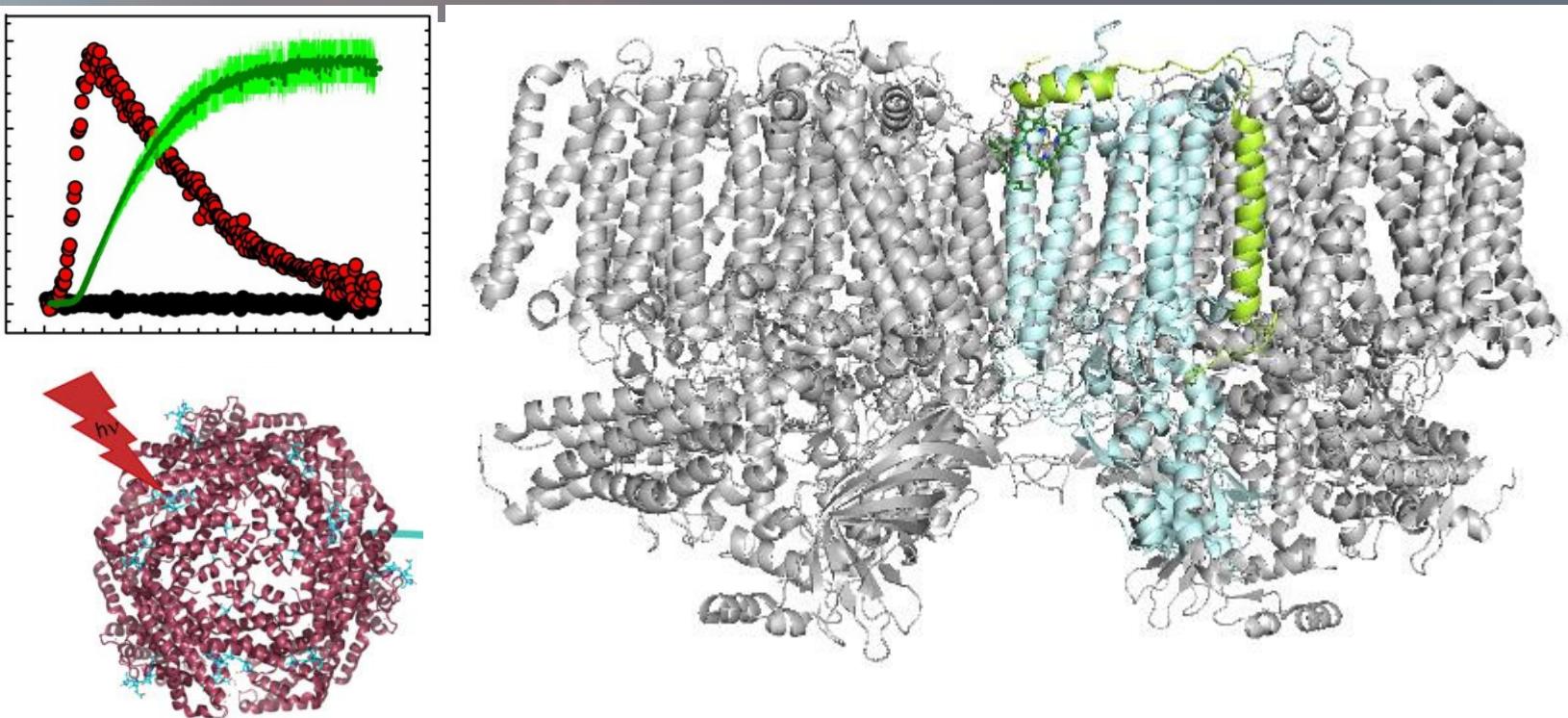


# 50th Midwest/Southeast Photosynthesis Conference

## Agenda and Abstracts



October 25-27 2024  
Turkey Run Inn

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# Program

## Friday, October 25

4:00–6:00pm	Registration and Poster Hanging		
6:00pm	Dinner		
7:30–7:40pm	Introduction and Welcome		
7:40–8:20pm	Keynote	<b>Govindjee</b> University of Illinois at Urbana-Champaign	Photosynthesis: stories from the past
8:20–8:40pm		<b>Chris Gisriel</b> University of Wisconsin	Structure and evolution of Photosystem I in the early-branching cyanobacterium <i>Anthocerotibacter panamensis</i>
8:40–9:00pm		<b>Nina Ponomarenko</b> Argonne National Laboratory	Structural Analysis of Platinum Nanoclusters Assembled on Photosystem I by Light-Driven Chemistry
9:00–9:20pm		<b>Rajnandani Kashyap</b> St. Louis University School of Medicine	Cryo-EM reveals a bi-copper cluster coordinating asymmetric electron transfer in the nitrogenase-like DPOR complex
9:30pm	Mixer and Poster Session		

## Saturday, October 26

7:00am	Breakfast		
9:00–9:40am	Keynote	<b>Lauren Ann Metskas</b> Purdue University	Rubisco polymerization inside alpha-carboxysomes - but how and why?
9:40–10:00am		<b>Rees Rillema</b> Michigan State University	Gas exchanges measurements of carboxysome mutants reveal conditional phenotypes and insights into cyanobacteria carbon concentrating mechanism
10:00–10:20am		<b>Chetna Sharma</b> University of Florida	KDPG aldolase modulates the photosynthetic carbon yield in <i>Synechococcus elongatus</i> PCC 7942
10:20–10:40am	Coffee		
10:40–11:00am		<b>Alizeé Malnoë</b> Indiana University Bloomington	qH-energy dissipation in photosystem II antennae
11:00–11:20am		<b>Grant Steiner</b> Loyola University Chicago	Extreme light acclimation reveals inherent photosystem II photoprotection in a natively low-light <i>Chlorella</i>

