECONSTRUCTED THYLAKOID MEMBRANES IN PHOTOBIOELECTROCHEMICAL CELL**

Roman VOLOSHIN<sup>1\*</sup>, Sergey ZHARMUKHAMEDOV<sup>2</sup>,  
Suleyman I. ALLAKHVERDIEV<sup>1</sup>

<sup>1</sup> K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia

<sup>2</sup> Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia

\*Corresponding author: [voloshinr@ifr.moscow](mailto:voloshinr@ifr.moscow)

The components comprising the photosynthetic apparatus possess a remarkable attribute: a high quantum yield of photoinduced charge separation. This distinctive characteristic renders them prominent sensitizers for photobioelectrochemical cells (PBEC). However, the isolated photosystems or membrane fragments exhibit low stability when integrated into the devices.

Within the photosynthetic apparatus, the oxygen evolving complex (OEC) containing a Mn-cluster emerges as the most delicate constituent. The enhancement of stability and efficiency in biohybrid devices based on the photosystem II contained membrane fragments can be achieved through the substitution of the native OEC with a stable and effective artificial analogue. To pave the way for the development of suitable artificial analogues, a preliminary investigation is required. Specifically, the Mn-depleted thylakoids photoactivated with  $MnCl_2$ , necessitate thorough examination in PBEC.

In this study, Mn-depleted thylakoid membranes were obtained using hydroxylamin treatment. A PBEC was built to investigate these thylakoids in the presence of artificial electron acceptor. Photoactivated electron transfer in the Mn-depleted membranes was studied in the presence of an exogenous electron donor diphenyl carbazide in both PBEC and fluorimeter. Also, Mn-depleted membranes photoactivated with exogenous manganese was studied in PBEC and Clark cell.

*This work was supported by grants from the Russian Science Foundation (No: 19-14-00118) and by the state contract of the Ministry of Science and Higher Education of the Russian Federation (Project No. 122050400128-1).*

T1.8

## **FATTY ACIDS PROFILES OF MICROALGAE STRAINS FROM THE IPPAS CULTURE COLLECTION**

Anastasia A. KRAPIVINA\*, Elena V. ZADNEPROVSKAYA, Maria A. SINETOVA, Alexander Y. STARIKOV, David A. GABRIELIAN, Suleyman I. ALLAKHVERDIEV

K.A. Timiryazev Institute of Plant Physiology RAS, Moscow, Russia

\*Corresponding author: [a.krapivina35@gmail.com](mailto:a.krapivina35@gmail.com)

Microalgae are unicellular microscopic organisms that use sunlight to convert carbon dioxide and water into organic matter through the process of photosynthesis. There has been a considerable increase in interest in microalgae as fast-growing organisms that are able to produce valuable compounds such as proteins, carbohydrates, pigments, and lipids and have a wide range of applications in biotechnology.

This study aims to find strains of microalgae capable of accumulating triacylglycerols with a high content of long-chain polyunsaturated fatty acids. For this purpose, the composition of fatty acids in 15 strains of microalgae belonging to Chlorophyta, Charophyta, Rhodophyta, Ochrophyta, and Bacillariophyta was studied. Microalgae strains were obtained from the collection of microalgae and cyanobacteria IPPAS (K.A. Timiryazev Institute of Plant Physiology RAS, Moscow, Russia). The  $\omega$ -3 eicosapentaenoic acid was found in *Spirogyra* sp. IPPAS C-2071 (Charophyta), *Porphyridium* spp. IPPAS P-271, IPPAS P-273, IPPAS P-293, IPPAS P-519, IPPAS P-520 (Rhodophyta), *Nannochloropsis* sp. IPPAS D-734 (Ochrophyta), *Tribonema vulgare* IPPAS H-150 (Ochrophyta), *Lobosphaera* sp. IPPAS C-1540 (Chlorophyta), *Tetraselmis* sp. IPPAS C-2069 (Chlorophyta), and *Halamphora* sp. IPPAS H-1541 (Bacillariophyta). The  $\omega$ -3 docosapentaenoic acid was detected in *Pseudopleurococcus* sp. IPPAS U-1510 (Chlorophyta), and *Mallomonas* sp. IPPAS H-2051 (Ochrophyta). The  $\omega$ -6 arachidonic acid (ARA) was present in IPPAS H-150, IPPAS D-734, IPPAS P-271, IPPAS P-273, IPPAS P-293, IPPAS P-519, IPPAS P-520, IPPAS H-1541, and IPPAS H-150. In this research, a distinctively high ARA content was observed in *Lobosphaera* sp. IPPAS C-1540.



*This study was supported by the Russian Science Foundation (no. 22-44-08001).*

T1.9

**TEMPERATURE-INDUCED REVERSIBLE CHANGES IN PHOTOSYNTHESIS  
EFFICIENCY AND ORGANIZATION OF THYLAKOID MEMBRANES FROM PEA  
(PISUM SATIVUM)**

Jyoti Ranjan RATH a,1, Jayendra PANDEY a,1, Ranay Mohan YADAV a,1, Mohammad Yusuf  
ZAMAL a, Pavithra RAMACHANDRAN a, Nageswara Rao MEKALA a, Suleyman I.  
ALLAKHVERDIEV b, Rajagopal SUBRAMANYAM a,\*

a Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad,  
500046, India

b K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya St.  
35, Moscow, 127276, Russia

High temperature can induce a substantial adverse effect on plant photosynthesis. This study addressed the impact of moderately high temperature (35°C) on photosynthetic efficiency and thylakoid membrane organization in *Pisum sativum*. The Chl a fluorescence curves showed a significant change, indicating a reduction in photosynthetic efficiency when pea plants were exposed to moderate high-temperature stress. The pulse-amplitude modulation measurements showed decreased non-photochemical quenching while the non-regulated energy dissipation increased in treated compared to control and recovery plants. Both parameters indicated that the photosystem (PS)II was prone to temperature stress. The PSI donor side limitation increased in treated and recovery plants compared to control, suggesting the donor side of PSI is hampered in moderate-high temperature. Further, the PSI acceptor side increased in recovery plants compared to control, suggesting that the cyclic electron transport is repressed after temperature treatment but revert back to normal in recovery conditions. Also, the content of photoprotective carotenoid pigments like lutein and xanthophylls increased in temperature-treated leaves. These results indicate the alteration of macro-organization of thylakoid membranes under moderately elevated temperature, whereas supercomplexes restored to the control levels under recovery conditions. Further, the light harvesting complex (LHC)II trimers, and monomers were significantly decreased in temperature-treated plants. Furthermore, the amount of PSII reaction center proteins D1, D2, PsbO, and

Cyt b6 was reduced under moderate temperature, whereas the content of LHC proteins of PSI was stable. These observations suggest that moderately high temperature can alter supercomplexes, which leads to change in the pigment-protein organization.

T1.9

**RIBOFLAVIN GENERATES SUPEROXIDE AND H<sub>2</sub>O<sub>2</sub> IN CHLOROPLASTS AND MITOCHONDRIA TO PROMOTE STOMATAL CLOSURE WITHOUT COMPROMISING THE QUANTUM EFFICIENCY OF PSII IN LEAVES OF ARABIDOPSIS THALIANA**

Shashibhushan GAHIR\*, Pulimamidi BHARATH, Jyoti Ranjan RATH, Subramanyam RAJAGOPAL, Gudipalli PADMAJA and Agepati S. RAGHAVENDRA

Department of Plant Sciences, School of Life Sciences  
University of Hyderabad, Hyderabad 500046, India  
\* *Corresponding author Email: shashigahir18@gmail.com*

Riboflavin (vitamin B<sub>2</sub>, a precursor of FAD and FMN) is an essential element for plant processes and is known to protect plants from abiotic/biotic stresses. Riboflavin generated superoxide radicals and H<sub>2</sub>O<sub>2</sub> on exposure to even low light in leaves. Treatment with riboflavin at nanomolar concentrations, promoted stomatal closure in epidermis of *Arabidopsis thaliana* by elevating the levels of superoxide and H<sub>2</sub>O<sub>2</sub> in the guard cells, as indicated by the fluorescence of BES-So-AM and CM-H<sub>2</sub>DCFDA respectively. Further, the closure by riboflavin was reversed by tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid, superoxide anion scavenger) and catalase (ROS scavenger) highlighting the importance of reactive oxygen species (ROS) in guard cell signal transduction. The *fsd3* mutant (deficient in chloroplastic FeSOD3) exhibited reversal of closure, indicating that the major site of ROS generation by riboflavin might be at chloroplast. Co-localization studies corroborated that the major sites of superoxide and H<sub>2</sub>O<sub>2</sub> generation in response to riboflavin were chloroplasts and mitochondria, respectively. Diphenyleiodonium chloride (NADPH oxidase inhibitor) and salicylhydroxamic acid (peroxidase inhibitor) reversed stomatal closure, indicating that both NADPH oxidase and peroxidase played a key role during riboflavin-induced stomatal closure. Studies on O-J-I-P transients revealed that riboflavin treatment did not alter the F<sub>v</sub>/F<sub>m</sub> ratios unlike the decrease caused by ABA that induces stomatal closure. We propose that riboflavin could be an excellent priming agent to impart abiotic or biotic stress tolerance by modulating stomatal closure.