11:20-11:40am		<b>Amala Phadkule</b> Purdue University	Tuning the low-energy fluorescence state in photosystem II
11:40-12:00pm		<b>Mohamed Elrefaiy</b> The University of Texas at Austin	Computational prediction and experimental validation of pKa shifts in the Q57D mutant of <i>Lepidium virginicum</i> WSCP: implications for tailored chlorophyll protein design
12:00-12:20pm		<b>Jasleen Bindra</b> Argonne National Laboratory	Coherences of photo-induced electron spin qubit pair states in natural photosynthetic proteins
12:30pm	<b>Lunch</b>		
Afternoon	<b>Free to explore Turkey Run State Park</b>		
4:00-6:00pm	<b>Poster Session</b>		
6:00pm	<b>Dinner</b>		
7:30-8:10pm	Keynote	<b>David Vinyard</b> Louisiana State University	A Photosynthetic Variant of <i>Synechocystis</i> sp. PCC 6803 Sacrifices a Stress Response Pathway to Outcompete its Peers under Optimal Growth Conditions
8:10-8:30pm		<b>K. V. Lakshmi</b> Rensselaer Polytechnic Institute, New York	Understanding the mechanism of substrate delivery and binding in the oxygen-evolving complex of photosystem II
8:30-8:40pm	Remem-berance	<b>Colin Gates</b> Loyola University Chicago	Remembering Jeffery Cameron
8:40-8:50pm	Remem-berance	<b>Chris Gisriel</b> University of Wisconsin	Remembering Donald Bryant
9:00pm	<b>50th Anniversary Cake, Campfire, Drinks, and Poster Session</b>		

## Sunday, October 27

7:00am	<b>Breakfast</b>		
9:00-9:40am	Keynote	<b>Wim Vermaas</b> Arizona State University	50 years of cyanobacteria and photosynthesis
9:40-10:00am		<b>Christopher Jones</b> Washington University, St. Louis	ACCESSing the carbon uptake dynamics of cyanobacteria
10:00-10:20am		<b>Harvey Hou</b> Alabama State University	Tri-institutional effort to probe cyanobacterial metabolic overflow: from research to training
10:20-10:40am		<b>Ashraf Mohamed</b> The University of Texas at Austin	A computational framework for simulating protein organization in thylakoid membranes
10:40-11:00am	<b>Coffee</b>		
11:00am	<b>Awards Presentations</b>		

# Talk Abstracts

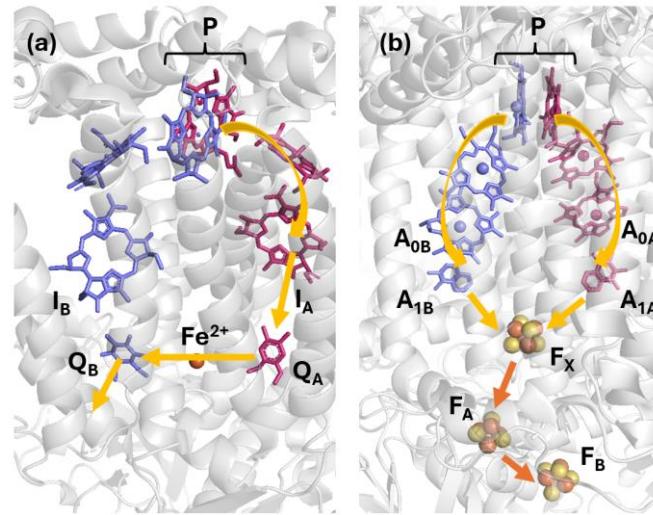
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# Coherences of Photo-Induced Electron Spin Qubit Pair States in Natural Photosynthetic Proteins

Jasleen K Bindra,<sup>1</sup> Jens Niklas,<sup>1</sup> Yeonjun Jeong,<sup>1</sup> Ahren W. Jasper,<sup>1</sup> Lisa M. Utschig,<sup>1</sup> and Oleg G. Poluektov<sup>1</sup>

<sup>1</sup>*Chemical Sciences and Engineering Division, Argonne National Laboratory, Lemont, IL 60439, USA, J.Bindra@anl.gov*

Photosynthetic proteins represent well-defined and experimentally tunable molecular systems, exhibiting complexities inspired by their functional roles. Due to these characteristics, they serve as ideal model systems for investigating spin coherences. The objective of this study is to unravel how nature manages coherence and spin entanglement in photosynthesis. Despite their significance, critical aspects, like coherence spatial lengths, lifetime, dephasing, decoherence mechanisms, and their interaction with the local and global protein structure, remain poorly understood, hindering a detailed understanding of decoherence in this context. This work presents the first comprehensive experimental study on decoherences in photoinduced electron spin states, in both Type II and Type I reaction centers. High-frequency electron paramagnetic resonance (EPR) spectroscopy operating at 130 GHz and 4.6 T was used to measure coherences through the decay of two-pulse electron spin echo signals and Rabi oscillations. The phase memory times (TM) recorded at various temperatures show that TM exhibits minimal dependence on biological species, biochemical treatment, and paramagnetic species. Nuclear spin diffusion and instantaneous diffusion mechanisms alone cannot explain the observed decoherence. Instead, the low-temperature dynamics of methyl groups surrounding the unpaired electron spin centers are suggested as the main factor governing loss of coherence. Understanding these intricate dynamics holds the key to enhancing our comprehension of photosynthetic processes and their potential applications in achieving more efficient solar energy conversion.



**Figure 1.** Figure 1: Schematic structure and ET pathways in photosynthetic RCs of Type II (a) and Type I (b).

# Computational Prediction and Experimental Validation of pKa Shifts in the Q57D Mutant of *Lepidium virginicum*WSCP: Implications for Tailored Chlorophyll Protein Design

Nicolas de Cordoba<sup>1</sup>, Mohamed A. Elrefaiy<sup>2</sup>, Andres S Urbina<sup>1</sup>, Gehan A. Ranepura<sup>3</sup>, M. R. Gunner<sup>3</sup>, Lyudmila Slipchenko<sup>1</sup>, Doran Raccah<sup>2</sup> and Mike Reppert<sup>1</sup>

<sup>1</sup> Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.

<sup>2</sup> Department of Chemistry, The University of Texas at Austin, Austin, TX 78712, USA.

<sup>3</sup> Department of Physics, City College of New York, New York, NY 10031, USA.

Engineering chlorophyll-binding proteins with customized optical properties is essential for advancing photobiology and renewable energy applications. Achieving this goal requires a thorough understanding of how the chlorophyll's local environment within protein complexes influences its spectral characteristics. A previous study demonstrated that a simple electrostatic model accurately predicted mutation-induced frequency shifts for eight out of nine single-point mutants of the Water-Soluble Chlorophyll Protein from *Lepidium virginicum* (LvWSCP). However, the Q57D mutant exhibited a small red shift typical of neutral amino acids instead of the anticipated large blue shift expected from negatively charged residues.

To elucidate the anomalous behavior of the Q57D mutant, we integrated computational predictions with experimental validations. Using Multi-Conformation Continuum Electrostatics (MCCE), we predicted the pKa values of ionizable residues and employed a simple electrostatic model using the Charge Density Coupling (CDC) method to calculate the expected absorption peak shifts. Our computational models predicted that all four Q57D residues in the LvWSCP tetramer have shifted pKa values, leading to their protonation at physiological pH (~7). We experimentally confirmed the protonation of all four Q57D residues at physiological pH through site-directed mutagenesis and spectroscopic analyses, including absorption and fluorescence spectroscopy.

Our findings underscore the critical importance of considering local pH environments and pKa shifts in engineering light harvesting proteins. By demonstrating the effectiveness of a combined computational and experimental approach, our study paves the way for the rational design of chlorophyll-binding proteins with tailored optical properties.

# Structure and evolution of Photosystem I in the early-branching cyanobacterium *Anthocerotibacter panamensis*

Christopher J. Gisriel<sup>1,2</sup>, Han-Wei Jiang<sup>3</sup>, David A. Flesher<sup>1</sup>, Gary W. Brudvig<sup>1</sup>, Tanai Cardona<sup>4</sup>, Ming-Yang Ho<sup>3</sup>

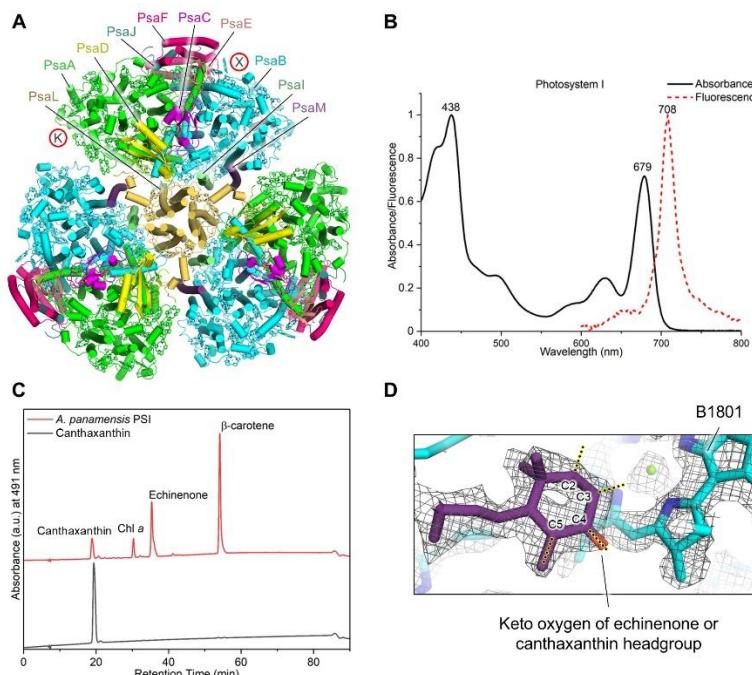
<sup>1</sup>Department of Chemistry, Yale University, New Haven, CT, USA

<sup>2</sup>Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA

<sup>3</sup>Department of Life Science, National Taiwan University, Taipei, Taiwan

<sup>4</sup>School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK

Thylakoid-free cyanobacteria are thought to preserve ancestral traits of early-evolving organisms capable of oxygenic photosynthesis. However, and until recently, photosynthesis studies in thylakoid-free cyanobacteria were only possible in the model strain *Gloeobacter violaceus*. Here, we report the isolation, biochemical characterization, cryo-EM structure, and phylogenetic analysis of photosystem I from a newly discovered thylakoid-free cyanobacterium, *Anthocerotibacter panamensis*, a distant relative of the genus *Gloeobacter*. We find that *A. panamensis* PSI exhibits a distinct carotenoid composition and has one conserved low-energy chlorophyll site, which was lost in *G. violaceus*. These features explain the *A. panamensis* capacity to grow under high light intensity, unlike other *Gloeobacter* bacteria. Furthermore, we find that while at the sequence level PSI in thylakoid-free cyanobacteria has changed to a degree comparable to that of other strains, its subunit composition and oligomeric form might be identical to that of the most recent common ancestor of cyanobacteria.



**Fig. 1.** *A. panamensis* PSI characteristics. (A) Structure of *A. panamensis* PSI. Subunits PsaK and PsaX are not present. (B) Room temperature absorbance and 77 K fluorescence of *A. panamensis* PSI. (C) HPLC chromatogram of pigments present in *A. panamensis* PSI and a canthaxanthin standard. (D) Cryo-EM map near a keto-carotenoid and positions that were quantitatively assessed.

# Tri-institutional Effort to Probe Cyanobacterial Metabolic Overflow: From Research to Training

Harvey J.M. Hou<sup>1,\*</sup>, Himadri Pakrasi<sup>2</sup>, and Jianping Yu<sup>3,\*</sup>

<sup>1</sup>Laboratory of Forensic Analysis and Photosynthesis, Department of Physical Sciences, Alabama State University, Montgomery, Alabama 36104; <sup>2</sup>Department of Biology, Washington University in St. Louis, St. Louis, Missouri 63130. <sup>3</sup>Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401. \*Email: hhou@alasu.edu, jianping.yu@nrel.gov

Understanding energy metabolism in cyanobacteria has potential to guide new strategies for biofuel and chemicals production. Metabolite overflow is an alternative energy dissipation mechanism identified in a glycogen mutant of cyanobacteria (Cano et al 2018). Recently, the deletion of the polyphosphate kinase gene ( $\Delta$ ppk) increased laboratory productivity by re-wiring energy distribution (Sebesta et al 2024). We hypothesize that the metabolite overflow plays a dynamic role in carbon sink and energy management in cyanobacteria. Here we present data in metabolite overflow in the cyanobacterium *Synechocystis* 6803 and *Synechococcus* 7942. (1) HPLC and LCMS analysis showed that WT *Synechocystis* can overflow alpha-ketoglutarate (AKG) and pyruvate in low quantities at normal growth conditions. (2) Mutant *Synechocystis* with the polyphosphate deletion maintains robust Ci fixation capability and secrets about three-fold more AKG and pyruvate than the WT strain. (3) HPLC and FTIR analysis showed that the growth medium of *Synechocystis* 6803 has different time-dependent overflow metabolite profiles. Distinctive time-dependent IR profiles in the cells of *Synechocystis* 6803 and *Synechococcus* 7942 were also observed. The preliminary lines of evidence support that the metabolite overflow may be an energy management strategy in cyanobacteria and may have potential applications in the forensic community. The use of such research activities in teaching at Alabama State University will be discussed (Figure 1). This work was supported by the U.S. Department of Energy, Office of Science Energy Earthshot Initiative, as part of the Science Foundations for Energy Earthshot (DOE SFEE) Projects under grant number DE-SC0024702.