**PHOTOSYNTHETIC ELECTRON FLOWS IN HORDEUM VULGARE LEAVES OF  
DIFFERENT AGE UNDER HEAT STRESS**

Natallia PSHYBYTKO<sup>1,\*</sup>, Jerzy KRUK<sup>2</sup>, Eugene LYSENKO<sup>3</sup>, Kazimierz STRZALKA<sup>2</sup>, Vadim DEMIDCHIK<sup>1</sup>

<sup>1</sup> Belarusian State University, Minsk, Belarus

<sup>2</sup> Jagiellonian University, Kraków, Poland

<sup>3</sup> Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia

\*Corresponding author: E-mail: [pshybytko@bsu.by](mailto:pshybytko@bsu.by)

Temperature is one of the major environmental constraints that limits photosynthetic activity, affecting plant growth and productivity. One of the unresolved issues in studies of thermal adaptation of plants is the question of the difference in adaptive strategies to heat of the photosynthetic apparatus at different stages of development. The aim of this study was to investigate how photosynthetic electron flows respond to hyperthermia (40 °C, 3 h) at different stages of leaf development (four-, seven- and eleven-day-old primary leaves of *Hordeum vulgare* L.). Chlorophyll fluorescence parameters, characteristics of the redox state of P700 and plastoquinones and ferredoxin as well as transcription of *ndhA* and *ndhF* genes were examined. It was shown that heat inhibited the ferredoxin-plastoquinone reductase (FQR)-dependent cyclic electron flow (CEF) in all tested variants. In eleven-day-old leaves, an inhibition of the linear electron flow (LEF) was also detected. Heat-induced decrease of CEF and LEF in seven- and eleven-day-old plants (but not in four-day-old seedlings) was compensated by the activation of ‘NADH dehydrogenase-like complex’ (NDH)-dependent electron flow as well as increase of *ndhA* and *ndhF* transcription (genes encoding NDH). A hyperthermia decreased the level of plastoquinone pool reduction in all tested leaves. In seven- and eleven-day-old leaves, exposure to heat decreased the photoactive and increased the non-photoactive plastoquinone pools, respectively. Infiltration of leaves by 2,6-dichlorophenolindophenol (DPIP) prevented the decrease of the plastoquinone reduction. It also inhibited the FQR-dependent CEF and qE changes. At the same time, the DPIP treatment did not affect the heat-induced suppression of LEF and the redistribution of plastoquinones from the photoactive to the non-photoactive pool. Based on the data obtained, it can be concluded that a decrease in the efficiency of electron donation from plastocyanin to PSI and electron acceptance by ferredoxin could be the reason of heat-induced suppression of FQR-dependent CEF, while a decrease in the size of photoactive plastoquinone pool is potentially a reason of heat-induced LEF inhibition.

T1.9

## HEAVY METAL AND SINGLET OXYGEN SENSING IN SYNECHOCYSTIS PCC 6803 CYANOBACTERIA

Gábor PATYI<sup>1\*</sup>, Barbara HÓDI<sup>1</sup>, Péter B. KÓS<sup>1</sup>, Imre VASS<sup>1</sup>

<sup>1</sup> Institute of Plant Biology, Biological Research Centre of the Eötvös Lóránd Research Network,  
Szeged, Hungary

\*Corresponding author: E-mail: patyi.gabor@brc.hu

The *Synechocystis* cyanobacterium is an ideal chassis for constructing whole cell bioreporters assessing various molecules and stress factors. Among those, heavy metals (HM) and singlet oxygen (<sup>1</sup>O<sub>2</sub>) are very important stress factors in *Synechocystis* cells, therefore detecting their exact concentration in a continuous, non-destructive way is vital for research. The detection of these stress factors is complicated due to various issues.

The limited sensitivity of HM bioreporters may prevent their wide-spread application. Therefore, we created constructs with increased sensitivity and high specificity.

In the last decades, the recognition that <sup>1</sup>O<sub>2</sub> can participate in signal transduction has received increasing attention, but monitoring of this process is very difficult. To solve this problem, we used the promoter of a <sup>1</sup>O<sub>2</sub>-inducible gene to create a <sup>1</sup>O<sub>2</sub>-specific whole-cell biosensor.

Our results enabled the specific *in situ* and *in vivo* <sup>1</sup>O<sub>2</sub> detection, and in addition improved the sensitivity of bacterial HM biosensors.

T1.9

## PHOTOSYNTHETIC ACTIVITY OF HAEMATOCOCCUS PLUVIALIS REVEALED BY FLASH-INDUCED FLUORESCENCE RELAXATION AND SINGLE CELL CHLOROPHYLL FLUORESCENCE STUDIES

Priyanka Pradeep PATIL<sup>1,2,\*</sup>, Milán SZABÓ<sup>1</sup> and Imre VASS<sup>1</sup>

<sup>1</sup> Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged,  
Hungary

<sup>2</sup> Institute of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

\*Corresponding author : E-mail: [patil.priyanka@brc.hu](mailto:patil.priyanka@brc.hu)

*Haematococcus pluvialis* has been in the focus of intensive research in the past decades due to its capability of accumulating a massive amount of the valuable carotenoid, astaxanthin (Ast). Ast has a high commercial value in pharmaceutical, nutraceutical and aquaculture industries. It has been shown that a transient upregulation in cell metabolism and energy-dependent photoprotective mechanisms occurred during transformation of green cells to red cells, to meet the energy demand of Ast and fatty acid synthesis. However, the alternative electron transfer pathways remain largely unknown in this species. Using flash-induced fluorescence relaxation, we revealed that red cells exhibited a characteristic wave phenomenon, which was related to the operation of type II NAD(P)H dehydrogenase (NDH-2), whereas green cells did not show the wave phenomenon. We also demonstrated the PSII efficiency  $F_v/F_m$  was relatively unchanged during green to red cell transition, however characteristic changes appeared in transient fluorescence kinetics, analyzed at single cell level. *H. pluvialis* therefore displays condition-specific Chl fluorescence phenomena, which could be used as non-invasive markers of metabolic changes under the induction of Ast production.

This work was supported by National Research, Development and Innovation Office (NKFIH FK 128977).

T1.9

### **DIFFERENCES IN HEAT SUSCEPTIBILITY AND INVOLVEMENT OF NDH-2 IN ALTERNATIVE ELECTRON TRANSPORT IN CORAL ENDOSYMBIONT ALGAE, SYMBIODINIACEAE**

Sabit MOHAMMAD ASLAM<sup>1,2,\*</sup>, Imre VASS<sup>1</sup> and Milán SZABÓ<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

<sup>2</sup>Doctoral School of Biology, University of Szeged, Szeged, Hungary

\*Corresponding author: E-mail: [sabit.mohammad@brc.hu](mailto:sabit.mohammad@brc.hu)

Symbiodiniaceae is a family of unicellular, dinoflagellate algae, found mostly living in symbiotic relationships with cnidarians. They carry out the vital photosynthetic process, thus play a crucial role in supporting the energy need of host. Global climate change exerts significant threat on this symbiotic relationship. Rise in sea surface temperature causes the expulsion of these zooxanthellae from the host, leading to coral bleaching. However, the Symbiodiniaceae species harboured by the coral host, strongly determines the heat tolerance of corals. In our study, using a range of chlorophyll fluorescence methods we characterized the differences in response to acute heat stress and recovery within Symbiodiniaceae. We observed a wave like phenomenon of fluorescence relaxation, which is related to the decrease of the activity of Photosystem II (PSII) relative to the activity of Photosystem I (PSI) and the transient oxidation and re-reduction of plastoquinone (PQ) pool. *Symbiodinium* also showed increase in the re-reduction rate of the oxidized reaction centre of PSI, P700<sup>+</sup>, upon heat treatment as well as after chemical

inhibition of the Calvin-Benson cycle. Using different inhibitors on intact and partially digested *Symbiodinium* cells, we showed that linear electron transport plays a crucial role in the establishment of the wave phenomenon and also revealed the involvement of type II NAD(P)H dehydrogenase (NDH-2) in the alternative electron transport under stress conditions.

This work was supported by National Research, Development and Innovation Office (NKFIH FK 128977).

T1.9

## **DEVELOPMENT OF A MATHEMATICAL MODEL OF PHOTOSYNTHETIC PROCESSES UNDER TEMPERATURE STRESS**

Daria RATNITSYNA \*, Ekaterina SUKHOVA and Vladimir SUKHOV

<sup>1</sup>N.I. Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.

\*Correspondence: [Dasha-lola1997@mail.ru](mailto:Dasha-lola1997@mail.ru)

Constantly changing environmental conditions can affect the most of physiological processes of plants. Photosynthesis is the key plant process which can be the target of the influence of stressors. One of the important methods of studying this process is mathematical modeling which has a number of advantages over experimental methods.

One of the most famous models of dark reactions of photosynthesis is the Farquhar-von Caemmerer-Berry (FvCB) model which also describes light reactions in a simplified way. The main assumption of the FvCB model is that the steady state rate of CO<sub>2</sub> assimilation is described as being equal to the slowest of the three main photosynthetic processes: (i) carboxylation/oxygenation related RuBP activity; (ii) regeneration of RuBP associated with activity of the electro-transport chain; (iii) outflow of triose phosphates from the Calvin cycle. The third process is not considered in the model at simulated times. The effect of stressors on photosynthetic reactions is considered in the developed model by introducing damage coefficients of light reactions. The model also considers the temperature dependences of the rates of light and dark reactions of photosynthesis. The developed model was parameterized in detail based on experimental data using pea plants.

Analysis of the developed model showed the development of photodamage even at low light intensity. This effect is consistent with experimental data. Then, an analysis was made of the development of light damage under various temperature conditions. The analysis of the mathematical model shows the suppression of photosynthetic processes when the temperature rises above the optimum. This effect is shown at all light intensities, both low and high. It is important to note that the model shows a decrease in the magnitude of thermal damage to photosynthetic processes after the temperature rises to a certain level. An important assumption of the model is the absence of protein destruction under the action of stressors. This assumption of the model imposes a temperature restrictions for the applicability of the model.

The investigation was funded by the Russian Science Foundation, project number 23-14-00127.