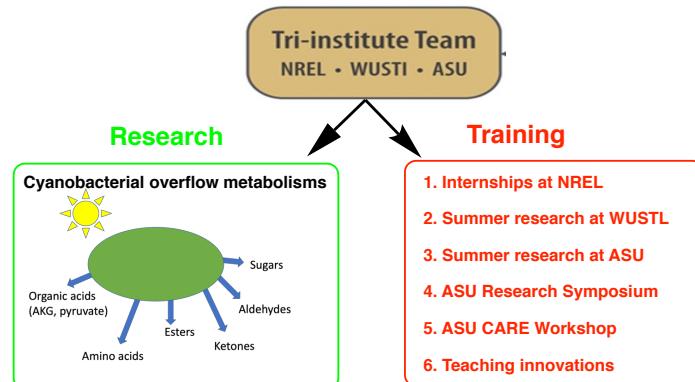


Figure 1. Joint efforts of the tri-institutional team (NREL-WUSTL-ASU) to probe cyanobacterial metabolic overflow. (a) fundamental research (left) and (b) training and teaching (right).

Cano et al (2018) Manipulation of glycogen and sucrose synthesis increases photosynthetic productivity in cyanobacteria, *Front Microbiol*. 14: 1124274.

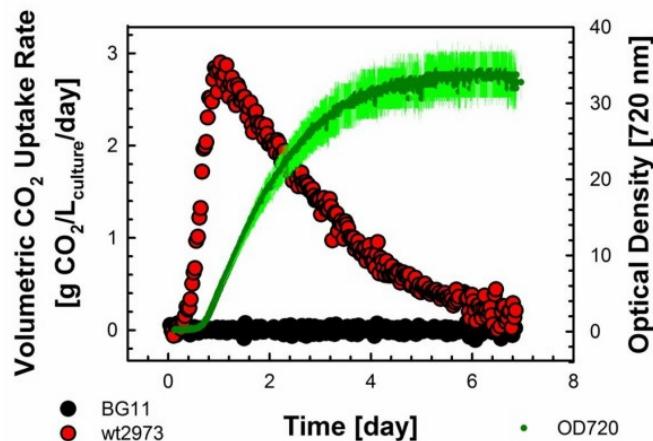
Sebesta et al (2024) Polyphosphate kinase deletion increases laboratory productivity in cyanobacteria. *Front. Plant Sci.* 15:1342496. doi: 10.3389/fpls.2024.1342496

# ACCESSing the Carbon Uptake Dynamics of Cyanobacteria

Christopher M. Jones and Himadri B. Pakrasi

*Department of Biology, Washington University, St. Louis, Missouri*

Quantification of CO<sub>2</sub> uptake rates in cyanobacteria is a vital parameter for assessing their biotechnological potential. However, high resolution and *in situ* CO<sub>2</sub> uptake measurements from active cyanobacterial cultures have so far remained elusive. To solve this problem, an automated carbon and CO<sub>2</sub> experimental sampling system (ACCESS) was developed. ACCESS is based on an Arduino-controlled mini-solenoid array that systematically diverts the off-gas from cyanobacterial cultures towards a flow sensor and infrared gas analyzer which allows for high resolution quantification of CO<sub>2</sub> uptake rate as well as the total amount of fixed carbon. The accuracy of this novel system was independently verified by elemental analysis. Using four cyanobacterial strains under various light and CO<sub>2</sub> regimes, ACCESS data reveals that the CO<sub>2</sub> uptake rate of a cyanobacterial batch culture is dynamic, accelerating linearly to a maximum before decaying monotonically to cessation. This low-cost system simultaneously provides accurate CO<sub>2</sub> uptake rates, total amount of fixed carbon, instantaneous specific growth rates, and specific CO<sub>2</sub> uptake rates, thus illuminating vital parameters to assess the dynamics of carbon uptake by cyanobacteria.



**Figure 1.** ACCESS data for wild type *Synechococcus* UTEX 2973 grown in BG11 media at 1000  $\mu\text{E}$  illumination and fed 1% CO<sub>2</sub>

*Supported by the U.S. Department of Energy, Office of Science, Award Number DE-SC0024702*

# Cryo-EM reveals a bi-copper cluster coordinating asymmetric electron transfer in the nitrogenase-like DPOR complex

Rajnandani Kashyap<sup>1</sup>, Jaigeeth Deveryshetty<sup>1</sup>, Natalie Walsh<sup>1</sup>, Monika Tokmina-Lukaszewska<sup>2</sup>, Brian Bothner<sup>2</sup>, Brian Bennett<sup>3</sup>, and Edwin Antony<sup>1,\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104. Email: [rajnandani.kashyap@health.slu.edu](mailto:rajnandani.kashyap@health.slu.edu)

<sup>2</sup>Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT

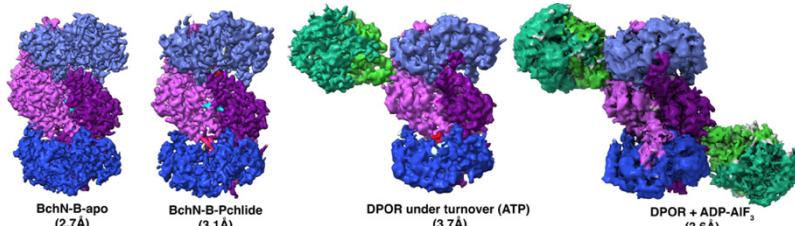
<sup>3</sup>Department of Physics, Marquette University, Milwaukee, WI

Anoxygenic bacteria undergo bacteriochlorophyll biosynthesis in the absence of oxygen and utilize a unique series of enzymes to convert the tetrapyrrole substrate to the chlorophyll. One key enzyme in the biosynthesis pathway is the dark-operative protochlorophyllide oxidoreductase (DPOR). It catalyzes the stereo-specific reduction of the C17=C18 double bond of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). DPOR is widely thought to function as a structurally symmetric octameric complex that consists of an electron donor (BchL) and an electron acceptor/substrate binding (BchN-BchB) component protein. Both contain Fe-S clusters and form a transient complex in the presence of ATP. We recently showed asymmetry in electron transfer between the two identical halves of DPOR (Danyal et. al. PNAS 2016).

These findings raise several interesting questions about the structure-function relationships in such electron transfer enzymes that function as higher order complexes. This higher order complex functions with a cascade of events like ATP hydrolysis, protein-protein interactions, and catalysis.

DPOR also has an intricate structure assembly of two identical functional halves featuring homodimer of BchL and heterotetramer of BchN-BchB. Our overall objective is to understand how does this electron transfer protein complex relays information over long range distances and provide exquisite allosteric control over the other half of the complex.

Using Cryo-EM we capture snapshots of the DPOR enzyme during substrate recognition and turnover. The structures reveal that asymmetry is enforced upon substrate binding and leads to an allosteric inhibition of protein-protein interactions and electron transfer in one half. Residues that form a conduit for electron transfer are aligned in one half while misaligned in the other. An ATP-turnover coupled switch is triggered once electron transfer is accomplished in one half and relayed through a bi-copper cluster at the oligomeric interface, leading to activation of enzymatic events in the other. The findings provide a mechanistic blueprint for regulation of asymmetric long-range electron transfer.



**Figure 1.** Cryo-EM structures of DPOR protein component BchN-BchB, BchN-BchB with Pchlde, DPOR complex under turnover conditions (ATP) clearly depicting asymmetrical binding of the electron donor component protein BchL and the DPOR complex in the presence of ADP-AlF3 depicting symmetrical binding just like the X-ray structure (PDB: 2YNM).

# **Understanding the mechanism of substrate delivery and binding in the oxygen-evolving complex of photosystem II**

**K. V. Lakshmi,<sup>1</sup> V. Kalendra,<sup>1</sup> G. Banerjee,<sup>2</sup> I. Ghosh,<sup>2</sup> K. Reiss,<sup>2</sup> K. Yang,<sup>2</sup> V. S. Batista<sup>2</sup> and G. W. Brudvig<sup>2</sup>**

<sup>1</sup>*Department of Chemistry and Chemical Biology and The Baruch '60 Center for Biochemical Solar Energy Research, Rensselaer Polytechnic Institute, Troy, NY 12180, USA  
(Lakshk@rpi.edu)*

<sup>2</sup>*Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06511, USA*

The solar water-splitting protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in Nature by using light energy to drive a catalyst capable of oxidizing water. The water oxidation reaction is catalyzed by the Mn<sub>4</sub>Ca-oxo cluster in the oxygen-evolving complex (OEC) which cycles through five light-driven S-state intermediates (S<sub>0</sub>-S<sub>4</sub>) as it accumulates charge equivalents to split water. However, a detailed mechanism of the reaction remains elusive as it requires knowledge of the binding of substrate water in the higher S-state intermediates of the OEC. In particular, the binding of substrate in the S<sub>2</sub> to S<sub>3</sub> state transition of the OEC that leads to O-O bond formation is poorly understood because of the inability of conventional methods to probe water molecules. We are using two-dimensional (2D) hyperfine sublevel correlation spectroscopy and density functional theory methods to determine the binding of water in the S state intermediates of the OEC. In this presentation, we will describe ongoing studies that employ small molecule analogs and site-directed mutagenesis of PSII to elucidate the mechanism of the delivery and binding of substrate at the Mn<sub>4</sub>Ca-oxo cluster in the S<sub>2</sub> and S<sub>3</sub> states. These studies have important implications on the mechanistic models for water oxidation in PSII.

<sup>†</sup>This study was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Photosynthetic Systems Program under the contract DE-FG02-07ER15903 (KVL), DE-FG0205ER15646 (GWB) and DE-SC0001423 (VSB).

# qH-Energy Dissipation in Photosystem II Antennae

Alizée Malnoë<sup>1,2</sup>, Pierrick Bru<sup>1,2</sup>, Zeno Guardini<sup>3</sup>, Roberto Bassi<sup>3</sup>, Luca Dall’Osto<sup>3</sup>,  
Pascal Albanese<sup>4</sup>, Cristian Ilioiaia<sup>5</sup>, Andrew Pascal<sup>5</sup>, Bruno Robert<sup>5</sup>, Edel Cunill  
Semanat<sup>6</sup>, Elisabet Romero<sup>6</sup>, Madeline Hoffmann<sup>7</sup>, Gabriela Schlau-Cohen<sup>7</sup>, Aurélie  
Crepin<sup>2,8</sup>

<sup>1</sup>Dept. of Biology, Indiana University Bloomington, USA. [amalnoe@iu.edu](mailto:amalnoe@iu.edu).

<sup>2</sup>Dept. of Plant Physiology, Umeå University, Sweden.

<sup>3</sup>Dept. of Biotechnology, University of Verona, Italy.

<sup>4</sup>IRIG, CEA-Grenoble, France.

<sup>5</sup>SB2SM, CEA-Saclay, France.

<sup>6</sup>ICIQ, Tarragona, Spain.

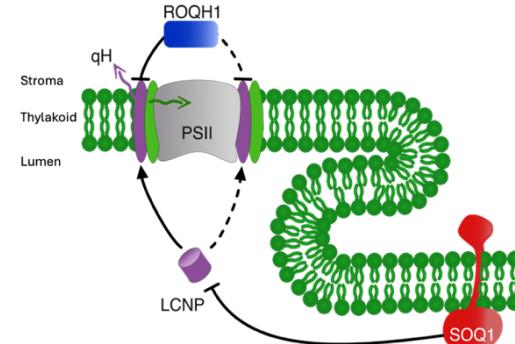
<sup>7</sup>Dept. of Chemistry, MIT, USA.

<sup>8</sup>LGBP, BIAM, Aix-Marseille University, France.