## T1.9

### **ABSCISIC ACID-INDUCED STOMATAL CLOSURE INVOLVES CYTOSOLIC ALKALINIZATION MEDIATED BY BOTH PM H<sup>+</sup>-ATPASE AND VACUOLAR H<sup>+</sup>-ATPASE AND IS FOLLOWED BY A RISE IN ROS/NO IN ARABIDOPSIS THALIANA GUARD CELLS**

P. BHARATH\*, Shashibhushan GAHIR, Gudipally PADMAJA, and Agepati S. RAGHAVENDRA

Department of Plant Sciences, School of Life Sciences  
University of Hyderabad, Hyderabad 500046, India

\*Corresponding author: E-mail: [bharathsjgc@gmail.com](mailto:bharathsjgc@gmail.com)

Stomatal closure by abscisic acid (ABA) is one of the early responses initiated by plants under abiotic or biotic stress conditions. Stomatal closure by ABA not only regulates the rates of photosynthesis/transpiration but also provides immunity to plants. ABA-induced closure was initiated by alkalization of cytosol, followed by an increase in reactive oxygen species (ROS), and nitric oxide (NO) in guard cells of Arabidopsis. External addition of methylamine (MA, a weak base) caused alkalization of guard cells and promoted stomatal closure, suggesting pH changes would be important for stomatal closure. Both ABA and MA elevated the fluorescence levels corresponding to ROS and NO during closure. The presence of ROS modulators (DPI, SHAM, and catalase) or NO modulators (cPTIO, L-NAME, and tungstate) nullified the effects of ABA and MA, confirming the importance of both ROS and NO. Specific H<sup>+</sup>-ATPase inhibitors and their mutants were used to assess the origin of cytosolic pH. Vanadate, a plasma membrane H<sup>+</sup>-ATPase (PM H<sup>+</sup>-ATPase) inhibitor, and concanamycin A1, a vacuolar H<sup>+</sup>-ATPase (V-ATPase) inhibitor restricted ABA-induced stomatal closure. Both H<sup>+</sup>-ATPase inhibitors, besides preventing ABA-induced stomatal closure, reduced the levels of cytosolic pH, ROS, and NO, suggesting that the generation of ROS and NO was dependent on the PM- and V-ATPase activities. The inability of ABA to induce stomatal closure in Arabidopsis mutants deficient in PM H<sup>+</sup>-ATPase (*aha*) and V-ATPase (*vha*) confirmed the functional need for both PM- and V-ATPase during stomatal closure. Reduced levels of pH, ROS, and NO in guard cells of *aha* and *vha* mutants validate the essential role of cytosolic alkalization. Thus, both PM H<sup>+</sup>-ATPase and V-ATPase might be mediating the cytosolic alkalization, an early signal, followed by the generation of ROS and NO during stomatal closure induced by ABA.

## T1.9

### **ROOT AND LEAF AQUAPORINS INVOLVEMENT IN ADAPTATION OF HYDRAULIC SYSTEM OF MAIZE PLANTS TO WATER STRESS**

Maksim SUSLOV<sup>1\*</sup>, Julus Khan EGOROV<sup>1,2</sup>

<sup>1</sup>Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russia

<sup>2</sup>Kazan (Volga Region) Federal University, Kazan, Russia

\*Corresponding author: E-mail: [makscom87@mail.ru](mailto:makscom87@mail.ru)

As part of the study of coordination mechanisms of plant hydraulic system components during plant adaptation to abiotic stresses, the contribution of maize plant roots and leaves aquaporins to plants adaptation to water stress was investigated. In particular, the dynamics of radial water transport in roots and transpiration rate in leaves of intact maize plants as well as gene expression and localization of aquaporins in roots and leaves were investigated. In addition, physiological parameters such as root and shoot growth rate, leaf water content, and xylem sap pH were examined. Water transport parameters in roots and transpiration rate in leaves were measured in intact plants continuously for a long time from under water stress impact at controlled environmental conditions. It was shown that during the first minutes after 10% PEG 6000 induced water stress impact, the intensity of cell-to-cell water transport in the roots decreases with a parallel short-term increase in the rate of transpiration in leaves and, presumably, apoplastic transfer in roots. Further, after decrease in transpiration rate, the intensity of cell-to-cell water transport was restored approximately to the initial values and was accompanied by parallel over-expression of some PIP aquaporin genes in roots and leaves, changes in aquaporin localization in root tissues and changes in xylem sap pH. Under water stress conditions cell-to-cell water transport in roots becomes dominant. It is assumed that change in xylem sap pH may be a signal involved in the mechanisms of coordination of the components of the plant hydraulic system, in particular, roots and shoots, in response to water stress.

This study was supported by the Russian Science Foundation (project number 22-74-10087, <https://rscf.ru/en/project/22-74-10087/>).

T.1.9

#### **RELATIONSHIP BETWEEN SHORT- AND LONG-TERM ADAPTIVE MECHANISMS OF THE PHOTOSYNTHETIC APPARATUS OF HIGHER PLANTS AT HIGH LIGHT**

Daria VETOSHKINA\*, Nikolay BALASHOV, Ilya NAYDOV, Maria Borisova-MUBARAKSHINA

<sup>1</sup>Institute of Basic Biological Problems, Federal Research Center «Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences» Pushchino, Russia

\*Corresponding author: E-mail: [vetoshkinadv@gmail.com](mailto:vetoshkinadv@gmail.com)

Changes in illumination trigger various short-term and long-term adaptation mechanisms, including those at the level of the light-harvesting antenna of photosystem II (PSII). Redistribution of absorbed solar energy between photosystems (PS), called state transition, is one of the mechanisms of short-term adaptation. The first step in this process is the activation of the STN7 kinase, leading to phosphorylation of Lhcb1 and Lhcb2 proteins that are part of the trimers of the PS II light-harvesting antenna (LHCII). As a result, these trimers with phosphorylated proteins detach from PSII and bind to PSI. The long-term adaptation mechanism is activated after prolonged exposure to high light, and is represented by the decrease of the size of the PS II light-harvesting antenna, mostly owing to decrease in the amount of the trimers. Under high light conditions, state transitions are inhibited. However, the exact mechanism of this inhibition has remained unclear.

When the intensity of light increases, reactive oxygen species are generated in plant cells primarily within chloroplasts. Previous studies have revealed that hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) acts as a specific



signaling molecule, initiating the regulation of the size of the light-harvesting antenna of PSII under prolonged exposure to high light.

The aim of this study was to investigate the impact of H<sub>2</sub>O<sub>2</sub> on the activity of STN7 kinase. It has been demonstrated that arabidopsis plants lacking STN7 kinase are unable to adapt to high light using the same long-term adaptation mechanism as wild type plants. Furthermore, using isolated thylakoid membranes, it was demonstrated that the addition of H<sub>2</sub>O<sub>2</sub> at concentrations of 250 μM and 500 μM leads to the inhibition of STN7 kinase activity under low light illumination. Thus, an increase in the amount of H<sub>2</sub>O<sub>2</sub> in chloroplasts with increasing illumination leads to the inhibition of STN7 kinase and the return of LHCI to PSII. This may be important for the Lhcb 1 and Lhcb 2 proteolysis during prolonged exposure to increased illumination. Therefore, H<sub>2</sub>O<sub>2</sub> may represent a link between short-term and long-term adaptation processes inhibiting the former and activating the second.

The study was supported by the Russian Science Foundation (project no. 22-74-10088).

T.1.9

#### **INFLUENCE OF CHANGES IN MITOCHONDRIAL ALTERNATIVE OXIDASE CONTENT ON PHOTOSYNTHESIS AT LOW TEMPERATURE**

Anastasiia BRAZHNIKOVA<sup>1,2\*</sup>, Nikolay BALASHOV<sup>1</sup>, Ilya NAYDOV<sup>1</sup>, Maria Borisova-MUBARAKSHINA<sup>1</sup>

<sup>1</sup>Institute of Basic Biological Problems, RAS, Pushchino, Russia

<sup>2</sup>Lomonosov Moscow State University, Department of biotechnology, Moscow, Russia

\*Corresponding author. E-mail: [brazhnikovanastasia@yandex.ru](mailto:brazhnikovanastasia@yandex.ru)

It is known that mitochondria of many organisms contain a cyanide-insensitive alternative oxidase (AOX) whose function is the reduction of oxygen to water using reduced ubiquinone as an electron donor. It is believed that alternative electron transport through AOX is enhanced under stress conditions that prevents increasing generation of reactive oxygen species in the mitochondrial electron transport chain and, therefore, the development of oxidative stress in mitochondria. To date, the AOX role in thermogenesis in a number of plants has been proven, but the AOX role in protecting plants against low temperature is still not fully understood.

In the present work, using Arabidopsis wild type and AOX overexpressed or AOX antisense plants, the significance of mitochondrial alternative oxidase functioning on maintaining photosynthetic efficiency under low temperature (6°C) was evaluated. In wild type plants, a decrease in quantum yield of photosystem II (PS II) was observed after 12 days at low temperature, but in mutant plants with altered AOX content the PS II quantum yield remained at the same level as under control conditions. Moreover, the ability of wild type plants to dissipate energy into heat also decreased at low temperature, while in both mutant lines it remained at the same level observed under control conditions at 21 °C. Along with it, in all the studied plants the cyclic electron flow around PS I was enhanced. The H<sub>2</sub>O<sub>2</sub> content in the leaves of mutant plants remained almost unchanged after 12 days at low temperature, while the amount of H<sub>2</sub>O<sub>2</sub> increased approximately twice in the leaves of wild type plants. In addition, we have analyzed changes in the amount of AOX in plants with insight into the changes in the amount of reduced and oxidized fractions of AOX at low temperature.

It has been concluded that the alteration of AOX content represents an important signal to trigger acclimatory response of plants to low temperature. Summarizing, the studied plants adapt to the stress conditions by enhancing the AOX content and the cyclic electron flow around PS I. Wild type plants additionally activate alternative electron transport to oxygen probably because of their reduced capacity to dissipate energy into heat, in contrast to mutant plants with altered AOX content that retain this capacity.

This work was supported by the Russian Science Foundation (Grant No. 23-14-00396).

T.1.9

### THE PARTICIPATION OF THYLAKOID CARBONIC ANHYDRASES IN PHOTOSYNTHESIS OF HIGHER PLANTS

Natalia RUDENKO<sup>1\*</sup>, Natalya PERMYAKOVA<sup>2</sup>, Marina KOZULEVA<sup>1</sup>, Valentina GORBACHEVA<sup>1</sup>, Anastasia NEVZOROVA<sup>1</sup>, Lyudmila IGNATOVA<sup>1</sup>, Tatyana KHOROSHAeva<sup>1</sup>, Elena DEINEKO<sup>2</sup>, Boris IVANOV<sup>1</sup>

<sup>1</sup>Institute of Basic Biological Problems, Federal Research Center «Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences» Pushchino, Russia

<sup>2</sup>Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

\*Corresponding author: E-mail: nataliacherry413@gmail.com

Carbonic anhydrases (CA) are the enzymes, which catalyze the reversible hydration of carbon dioxide to form bicarbonate and proton. Most of the higher plant species possess dozens of the genes encoding CAs of three families  $\alpha$ ,  $\beta$  and  $\gamma$ , and the location and physiological role of many of them remains unclear. In *Arabidopsis thaliana* among the products of 19 genes encoding CAs  $\alpha$ CA5 was found in stromal thylakoids enriched with PSI and ATP synthase complexes and the presence of  $\alpha$ CA4 was established in granal thylakoids enriched with PSII.

*A. thaliana* plants with knocked out  *$\alpha$ CA4* gene, obtained both by T-DNA insertion mutagenesis and by CRISPR/Cas9 genome editing system demonstrated a decrease in the value of the energy-dependent component of non-photochemical quenching of chlorophyll fluorescence (NPQ) as well as a decrease in pH gradient across the thylakoid membrane vs. wild type plants (WT). These differences increased with a growth of light intensity during the measurements.  *$\alpha$ CA4* gene knockout had no effect on electron transport rate in isolated thylakoids in functionally isolated PSI, whereas the rate of electron transport in PSII from water to  $Q_A$  significantly increased if compared with WT. In these mutants the content of D1, the main protein of PSII, decreased, while the content of the proteins, regulating NPQ, PsbS and STN7 kinase, increased significantly vs. the WT.

The inhibition by iodonitrotimol 2,4-dinitrophenyl ether (DNP-INT) of the proton release into the lumen during plastohydroquinone oxidation in cytochrome *b6f* complex was enhanced after bicarbonate addition under uncoupling conditions in thylakoids from WT plants; this effect was more pronounced in thylakoids from  $\alpha$ CA4 mutants, and it was significantly weaker in thylakoids from  $\alpha$ CA5 mutants.

T1.9

### **STUDY OF SALINITY TOLERANCE IN SWEET SORGHUM GENOTYPES USING JIP TEST**

Marek KOVÁR<sup>1\*</sup>, Marek ŽIVČÁK<sup>1</sup>, Mária BARBORIČOVÁ<sup>1</sup>, Marián BRESTIČ<sup>1</sup>

<sup>1</sup>Institute of Plant and Environmental Sciences, Slovak University of Agriculture, Nitra, Slovakia

*\*Corresponding author: e-mail: [marek.kovar@uniag.sk](mailto:marek.kovar@uniag.sk)*

With an increase of the Earth's population, a significant proportion of grasslands or wetlands are being exploited through intensive cultivation and management. The combination of salinity with drought further adds to the nutritional constraints for agriculture productivity. The efficiency of photosynthetic machinery under stressful conditions has been identified as a key target for crop improvement. The effect of salinity on primary photochemical reactions in six sweet sorghum genotypes was tested. An increase in salt concentrations significantly induced the accumulation of proline and caused a decline in leaf osmotic potential, which induced an increase in the capacity for osmotic adjustment of cells. Salinity-induced changes in sorghum phenotype were identified at a later stage of the plant's life cycle. Salinity significantly decreased chlorophyll content and photosynthetic efficiency of plants. Increasing salinity led to a higher accumulation of QB-nonreducing PSII reaction centres. K-step in OJIP fluorescence transient was observed for the most sensitive genotypes under the high NaCl concentration. The studied sorghum genotypes responded differently to salinity stress. Thus, the study helps understand the plant tolerance mechanisms of different sweet sorghum genotypes to increasing salinity stress. The study also confirmed that the use of JIP-test is suitable for the identification of sorghum genotypes according to their growth under salinity stress.

Supported by the projects VEGA 1-0664-22, VEGA-1-0425-23 and APVV-18-0465.

T1.9

### **UV-B INDUCE CHANGES OF PRIMARY PHOTOCHEMICAL EFFICIENCY OF PSII IN LETTUCE PLANTS WITH DIFFERENT FLAVONOIDS CONTENT**

Dominika Mlynárikova VYSOKÁ<sup>1\*</sup>, Marek KOVÁR<sup>1</sup>, Marek ŽIVČÁK<sup>1</sup>, Mária BARBORIČOVÁ<sup>1</sup>,  
Marián BRESTIČ<sup>1</sup>

<sup>1</sup>Institute of Plant and Environmental Sciences, Slovak University of Agriculture, Nitra, Slovakia

*\*Corresponding author: e-mail: [dominika.vysoka@uniag.sk](mailto:dominika.vysoka@uniag.sk)*

The photosynthetic activity of plants is light wavelength dependent. Ultraviolet-B radiation (UV-B) is an important component of the environment acting as an eco-physiological factor with the potential to alter plant growth and photosynthesis. However, the effects of UV-B radiation on biological processes

are highly dependent on plant species, further on the doses of the radiation, and the acclimation level of the plants. The main reason for the harmful effects of UV-B radiation is initiations of photochemical reactions, including production of reactive oxygen species (ROS), which damages biologically active molecules. The objective of this study was to evaluate how UV-B radiation influence the morphology, antioxidative status of cells and photosynthetic behaviour of both green- and red-leaf lettuce plants (*Lactuca sativa* L., cvs. Lento and Rosemary). Using complex of biophysical (JIP test), biochemical (contents of MDA, photosynthetic pigments, anthocyanins and polyphenols), physiological and morphological (dry matter, leaf area) traits were analysed effects of stress acclimation and level of UV-B induce photodamage. UV-B radiation increased the content of flavonoids but not anthocyanins. Rising UV-B doses did not lead to a further increase in the content of biologically valuable substances but caused numerous harsh effects. The red genotype showed higher resistance to the increasing intensity of UV-B radiation, mainly by maintaining a higher efficiency of primary photosynthetic reactions. This trait significantly correlate with both anthocyanins and flavonoids content, as well as with the activity of antioxidant enzymes. The results confirmed the assumption that the spectral composition of light influenced physiological responses both at the level of functional and structural parameters of the photosynthetic apparatus, at the level of growth and morphological signs, as well as selected biochemical indicators.

Supported by the projects VEGA 1-0664-22, VEGA-1-0425-23 and APVV-18-0465.

T1.9

**THE LOW TEMPERATURE-ACCLIMATED GREEN MICROALGAE *LOBOSPHAERA INCISA* REVEALS SIGNIFICANT NONPHOTOCHEMICAL QUENCHING, MINOR CHANGES IN VAZ POOL SIZE, PROLONGED EXPRESSION OF PSBS-ENCODING GENE AND DECREASED ROS PRODUCTION**

Vasily V. PTUSHENKO<sup>1,2,\*</sup>, Grigorii N. BONDARENKO<sup>3</sup>, Olga B. CHIVKUNOVA<sup>4</sup>, Elena S. GLAGOLEVA<sup>1,4</sup>, Olga V. KARPOVA<sup>4</sup>, Elena S. LOBAKOVA<sup>4</sup>, Elizaveta N. VINOGRADOVA<sup>4,5</sup>, Oxana S. PTUSHENKO<sup>1,4</sup>, Karina A. SHIBZUKHOVA<sup>1,4</sup>, Alexei E. SOLOVCHENKO<sup>4</sup>, and Boris V. TRUBITSIN<sup>6</sup>

<sup>1</sup>Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119234 Moscow, Russia; <sup>2</sup>Emanuel Institute of Biochemical Physics of Russian Academy of Sciences,

Kosygina 4, 119334 Moscow, Russia

<sup>3</sup>Faculty of Chemistry, Lomonosov Moscow State University, 119234 Moscow, Russia

<sup>4</sup>Faculty of Biology, Lomonosov Moscow State University, 119234 Moscow, Russia

<sup>5</sup>National Research Center “Kurchatov Institute”, 123182 Moscow Russia

<sup>6</sup>Faculty of Physics, Lomonosov Moscow State University, 119234 Moscow, Russia

\*Corresponding author. Email: [ptush@belozersky.msu.ru](mailto:ptush@belozersky.msu.ru)

Green microalgae *Lobosphaera incisa* IPPAS C-2047 incubated at moderate irradiance (50  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) was subjected to low positive temperature ( $\sim 0^\circ\text{C}$ ) within 9 days. During the first 4–5 days, the maximal PSII efficiency, Fv/Fm, substantially decreased (to 0.2–0.3). Simultaneously, the intensity of the absorbed light energy dissipation increased. Light curves of both PSII operating efficiency and oxygen evolution of *L. incisa* cells revealed the high light stress. Within this period,

neither chlorophyll (Chl) and carotenoids (Car) content and composition, nor stoichiometry of the photosystems has changed remarkably. Conversely, the PsbS and LhcSR mRNA content, especially the former one, increased significantly. During the entire period, PsbS mRNA content changed only slightly and remained ca. 1000-fold higher than before the acclimation. The ROS production by low temperature acclimated cells was significantly lower compared with control ones. Since the 4<sup>th</sup>–5<sup>th</sup> day, both Car content (including the VAZ pool size) and Chl a/b ratio increased, while the PsbS mRNA content decreased, and Fv/Fm as well as the intensity of the absorbed light energy dissipation did not decrease further remarkably.