Photoprotection includes several processes quenching chlorophyll fluorescence emission. Although short- or mid-term regulation is well-studied, we lack a detailed understanding of how photosynthesis acclimates to longer stress. The “sustained quenching” mechanism qH, for its induction and relaxation timescales of tens of minutes to hours, is independent of previously known regulatory proteins and triggers of energy dissipation. Its activation requires neither lumen acidification, nor xanthophyll pigments, or phosphorylation. Instead, several effectors have been described in *Arabidopsis*, whose exact role in qH remains to be characterized. These include the luminal effector LCNP (LIPOCALIN IN THE PLASTID), SOQ1 (SUPPRESSOR OF QUENCHING 1), a membrane multi-domain protein that operates upstream of LCNP and prevents quenching, and ROQH1 (RELAXATION OF qH 1), a short-chain dehydrogenase-reductase.

Recently, we have been investigating the targets and mechanism of qH combining genetics, biochemistry, and fluorescence studies, and showed that light harvesting complex II (LHCII) trimers, the major antenna of photosystem II (PSII), serve as a qH site. We now provide evidence that the minor, monomeric antennae of PSII can also be quenched in qH-active conditions and may represent a secondary target in cases of LHCII depletion or stress persistence.

Further, we aimed to characterize the qH-quenched state in LHCII antennae by combining structural biology, biophysical and biochemical approaches on isolated particles. The results exclude the occurrence of a substantial conformational change in qH-quenched particles. They rather point at a small, yet unidentified modification of a subpopulation of antennae affecting the environment of the neoxanthin pigment and surrounding cluster of chlorophylls, enhancing charge-transfer and blinking in the quenched particles.



**Figure 1: Schematic of qH working model.**  
Green arrow, light harvesting; purple arrow,  
energy dissipation taking place in LHCII.

# Rubisco polymerization inside alpha-carboxysomes - but how and why?

Lauren Ann Metskas<sup>1</sup>

*<sup>1</sup>Department of Biological Sciences, Purdue University. lmetskas@purdue.edu.*

Functional protein lattices are found throughout the domains of life, and yet many basic questions about their assembly and mechanisms of action remain unanswered. The Metskas lab studies large protein assemblies in viruses and bacteria, targeting the relationships between structure, function, and chemical environment with correlated cryo-electron tomography and cryo-fluorescence imaging.

Roughly 20% of bacteria employ microcompartments to sequester dangerous or inefficient enzymatic processes inside a semi-permeable protein shell. One of the best-studied microcompartments is the alpha-carboxysome, which is responsible for carbon fixation independently of photosynthesis. We employ cryo electron tomography to image intact alpha-carboxysomes, characterizing protein arrangements and biophysical characteristics. High-resolution processing has enabled us to solve an *in situ* structure of the primary cargo enzyme, Rubisco; identify its interactions; characterize a novel ultrastructure; and calculate its binding affinity *in situ*. I will discuss these technical advances and their biological results, and present ongoing methods development in correlative techniques to add non-structural information to our *in situ* protein visualization.

# A Computational Framework for Simulating Protein Organization in Thylakoid Membranes

Ashraf Mohamed<sup>1</sup>, Doran I. G. B. Raccah<sup>1</sup>

<sup>1</sup>*Department of Chemistry, The University of Texas at Austin. Austin, Texas 78712, USA.*  
[ashraf.mohamed@austin.utexas.edu](mailto:ashraf.mohamed@austin.utexas.edu).

In the grana membranes of plants, the efficiency of photosynthetic light harvesting and electron transport depends on the spatial arrangement of proteins, collectively referred to as the “protein landscape.” This landscape is tightly regulated to ensure optimal photosynthetic efficiency. Therefore, a robust modeling tool is essential for understanding and potentially engineering photosynthetic regulation. In this study, we present a novel computational model to simulate the organization of different types of proteins in thylakoid membranes. Using Monte Carlo sampling, we simulate membranes of various sizes and protein densities. The model exploits the precise atomic structures of proteins and enforces volume exclusion to capture realistic protein packing on the 100 nm length scale. As a proof of concept, we applied our Monte Carlo membrane model to the protein landscape of the grana membrane in *Arabidopsis thaliana*. We performed Monte Carlo simulations on the particle maps measured by freeze-fracture cryo-electron tomography and revealed protein identities and orientations. We also demonstrated that when only accounting for volume exclusion, the simulated membrane packing at biological protein densities the model is capable for describing the overall protein landscape for real-world membranes. Looking forward, this model is adaptable and will provide a quantitative platform for comparing experimental data with theoretical models of thylakoid membranes of different sizes and with various protein densities.

# Tuning the Low-Energy Fluorescence State in Photosystem II

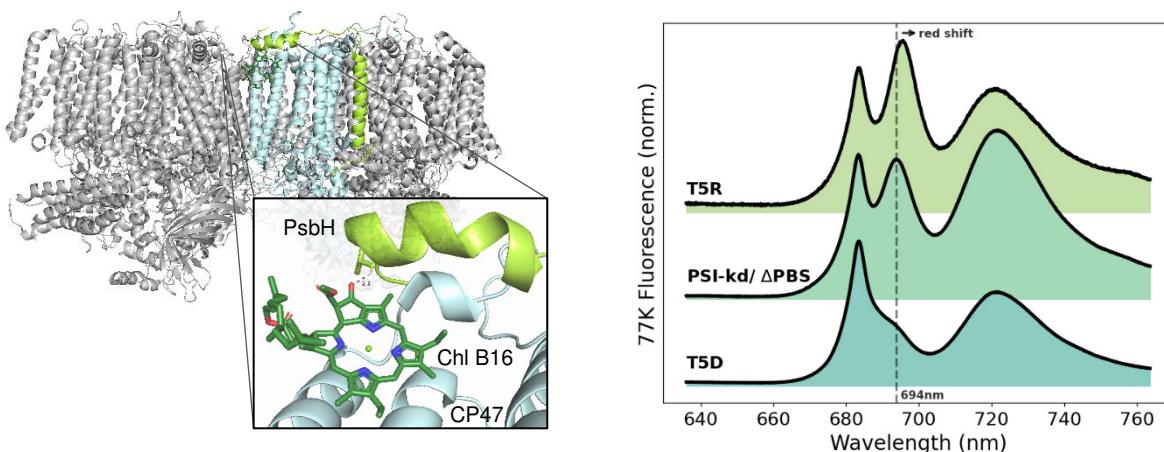
Amala Phadkule<sup>1</sup>, Amit Srivastava<sup>1†</sup>, and Mike Reppert<sup>1</sup>

1 Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

† Current affiliation - Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Třeboň, 379 01 Czech Republic

aphadkul@purdue.edu, srivastava@alga.cz, and reppertm@purdue.edu

Photosystem II (PSII) is a multi-subunit pigment-protein complex that hosts the reaction center where water splitting occurs. The reaction center is surrounded by two core antennas, CP43 and CP47, which possess low-energy fluorescence states. In fluorescence, the CP47 low-energy state results in a 695 nm band that is hypothesized to act as a trap state, but the exact function is unknown. Initially, both, mutagenesis of chlorophyll ligands in CP47 done by Shen and Vermaas and spectroscopic investigation of the isolated CP47 by de Weerd et al. suggested that the chlorophyll ligated to His 114 (Chl B16) is responsible for the low-energy state. Subsequent theoretical and spectroscopic studies variously support or dispute this assignment. A factor for ambiguity in previous mutagenesis studies is the large perturbation in the system, e.g. mutation of the residue ligated to chlorophyll or deletion of the PsbH subunit. This study suggests an *in vivo* approach using site-directed mutagenesis in *Synechocystis sp.* PCC 6803 to perturb the hydrogen bond between Thr5 in the PsbH subunit and Chl B16. To minimize spectral congestion, we developed a PSI knockdown strain combined with phycobilisome deletion (PSI-kd/ΔPBS). We used 77K fluorescence for whole cells to study the site mutations made in the PSI-kd/ΔPBS background. The spectra show an isolated PSII signal that yields conclusive results about the perturbations caused by the change in hydrogen bonding. We observe a red shift in the low-energy band when the Thr5 is replaced by Arg, weakening the hydrogen bond and a blue shift when the Thr5 is replaced by Ala forming no hydrogen bond. 77K emission spectra show that the site mutations made in the PsbH subunit tune the 695nm band suggesting it is Chl B16 responsible for the low-energy state.



**Figure 1:** Chl B16 bound to CP47 and PsbH subunit in photosystem II. 77K spectra for site mutants at Thr5 in PSI-kd/ΔPBS background after PSI suppression.

# Structural Analysis of Platinum Nanoclusters Accumulated by Photosystem I Photo-reduction via the X-Ray Scattering Techniques

Nina S. Ponomarenko,<sup>1</sup> Nestor J. Zaluzec,<sup>4</sup> Xiaobing Zuo,<sup>2</sup> Olaf J. Borkiewicz,<sup>2</sup> Justin M. Hoffman,<sup>2</sup> Alex B. F. Martinson,<sup>3</sup> Lisa M. Utschig,<sup>1</sup> and David M. Tiede<sup>1</sup>

<sup>1</sup>*Chemical Sciences and Engineering, <sup>2</sup>X-ray Science, and <sup>3</sup>Materials Science Divisions, Argonne National Laboratory*

<sup>4</sup>*Pritzker School of Molecular Engineering, University of Chicago*

Photosystem I (PSI) is one of the major protein complexes sustaining the photoinduced electron transfer reactions in the conversion of the energy of solar electromagnetic radiation to the energy of chemical bonds in the process of photosynthesis. The accumulated under the influence of light potential for reduction reaction in this protein-cofactor assembly can be redirected from natural electron carriers to alternative acceptors. Such deviation, while retaining the exceptional ability for electron transfer, imparts novel functionality to photosynthetic complexes. One of the most inspiring applications of the reducing ability of PSI is the photo-precipitation of metallic platinum from its solution and thus functionalization of this protein by a nanocluster complex capable of catalyzing the consequent hydrogen evolution reaction. After first demonstrated about 50 years ago [Elias Greenbaum, Platinized Chloroplast: A novel photocatalytic material. *Science* 1985, 230, 1373-1375], this approach has been further corroborated in succeeding works, and the utilizing it systems were found to be stable and viable.

While the light-induced effect of platinization was known and utilized for the catalysis of subsequent hydrogen evolution reactions, the atomic structure of formed abiotic clusters certainly needed investigation for innovative implementations. Since photo-reduction was expected to produce the non-crystalline or semi-crystalline substance in solution, its structural characterization by application of traditional diffraction methods, where a periodic arrangement of elements is the prerequisite for analysis, was very challenging and the rational explanation for the lack of atomic structural investigations up to now.

The development of X-ray total scattering and corresponding Pair Distribution Function analysis made a structural study of materials in solution feasible and they become powerful techniques for the atomic structure characterization of nanomaterials, including amorphous clusters. The application of this approach for the examination of Pt nanoclusters accumulated by photo-reaction provided adequate information for elucidation of the internal structure of produced nanodiscs and for the modeling to be carried out. The investigation of the photo-precipitation of platinum using X-ray scattering complemented by High-angle annular dark-field scanning transmission electron microscopy and Energy dispersive X-ray spectroscopy facilitated the search for insight into the bio-inorganic interfaces on which this reaction is taking place, the sites for reductive electron transfer chemistry on the PSI surface.

# **Gas exchanges measurements of carboxysome mutants reveal conditional phenotypes and insights into cyanobacteria carbon concentrating mechanism.**

Rees Rillema<sup>1</sup>, Anne Steensma<sup>1</sup>, Daniel Ducat<sup>1</sup>, and Berkley Walker<sup>1</sup>

<sup>1</sup>*Michigan State University. 612 Wilson Rd, Room 206a. rillemar@msu.edu.*