These data suggest a significant role of PsbS in the induction of nonphotochemical quenching and the protection of PSA in green algae. Note that previously only short-term PsbS expression was shown for green algae (Correa-Galvis et al. 2016, Tibiletti et al. 2016, Strenkert et al. 2019).

The work was supported by Russian Science Foundation (grant 22-24-00323).

T1.9

## **SUCROSE ACCUMULATION OF CYANOBACTERIAL CELLS UNDER STRESS CONDITIONS**

Broussos P-I<sup>1</sup>, Stamatakis K<sup>1</sup> \*

<sup>1</sup>Institute of Biosciences and Applications, NCSR Demokritos, Aghia Paraskevi, 15310 Attikis, Greece

\*Corresponding author: E-mail: [kstam@bio.demokritos.gr](mailto:kstam@bio.demokritos.gr)

Sucrose is one of the most important feedstocks for food industry and is widely used as a carbohydrate substrate for the production of clean fuels. In the present study, we use two standard laboratory organisms, the unicellular freshwater cyanobacteria *Synechococcus elongatus* PCC7942 (S7942) and *Synechocystis sp.* PCC6714 (S6714), which, when exposed to high salinity, synthesize sucrose as their main compatible osmolyte. We examined the optimal temperature during the accumulation of sucrose in those two organisms, in salted BG-11 medium (0,4M NaCl) and their cell proliferation rate under the same conditions. The sucrose production was favored by high temperature (above 31°C standard growth temperature) and was maximized at 35°C in both organisms. In particular, the intracellular sucrose content per chlorophyll a was increased by 33% and by 52%, in the case of S7942 cells and of S6714 cells, respectively. The cell proliferation rate of S7942 remained constant and that of S6714 declined slightly during the first 7 days of the upshock at 35°C, then both increased and remained positive for the rest of the incubation periods. Overall, the quantities of the sucrose produced by S7942 and S6714 were enhanced significantly and may be sufficient as a viable alternative (a) to sucrose synthesis, and (b) to fuel formation such as H<sub>2</sub> or bioethanol, outside the finite freshwater reservoirs, while reducing the ambient CO<sub>2</sub>.

**COMPROMISED CAPACITY FOR STATE I-STATE II TRANSITIONS IN THE  
ANTARCTIC ECOTYPE OF COLOBANTHUS QUITENSIS IMPLIES DIFFERENTIAL  
STRATEGIES TO COLD ACCLIMATION OF ANTARCTIC AND ANDES ECOTYPES**

León A. BRAVO<sup>1</sup>, Beth SZYSZKA-MROZ<sup>2</sup>, Dimitre A. IVANOV<sup>2</sup>, Alexander G. IVANOV<sup>2,3\*</sup> Luis  
J. CORCUERA<sup>4</sup> and Norman P.A. HÜNER<sup>2</sup>

<sup>1</sup> Laboratorio de Fisiología y Biología Molecular Vegetal, Instituto de Agroindustria, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Forestales, Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus. Universidad de La Frontera. Temuco, Chile.

<sup>2</sup> Department of Biology and The Biotron, University of Western Ontario, London, Ontario, Canada N6A 5B7

<sup>3</sup> Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia 1113, Bulgaria

<sup>4</sup> Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Correo 3, Concepción, Chile

\*Corresponding author: E-mails: [aivanov@uwo.ca](mailto:aivanov@uwo.ca), [aivanov@bio21.bas.bg](mailto:aivanov@bio21.bas.bg)

Excitation energy partitioning measurements indicated that the fraction of absorbed irradiance utilized via Photosystem II (PSII) photochemistry ( $\Phi_{PSII}$ ) in cold acclimated (CA) Antarctic plants is 1.6-fold higher compared to the Andes ecotype of *Colobanthus quitensis* (Kunth) Bartl. This was accompanied by a higher relative abundance of PSII reaction center protein D1(PsbA). Cold acclimation of both ecotypes resulted in an almost identical increase of Photosystem I (PSI) photochemistry measured via far-red light-induced P700 oxidation ( $P700^+$ ). However, the effective absorption section of PSI in the Antarctic ecotype was 2-fold higher than the Andes ecotype. This corresponds well with the observed higher abundance of Lhca1 in the Antarctic compared to the Andes plants. BN-PAGE profiles of the major chlorophyll-protein complexes also confirmed higher abundance of PSI-related bands in the Antarctic plants. Interestingly, the capacity for State I-State II transitions (Fr) in non-acclimated (NA) and CA Antarctic plants was 55% and 61% lower than the corresponding values in Andes ecotype. In addition, the kinetics of State I-State II transitions were much slower in Antarctic ( $t = 12.35 \pm 0.13$  s) compared to Andes ( $t = 6.44 \pm 0.02$  s) plants. The restricted capacity for state transitions corresponded with the lower phosphorylation status of Lhcb proteins in cold acclimated Antarctic than Andes plants. The ecotype differences are discussed in relation to the different climatic conditions of the two *Colobanthus* ecotypes.

T1.12

### **INNOVATIVE BIOLOGICAL FEEDBACK SYSTEM FOR REGULATING SUPPLEMENTAL LIGHTING IN GREENHOUSE**

*Corresponding author: E-mail: hazem@kalaji.pl*

The effects of different stressors on plants have been studied through the utilization of photosynthesis and chlorophyll fluorescence (ChFI). Originally, ChFI measurements were used to comprehend the response of plants to various biotic and abiotic stressors. Later, they were used to improve plant growth and yield, as well as increase food production and promote agricultural sustainability. Our research has extended this approach by demonstrating that ChFI measurements can be utilized to non-invasively monitor photosynthesizing organisms in diverse ecosystems and determine the nature of the stress. Recently, we have employed artificial intelligence and machine learning techniques to develop a biological feedback system that allows plants to regulate their growth conditions, including light quality and intensity.

T1.13

### **THE ROLE OF ALTERNATIVE ELECTRON FLOWS IN RESPONSE OF DISTINCT WHEAT GENOTYPES TO NITROGEN DEFICIENCY**

Andrej FILAČEK<sup>1\*</sup>, Marek ŽIVČÁK<sup>1</sup>, Mária BARBORIČOVÁ<sup>1</sup>, Marek KOVÁR<sup>1</sup>, Lucia JASENOVSKÁ<sup>1</sup>, Marián BRESTIČ<sup>1</sup>

<sup>1</sup>Institute of Plant and Environmental Sciences, Slovak University of Agriculture, Nitra, Slovakia

*\*Corresponding author: e-mail: xfilacek@uniag.sk*

The availability of N greatly affects the health and functioning of the photosynthetic machinery in leaves. Understanding and accurately assessing plant responses to nitrogen deprivation would help to improve NUE (nitrogen use efficiency) and provide a better picture of the appropriate usage of N fertilizers. In this regard, our study aims to contribute to the understanding of the photosynthetic acclimation to various nitrogen availability by performing the advanced analysis of CO<sub>2</sub> assimilation, PSII, and PSI photochemistry in two distinct wheat genotypes (cv. Enola and cv. Slomer) to investigate the effects of various nitrogen nutrition at three different leaf positions. We observe the variety of reactions related to photosynthetic acclimation and photoprotection. The loss of the photosynthetic capacity of the lower leaves was associated with a decrease in PSII photochemistry parameters and low photosynthesis was related to the decrease in the activity of alternative electron flows, with the exception of the cyclic electron flow, whose activity was increased in most samples with low photosynthesis. We found significant genotype-specific effects, with the older Slomer genotype showing improved alternative electron flow and photorespiratory capacity while exhibiting a lower rate of CO<sub>2</sub> assimilation which was in contrast with modern genotype Enola which responded to the decrease in photosynthesis with increased non-photochemical dissipation and cyclic electron flow. The significance of alternate electron fluxes for removing the excitation pressure at PSII is well-supported by our findings. In order to prevent the overreduction of the PSI acceptor side and the associated photooxidative damage of photosynthetic structures in leaves exposed to nitrogen deficiency, it is reasonable to assume that the

decrease in electron acceptor capacity was balanced by the structural and functional changes of the components of the electron transport chain.

Supported by the projects VEGA 1-0683-20, APVV-18-0465 and APVV-SK-CN-21-0045.

T1.13

### **BIOTECHNOLOGICAL POTENTIAL OF THE GREEN MICROALGAE *Coelastrella* sp. IPPAS H-626**

Elena V. ZADNEPROVSKAYA<sup>1,2\*</sup>, Anastasiya A. KRAPIVINA<sup>1</sup>, Maria A. SINETOVA<sup>1</sup>, Dmitry O. DUNIKOV<sup>2</sup>, Suleyman I. ALLAKHVERDIEV<sup>1,2</sup>

1. Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia
2. Joint Institute for High Temperatures, RAS, Moscow, Russia

\*Corresponding author: [zadneprovskaya@ifr.moscow](mailto:zadneprovskaya@ifr.moscow)

Green microalgae are widely used in biotechnological production due to their rapid growth, ability to produce large amounts of triglycerols (TAGs), and high content of fatty acids of various saturations. Metabolites of green microalgae can be used as raw materials for the production of biofuels, food, medicines, cosmetics, etc. It is also known that green microalgae are capable of producing biohydrogen under certain stressful conditions.

Mineral starvation is known to stress algal cells and lead to the accumulation of TAG, starch, and other metabolites. We investigated the effects of nitrogen and magnesium starvation on lipid and starch accumulation in cells of the green microalga *Coelastrella* sp. IPPAS H-626. Algae were grown under conditions of intense growth on modified Tamiya<sup>1/2</sup> medium (Tamiya<sup>1/2</sup>-N, Tamiya<sup>1/2</sup>-Mg), at 30°C and continuous illumination of 500 μmol m<sup>-2</sup>s<sup>-1</sup>, with continuous aeration with a gas–air mixture containing 1.5-2% CO<sub>2</sub>.

On the third day of the experiment, the total lipid content in the control culture was 81 mg/g of dry weight (g d.w.), under nitrogen starvation – 288 mg/g d.w., and in Mg free medium – 143 mg/g d.w. The major fatty acids (FA) of the strain H-626 are specific to green algae: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1Δ9), linoleic acid (18:2Δ9,12), α-linolenic acid (18:3Δ9,12,15). In the control culture, the major fatty acids accounted for 86.6% of the sum of all fatty acids.

The total carbohydrate content on Tamiya<sup>1/2</sup> medium reached 154 mg/g d.w. Nitrogen and magnesium starvation increased the carbohydrate content to 252 mg/g d.w. and 351 mg/g d.w., respectively.

*This work was funded by the Russian Science Foundation (No. 22-44-08001, and in particular 22-19-00516) and by the state contract of the Ministry of Science and Higher Education of the Russian Federation (Project No. 122050400128-1).*

T2.1

### **POTENTIAL CULTURES OF CYANOBACTERIA AS FEEDSTOCK FOR BIOHYDROGEN PRODUCTION**



Ardak KAKIMOVA<sup>1</sup>, Bolatkhan ZAYADAN<sup>1\*</sup>, Kenzhegul BOLATKHAN<sup>1</sup>, Asemgul SADVAKASOVA<sup>1</sup>, Nurzia AKMUKHANOVA<sup>1</sup>, Fariza SARSEKEEVA<sup>1</sup>, Bekzhan KOSSALBAYEV<sup>1</sup>, Suleyman ALLAKHVERDIEV<sup>2</sup>

<sup>1</sup> al-Farabi Kazakh National University, Almaty, Kazakhstan

<sup>2</sup> Faculty of Engineering and Natural Sciences, Bahcesehir University, Istanbul, Turkey

\*Corresponding author: E-mail: [zbolatkhan@gmail.com](mailto:zbolatkhan@gmail.com)

Molecular hydrogen has emerged as a promising future energy source, offering an environmentally friendly and renewable alternative to limited fossil fuels, with no CO<sub>2</sub> emissions during production and disposal. Cyanobacteria, known for their oxygenic photosynthesis, possess high metabolic potential, making them viable candidates for hydrogen production. This study aims to identify new strains of cyanobacteria with active hydrogen production and optimize cultivation conditions to enhance efficiency. The research employed various methodologies, including microbiological, algological, biotechnological, molecular genetic, physical, chemical, and statistical analyses.

All three cyanobacterial cultures examined, namely *Anabaena variabilis* A-1, *Synechocystis* sp. S-1, and filamentous cyanobacteria *Oscillatoria* sp. O-1, exhibited hydrogen production in dark conditions. Notably, *A. variabilis* A-1 demonstrated the highest hydrogen productivity, achieving a maximum accumulation of hydrogen (8.67  $\mu\text{mol H}_2 \text{ mg}^{-1} \text{ Chl a h}^{-1}$ ) after 72 hours of incubation. Comparatively, the other strains exhibited lower hydrogen-producing activity in the dark. In contrast, *Synechocystis* sp. S-1 displayed the most efficient hydrogen production in light conditions, with the highest accumulation rate of 2.35  $\mu\text{mol H}_2 \text{ mg}^{-1} \text{ Chl a h}^{-1}$  observed after 72 hours.

These findings highlight the promising prospects and practical significance of further investigating the cyanobacteria cultures *Anabaena variabilis* A-1 and *Synechocystis* sp. S-1 as biosystems capable of effectively converting light energy into molecular hydrogen. The study underscores the importance of biohydrogen research, specifically focusing on heterocysts, hydrogenase, nitrogenase, and dark conditions.

Keywords: biohydrogen, heterocyst, hydrogenase, nitrogenase, dark condition.

Acknowledgements: The work was supported by the grant AP09260785 “Development of technology for producing biohydrogen based on promising strains of cyanobacteria for the production of biofuels” (2021-2023) funded by the MSHE SC RK.

T2.3

## HYDROGEN PRODUCTION BY SOME NEW CYANOBACTERIAL STRAINS

Ayshat BOZIEVA<sup>1,3\*</sup>, Makhmadyusuf KHASIMOV<sup>2</sup>, Roman VOLOSHIN<sup>1</sup>, Maria SINETOVA<sup>1</sup>, Elena KUPRIYANOVA<sup>1</sup>, Sergey ZHARMUKHAMEDOV<sup>2</sup>, Dmitry DUNIKOV<sup>3</sup>, Suleyman ALLAKHVERDIEV<sup>1,2</sup>

<sup>1</sup> K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia

<sup>2</sup> Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia

<sup>3</sup> Joint Institute for High Temperatures, Russian Academy of Sciences, Izhorskaya st. 13 bld. 2, Moscow 125412, Russia

\*Corresponding author: E-mail: [ayshat2696@mail.ru](mailto:ayshat2696@mail.ru)

This study is aimed to investigate hydrogen-producing ability of previously unstudied cyanobacterial strains (*Cyanobacterium* sp. IPPAS B-1200, *Dolichospermum* sp. IPPAS B-1213, and *Sodalinema gerasimenkoae* IPPAS B-353) from the IPPAS Collection of Microalgae and Cyanobacteria of Institute of Plant Physiology RAS. To obtain molecular hydrogen the cultures of cyanobacteria were purged with argon. The strain *Dolichospermum* sp. IPPAS B-1213 is identified as the most promising H<sub>2</sub> producer. The H<sub>2</sub> production rate by this strain was 0.44 μmol H<sub>2</sub> (mg Chl *a* h)<sup>-1</sup>. The addition of DCMU had a positive effect on the release of H<sub>2</sub> and led to an increase in the rate of hydrogen release by almost 10 times. Since the enzyme nitrogenase is involved in hydrogen metabolism, the rate of hydrogen release by this culture may depend on the number of heterocysts. To test this assumption, the frequency of heterocysts was determined microscopically. The heterocyst frequency in *Dolichospermum* sp. IPPAS B-1213 obtained in this study can be considered high which is an auspicious characteristic for hydrogen production. The heterocyst strain *Dolichospermum* sp. IPPAS B-1213 showed the best results and should be used in further studies.

*This work was supported by grants from the Russian Science Foundation (No: 22-44-08001, and in particular No: 22-19-00516) and by the state contract of the Ministry of Science and Higher Education of the Russian Federation (Project No. 122050400128-1).*

T2.5

## **HYDROGEN PRODUCTION FROM RENEWABLE ENERGY UNDER DIFFERENT CLIMATIC CONDITIONS IN ALGERIA**

Lilia Aiche HAMANE<sup>1,\*</sup> Mustapha HAMANE<sup>2</sup>

<sup>1\*</sup>Department of renewable energy, Faculty of technology, University Blida1, Algeria

<sup>2</sup> Centre for development of renewable energies (CDER), Algeria

\*Corresponding author :E-mail: [l\\_aiche@yahoo.fr](mailto:l_aiche@yahoo.fr)

The use of hydrogen as a clean energy carrier is a promising alternative for reducing greenhouse gas emissions and dependence on fossil sources. There are several methods developed these days to separate hydrogen gas from water, biomass, or natural gas. However, electrolysis is considered to be safe for the environment, particularly, when the electricity used is renewable and clean.

In this study, we proposed to compare hydrogen production from wind and solar power in Algeria. In order to reduce the cost production, we recommended to use systems that already exist for electric production and redirect the excess of energy for electrolysis.

However, the intermittency of solar and wind make necessary to optimal sizing of each component of the whole system production, especially the electrolyser and the storage tank. Sizing depends strongly on the weather and the topography. This study aims to estimate and optimize the hydrogen system depending on geographical situation in Algeria which is characterized by a very varied climate and topography. Indeed, we note the existence of three types of climate. The mild Mediterranean climate of the coast, the transitional climate of the northern hills and mountains and finally, the desert climate.

Three configurations were compared. Wind-hydrogen, PV-Hydrogen. Wind-PV-hydrogen. The systems consist of wind turbine, PV solar panel, converter, advanced alkaline electrolyser and hydrogen storage. The results showed that wind-hydrogen system is more suitable in the south where the wind speed exceeds 7 m/s annually. The pv-hydrogen system is more efficient in the north.

## ANODIZED FENI ALLOY FOR OXYGEN EVOLUTION REACTION

Nader Akbari and Mohammad Mahdi Najafpour\*

Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, 45137-66731, Iran

\*Corresponding author: [mmnajafpour@iasbs.ac.ir](mailto:mmnajafpour@iasbs.ac.ir)

An efficient and durable oxygen-evolution reaction (OER) catalyst is critical for water splitting toward hydrogen production and energy conversion [1]. FeNi hydroxides are promising for OER under alkaline conditions [2-6]. Herein, a FeNi (Ni: Fe 1:1) alloy as foam, after anodizing at 50 V in a two-electrode system in KOH solution (1.0 M), was characterized by some methods and used as an efficient and durable OER electrocatalyst in KOH solution (1.0 M). The overpotential for the onset of OER based on extrapolation of the Tafel plot was 225 mV. The overpotentials for the current densities of 10, and 30 mA/cm<sup>2</sup> are observed at 270, and 290 mV, respectively. In addition, a low Tafel slope is observed, 38.0 mV per decade, for OER. Corrected chronoamperometry based on the electrochemical active surface area shows that for the anodized foam, the improvement in OER is related to the number of active sites rather than the change in active sites. In surface-enhanced Raman spectroscopy shows the presence of high valent Fe and Ni ions on the electrode surface during OER. These high-valent species are unstable and could oxidize water.

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## CALCINED NANOLAYERED MN OXIDES FOR WATER OXIDATION

Nader AKBARI<sup>1</sup>, Suleyman I. ALLAKHVERDIEV<sup>2,3\*</sup>, Mohammad Mahdi NAJAFPOUR<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

<sup>2</sup>K. A. Timiryazev Institute of Plant Physiology RAS Moscow, Russia

<sup>3</sup>Faculty of Engineering and Natural Sciences, Bahcesehir University, Istanbul, Turkey

\*Corresponding authors; [suleyman.allakhverdiev@gmail.com](mailto:suleyman.allakhverdiev@gmail.com); [mmnajafpour@iasbs.ac.ir](mailto:mmnajafpour@iasbs.ac.ir)

Hydrogen is presumed to be a promising energy carrier [1]. Electrocatalytic water splitting by green electricity would provide hydrogen with a minimal CO<sub>2</sub> formation [2]. The water-oxidation reaction (WOR) is a bottleneck and sluggish reaction for water splitting [2]. Thus, stable and efficient catalysts

for WOR are necessary. Among different compounds [2], Mn-oxide based compounds are promising because they are stable, low-cost, and environmentally friendly [3,4]. In addition, an Mn-oxide best structure is efficiently used by Nature [5] for the same task in plants, algae, and cyanobacteria. Herein, using the thermal decomposition of  $\text{KMnO}_4$ , layered Mn oxides have been synthesized at different calcination temperatures and characterized by some methods. Then, the WOR activities of the calcined Mn oxides were studied. The experiments show that layered Mn oxide, even after calcination at 800 °C is a catalyst for WOR. The calcined sample at 800 °C in cerium(IV) ammonium nitrate at concentrations of 0.30 M shows water-oxidation reaction with a maximum turnover frequency of  $4.2 \times 10^{-6}/\text{s}$ .

The work is supported in part by the Russian Science Foundation (No. 19-14-00118)

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T2.15

### **SOLAR HYDROGEN PANEL SELECTION FOR A MUNICIPAL HOSPITAL USING DECOMPOSED FUZZY WASPAS**

Elif HAKTANIR

Bahcesehir University, Istanbul, Turkey

*Corresponding Authorl: elif.haktaniraktas@eng.bau.edu.tr*

The demand for sustainable and renewable energy sources has led to the development of various technologies, including solar hydrogen panels, which are capable of converting solar energy into hydrogen, a clean and renewable fuel source. However, selecting the most appropriate solar hydrogen panel for a particular application is a complex and challenging task due to numerous criteria that need to be considered. To address this issue, this study proposes a novel multi-criteria decision-making approach namely Decomposed Fuzzy-Decision-making Trial and Evaluation Laboratory with Weighted Aggregated Sum Product Assessment (DF-WASPAS), a powerful tool for decision-making that allows for systematic and structured handling of uncertainty and imprecision. The proposed method is developed to select the best solar hydrogen panel for a municipal hospital by considering various criteria, including efficiency, reliability, safety, cost, space requirements, and environmental impact. To address the uncertainties and potential biases in the decision-making process, the concept of DF sets is utilized to model human thoughts and perceptions more realistically and in greater detail through optimistic and

pessimistic membership functions. The proposed approach provides decision-makers with a useful tool to select the best solar hydrogen panel for a particular application, taking into account multiple criteria and their relative importance to the decision-maker. The results of the analysis showed that the photovoltaic-electrolysis (PV-E) model is the most suitable solar hydrogen panel for the hospital, with a high score in all criteria considered. By utilizing DF-WASPAS, the proposed method provides a structured approach to decision-making that helps ensure that the selected solar hydrogen panel meets the requirements of the application and is the most suitable option in terms of the considered criteria.

T1.12

## **PHOTOSYNTHETIC EFFICIENCY AND TRANSCRIPTOME ANALYSIS OF *DUNALIELLA SALINA* UNDER HYPERSALINE: A RETROGRADE SIGNALING MECHANISM IN THE CHLOROPLAST**

Pavithra Ramachandran<sup>1</sup>, Naveen Kumar andey<sup>2</sup>, Ranay Mohan Yadav<sup>1</sup>, Praveena Suresh<sup>1</sup>, Aman Kumar<sup>2</sup>, Rajagopal Subramanyam<sup>1\*</sup>

<sup>1</sup>*Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad-500046, India*

<sup>2</sup>*Novelgene Technologies Pvt, Ltd., bioinfo@novelegenetech.com , F201, Sri Sairam towers, Hafeezpet, Hyderabad, Telangana 500049, India*

*\*Corresponding author: Rajagopal Subramanyam (srgsl@uohyd.ac.in)*

Understanding the molecular mechanisms of environmental salinity stress tolerance and acclimation strategies by photosynthetic organisms facilitates accelerating the genetic improvement of tolerant economically important crops. In this study, we have chosen the marine algae *Dunaliella* (*D.*) *salina*, a high-potential and unique organism that shows superior tolerance against abiotic stresses, especially hypersaline conditions. We have grown the cells in three different salt concentrations 1.5M NaCl(control), 2M NaCl, and 3M NaCl (hypersaline). Fast chlorophyll fluorescence analysis showed increased initial fluorescence ( $F_0$ ) and decreased photosynthetic efficiency, indicating hampered photosystem II utilization capacity under hypersaline conditions. Also, the ROS localization studies and quantification revealed elevated accumulation of ROS in the chloroplast in the 3M condition. Pigment analysis shows a deficit in chlorophyll content and increased carotenoid accumulation, especially lutein and zeaxanthin content. This study majorly explored the chloroplast transcripts of the *D. salina* cell as it is the major environmental sensor. Even though most of the photosystem transcripts showed moderate upregulation in hypersaline conditions in the transcriptome study, the western blot analysis showed degradation of the core as well as antenna proteins of both the photosystems. Among the upregulated chloroplast transcripts, chloroplast Tidi, flavodoxin IsiB, and carotenoid biosynthesis-related protein

transcripts strongly proposed photosynthetic apparatus remodeling. Also, the transcriptomic study revealed the upregulation of the tetrapyrrole biosynthesis pathway (TPB) and identified the presence of a negative regulator of this pathway, called the s-FLP splicing variant. These observations point towards the accumulation of TPB pathway intermediates PROTO-IX, Mg-PROTO-IX, and P-Chlide, those earlier reported as retrograde signaling molecules. Our comparative transcriptomic approach along with biophysical and biochemical studies in *D. salina* grown under control (1.5 M NaCl) and hypersaline (3M NaCl) conditions, unveil an efficient retrograde signaling mechanism mediated remodeling of photosynthetic apparatus.

**Keywords:** carotenoids, chloroplast, *Dunaliella salina*, photosystems, reactive oxygen species, transcriptome

T1.9

## CRYO-OPTICAL MICROSCOPY STUDY ON REGULATION MECHANISM OF PHOTOSYNTHETIC LIGHT HARVESTING

Yutaka Shibata

<sup>1</sup>Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-6-3 Aramaki, Aoba Ward, Sendai, Miyagi 980-0845, Japan

*\*Corresponding author: shibata@m.tohoku.ac.jp*

We have developed and continuously improved the cryogenic laser-scanning confocal microscope systems [Shibata et al. BBA (2014)]. The systems have been applied to the study of intracellular rearrangement of LHCs during the state transitions (ST) and the fluctuation of energy-transfer pathways within single PSI's [Zhang et al. Res.Sq. (2023)]. Recent advancement of the cryo-electron microscopy has increased the structural understanding of the ST. However, it is still elusive whether the LHCI detached from PSII in state2 all bind to PSI or partially remain isolated from both of the photosystems (PSs). The developed system provides the high lateral resolution (0.4  $\mu$ m) and the ability to detect cryogenic fluorescence spectrum at each pixel, enabling resolutions of the intracellular PSI-rich and PSII-rich domains. The information about the PS segregation is effective to evaluate the LHC rearrangement during the ST [Fujita et al. J.Photo.Photo.B (2018)]. Our improved system achieved the simultaneous detection of the fluorescence spectrum and lifetime at each pixel. Owing to this advancement, we captured key evidence for the free LHCs isolated from both of the PSs in ST2. The observation revealed that the free LHCs were in the highly quenched state and accumulated in the PSI-

rich domains [Fujita et al. J.Photo.Photo.B (2022)]. We also developed a microscope system which acquires both the excitation and emission spectra at each pixel. Observation of Chlamydomonas cells by this excitation spectral microscope at room temperature enabled the real-time visualization of the rearrangement of LHCs upon the ST. We surprisingly found that the ST is less active in the region around the pyrenoid, which is a subcellular compartment specialized for the CO<sub>2</sub> fixation [Zhang et al. PNAS (2022)].

T1.10

**SHORT- AND LONG-TERM PHOTOSYNTHETIC RESPONSES TO IN SITU WARMING OBEY DIFFERENTIAL LEAF MORPHOLOGICAL DETERMINANTS BETWEEN THE ANTARCTIC VASCULAR SPECIES.**

León A. BRAVO<sup>1</sup>, Dariel LOPEZ<sup>1</sup>, Patricia L. SÁEZ<sup>1,3</sup>, Byron ALTAMIRANO<sup>1</sup>, Lohengrin CAVIERES<sup>2,3</sup>

<sup>1</sup>Laboratorio de Fisiología y Biología Molecular Vegetal, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera. Temuco, Chile. [leon.bravo@ufrontera.cl](mailto:leon.bravo@ufrontera.cl)

<sup>2</sup>Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla, Concepción, Chile.

<sup>3</sup>Instituto de Ecología y Biodiversidad, Concepción, Chile

*\*Corresponding author: leon.bravo@ufrontera.cl*

Warming in Antarctic Peninsula has been reported as the second fastest on Earth. This region has been colonized by two vascular plant species (*Deschampsia antarctica* and *Colobanthus quitensis*). Using in situ short-term warming experiments, we have previously reported that *C. quitensis* responds increasing its growth, while *D. antarctica* seems to be more resilient. However, populations of *D. antarctica* have expanded more than *C. quitensis*. We hypothesize that in the short-term in situ warming favors gain in photosynthesis of *C. quitensis*, followed by a diminished long-term response, while *D. antarctica* needs longer term warming to increase photosynthesis. In both species the increase in photosynthesis is associated with leaf morphoanatomical adjustments induced by different term warming exposures. We compared plants exposed to warming using open top chambers (OTC) installed near to Arctowski Station (62°090S, 58°280W) in 2013 and 2020. Physiological and morphological traits were evaluated in the summer season 2022. A<sub>Ci</sub> curves were performed to evaluate the main photosynthetic limitations (biochemical and diffusional). Additionally, the effect of in situ warming in the leaf morphological traits such as LMA, LD and vascular leaf characteristic (i.e. leaf hydraulic diameter, Dh and theoretical leaf hydraulic conductivity, Kleaf) were associated with variation in photosynthetic capacity. *C. quitensis* increase net photosynthesis (AN) from 4.8 to 10 (μmolCO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) after two years in OTC, but returned to similar values of nonwarmed plants after 10 years in OTC. These changes were concomitant with adjustments in gm, Dh and Kleaf, both at short and long-term. In *D. antarctica* AN exhibited an increase

but only in the long term, consistent with an increase in gs, gm, Dh and Kleaf. We conclude that the homeostatic response of *C. quitensis*, which finally after several seasons of warming exposure returned to normal AN, and the long-term response of *D. antarctica* is consistent with the higher expansion of this species in Antarctica.

**Acknowledgments:** Fondecyt1201824; INACH FR 02\_20; Fondecyt 1211231, ACT 210038.

T.1.9

## THE PARTICIPATION OF THYLAKOID CARBONIC ANHYDRASES IN PHOTOSYNTHESIS OF HIGHER PLANTS

Natalia Rudenko<sup>1\*</sup>, Natalya Permyakova<sup>2</sup>, Marina Kozuleva<sup>1</sup>, Valentina Gorbacheva<sup>1</sup>, Anastasia Nevzorova<sup>1</sup>, Lyudmila Ignatova<sup>1</sup>, Tatyana Khoroshaeva<sup>1</sup>, Elena Deineko<sup>2</sup>, Boris Ivanov<sup>1</sup>

<sup>1</sup>Institute of Basic Biological Problems, Federal Research Center «Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences» Pushchino, Russia

<sup>2</sup>Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

\*Corresponding author: E-mail: nataliacherry413@gmail.com

Carbonic anhydrases (CA) are the enzymes, which catalyze the reversible hydration of carbon dioxide to form bicarbonate and proton. Most of the higher plant species possess dozens of the genes encoding CAs of three families  $\alpha$ ,  $\beta$  and  $\gamma$ , and the location and physiological role of many of them remains unclear. In *Arabidopsis thaliana* among the products of 19 genes encoding CAs  $\alpha$ CA5 was found in stromal thylakoids enriched with PSI and ATP synthase complexes and the presence of  $\alpha$ CA4 was established in granal thylakoids enriched with PSII. *A. thaliana* plants with knocked out  $\alpha$ CA4 gene, obtained both by T-DNA insertion mutagenesis and by CRISPR/Cas9 genome editing system demonstrated a decrease in the value of the energy-dependent component of non-photochemical quenching of chlorophyll fluorescence (NPQ) as well as a decrease in pH gradient across the thylakoid membrane vs. wild type plants (WT). These differences increased with a growth of light intensity during the measurements.  $\alpha$ CA4 gene knockout had no effect on electron transport rate in isolated thylakoids in functionally isolated PSI, whereas the rate of electron transport in PSII from water to  $Q_A$  significantly increased if compared with WT. In these mutants the content of D1, the main protein of PSII, decreased, while the content of the proteins, regulating NPQ, PsbS and STN7 kinase, increased significantly vs. the WT. The inhibition by iodonitrotimol 2,4-dinitrophenyl ether (DNP-INT) of the proton release into the lumen during plastoquinone oxidation in cytochrome *b6f* complex was enhanced after bicarbonate addition under uncoupling conditions in thylakoids from WT plants; this effect was more pronounced in thylakoids from  $\alpha$ CA4 mutants, and it was significantly weaker in thylakoids from  $\alpha$ CA5 mutants.

The study was supported by the Russian Science Foundation (project no. 23-14-00396)



T1.9

## **NEW INSIGHT INTO INHIBITORY ACTIVITY OF DINITROPHENYL ETHER OF IODONITROTHYMOL AND THE FUNCTIONAL PROPERTIES OF CYTOCHROME B<sub>6</sub>F COMPLEX**

Daria Vilyanen<sup>1,\*</sup>, Ilya Naydov<sup>1</sup>, Boris Ivanov<sup>1</sup>, Maria Borisova-Mubaraksina<sup>1</sup>, Marina Kozuleva<sup>1</sup>

<sup>1</sup>Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia

*\*Corresponding author: vilyadar@gmail.com*

Dinitrophenyl ether of iodonitrothymol (DNP-INT) is a competitive inhibitor of plastoquinone (PQH<sub>2</sub>) oxidation at Q<sub>o</sub>-site of cytochrome b<sub>6</sub>f complex. It was synthesized by Aachim Trebst in 1978, and since then it has been widely used in research of photosynthetic electron transport. Recently we demonstrated that inhibitory activity of DNP-INT depends on light intensity and H<sup>+</sup>-uptake of thylakoids. In our experiments with thylakoid membranes isolated from higher plants the inhibitory activity of DNP-INT increased with the increase of light intensity, and completely diminished after light cessation. To understand how light modulates the inhibitory activity of DNP-INT, we simulated high and low rates of electron transport at different light intensities by using methylviologen and diuron both in non-saturating concentrations. The inhibitory activity of DNP-INT was closely correlated with the rate of electron transport. However, Gramicidin D (GrD) led to a decrease of DNP-INT activity regardless of electron transport rate. The combination of GrD and Valinomycin (Val) led to a higher DNP-INT inhibitory activity in contrast to using GrD alone. Earlier it was proposed that GrD induces deprotonation of amino acids of cyt. b<sub>6</sub>f complex. DNP-INT cannot strongly bind to Q<sub>o</sub>-site when Glu78, the amino acid residue essential for PQH<sub>2</sub> binding, is charged negatively. The effect of Val and GrD may be caused by shielding of these negative charges by K<sup>+</sup>-Val complexes and prevention of electrostatic repulsion between Glu78 and nitro groups of DNP-INT. We propose that long-lived protonated state of Glu78 provides an increase in the affinity of DNP-INT. Thus, the increase of the inhibitory activity of DNP-INT is caused by the long-lived protonation state of Glu78 in the Q<sub>o</sub>-site of cyt. b<sub>6</sub>f complex at a high rate of electron transport. This result can help understand the mechanisms of photosynthetic control and PQH<sub>2</sub> oxidation at the cyt. b<sub>6</sub>f complex.

This work was supported by Russian Science Foundation (grant № 22-24-01074).

T.1.9

## **A VERSATILE MECHANISM OF LIGHT-HARVESTING REGULATION UNDER PROLONGED STRESS CONDITIONS IN HIGHER PLANTS**

Nikolai Balashov, Daria Vetoshkina, Roman Markin, Anastasia Brajnikova, Boris Ivanov, Maria Borisova-Mubarakshina

<sup>1</sup>Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia

\*Corresponding author: E-mail: [kbalashovv@mail.ru](mailto:kbalashovv@mail.ru)

Photosynthesis begins with the light quanta absorption by light-harvesting antenna of photosystem I and photosystem II. Higher plants can increase the size of PS II under prolonged shade conditions and, conversely, decrease the size of PS II under prolonged high light, thus protecting the photosynthetic electron transport chain from photoinhibition (Anderson, 1986). Changes in the PS II antenna size occur owing to regulation in the biosynthesis of Lhcb proteins, which are encoded by nuclear genes (Morosinotto et al., 2006; Borisova-Mubarakshina et al., 2014). We have shown that not the redox state of the PQ pool per se, but the amount of H<sub>2</sub>O<sub>2</sub> in photosynthetic cells plays a determining role in regulating the PS II antenna size (Borisova-Mubarakshina et al., 2015). Since the H<sub>2</sub>O<sub>2</sub> content is known to change under the action of many stress factors, it was suggested that regulating the PS II antenna may also occur under the action of other factors. Our results have shown that abiotic factors such as drought, salinity and chilling lead to an increase in the level of PQ pool reduction and in the H<sub>2</sub>O<sub>2</sub> content in leaves, resulting in downsizing the PS II antenna. In opposite, colonization of plants with the rhizosphere bacteria *Pseudomonas putida* BS3701, diminishes the level of PQ pool reduction and the level of H<sub>2</sub>O<sub>2</sub> content, leading to an increase in the size of the PS II antenna. Additionally, we examined changes in non-photochemical quenching (NPQ) and state transitions under these conditions. We have observed an inhibition of state transitions process and a decrease in NPQ for plants, subjected to prolonged drought and salinity as it was shown for prolonged high light (Borisova-Mubarakshina et al., 2014; Yang et al., 2022). Thus, the regulation of light harvesting antenna size of PS II represents one of the versatile mechanisms of changes in structural and functional organization of photosynthetic apparatus of higher plants under changing environmental conditions.

This work was supported by the Russian Science Foundation (Grant No. 23-14-00396)\

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