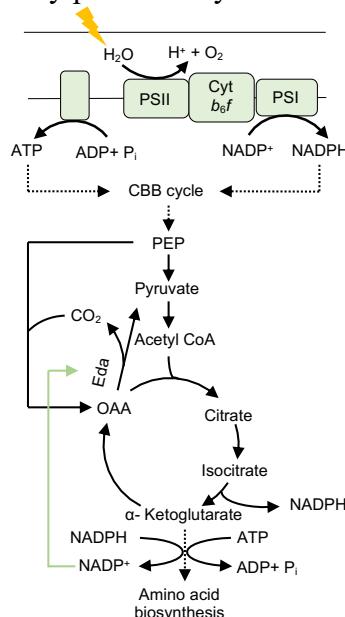
Gas exchange measurements in plants have improved the depth and resolution of photosynthesis and plant CO<sub>2</sub> assimilation rates, yet this technique is not widely adapted for cyanobacteria. Cyanobacteria are faster growing than plants, single-celled, and have a well-characterized carbon concentrating mechanism (CCM), but how individual components affect carbon utilization remains poorly understood. The central part of the CCM in cyanobacteria is a proteaceous bacterial microcompartment (BMC) called the carboxysome. Carboxysomes are unique among BMCs because they house rubisco, the critical metabolic enzyme in carbon-fixing autotrophs. This demand creates selective pressure on the carboxysome and associated proteins to evolve rapid responses to environmental stressors. Our knowledge of central carboxysome components is limited to their role in assembly and structure. More poorly understood is the function of accessory shell components widely found throughout carboxysome-containing bacteria phyla. Also, the field's current hypothesis of the carboxysome shell as a selectively impermeable barrier lacks direct evidence. We utilize novel gas exchange techniques adapted for liquid cultures to characterize the general photosynthetic performance within the cyanobacteria *Synechococcus elongatus* PCC7942 and elucidate accessory shell components' role in carbon fixation and carboxysome function. This research aids in our understanding of BMCs and improves future designs of synthetic BMCs.

# KDPG aldolase modulates the photosynthetic carbon yield in *Synechococcus elongatus* PCC 7942

Chetna Sharma<sup>1</sup>, Ningdong Xie<sup>1</sup> and Xin Wang<sup>1</sup>

<sup>1</sup>Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville-32603. [chetnasharma1@ufl.edu](mailto:chetnasharma1@ufl.edu)

Glycogen degradation during the night plays a crucial role in the survival and growth of cyanobacteria. Being single celled, photosynthesis and respiration are well connected in cyanobacteria and various glycolytic pathways are involved in the generation of energy and accumulation of metabolites. The oxidative pentose phosphate pathway has been known to modulate NADPH levels and provide intermediates to kickstart photosynthesis under light/dark growth conditions. However, the interconnection of respiratory pathways is still not entirely clear. In this study, we focused on the role of the Entner-Doudoroff (ED) pathway. Based on biochemical assay and Gas Chromatography-Mass spectrometry (GC-MS) metabolite analysis, we found that ED pathway does not operate in the cyanobacterium *Synechococcus elongatus* PCC 7942. These results were confirmed by the absence of enzyme 6-phosphogluconate dehydratase and lack of KDPG detection in crude cell lysates under both light and dark conditions. However, the mutant lacking KDPG aldolase (Eda) exhibits a bleaching phenotype under light, indicating a potential role for Eda in cyanobacterial metabolism. Therefore, we investigated the potential substrates of Eda. Through *in vitro* enzyme assay studies, we demonstrated that Eda functions as an oxaloacetate decarboxylating enzyme, with a  $K_m=0.473\text{mM}$ ,  $V_{max}=0.680\ \mu\text{mol min}^{-1}\text{mg}^{-1}$  and  $k_{cat}/K_m=437\text{s}^{-1}\text{M}^{-1}$  for oxaloacetate under *in vitro* conditions. This activity is further regulated by NADP<sup>+</sup> levels in cells to temporarily bypass the TCA cycle during periods of excess NADPH and ATP consumption through amino acid synthesis. This revelation of Eda's function and regulation contributes to our understanding of cyanobacterial metabolism and primary productivity under varying environmental conditions.



**Figure 1.** Proposed model showing KDPG aldolase regulation in photosynthetic yield and energy balance in *Synechococcus elongatus* PCC 7942.

# **Extreme light acclimation reveals inherent photosystem II photoprotection in a natively low-light *Chlorella***

Grant Steiner<sup>1</sup>, Devin Saini<sup>2</sup>, Arivu Kapoor<sup>1</sup>, Ambrose Krozel<sup>1</sup>, Colin Gates<sup>1,2</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry Loyola University Chicago, 1068 W. Sheridan Rd. 60660, Chicago IL.*

<sup>2</sup>*Department of Bioinformatics, Loyola University Chicago, 1032 W. Sheridan Rd. 60660, Chicago IL.*

The desert-native, extreme light (2,000  $\mu$ Ein)-adapted green alga *Chlorella ohadii* expresses exceptional photosystem II photoprotection, due in part to high levels of PSII-cyclic electron flow. This facilitates *C. ohadii* possessing the fastest recorded doubling time of any known phototroph, 1.4 hours, in high light and 2% CO<sub>2</sub>. When *Chlorella* NIES 642, a temperate, low light (20  $\mu$ Ein)-adapted alga is subjected to the same growth conditions as *C. ohadii*, we have observed various changes to the PETC indicative of high PSII-CEF and recorded a minimum doubling time of 1.6 hours. In extreme light grown NIES 642, PSII reaction centers do not fully saturate under conditions that can induce single-turnovers in organisms as recalcitrant as *C. ohadii*. 77K fluorometry and chlorophyll extraction suggest this may result from extensive minimization of chlorophyll pigments; in extreme light conditions the *C. ohadii* to *C. NIES 642* chlorophyll *a* ratio is  $1.82 \pm 0.18$ . Acclimated NIES 642 also shows various characteristics of high PSII-CEF on the acceptor side of PSII, including near-constant utilization of the plastoquinone pool ( $4.2 \pm 2.5\%$  performing no electron transfer) and a majority of centers with complexed oxidized Q<sub>B</sub> ( $59.7 \pm 2.9\%$ ), opposed to semiquinone ( $35.1 \pm 2.6\%$ ). P700 utilization monitored via  $\Delta A_{810\text{nm}}$  absorbance illustrates that extreme light conditions greatly diminish charge separation at PSI, and *C. ohadii* expresses more PSI, comparatively. Electrochromic shift measurements of the thylakoid membrane show a diminished trans-thylakoid proton gradient in the high PSII-CEF systems, strongly suggesting a large role of PSII-CEF in optimizing ATP production.

# 50 Years of Cyanobacteria and Photosynthesis

Wim Vermaas

*School of Life Sciences and Center for Bioenergy and Photosynthesis, Arizona State University,  
Tempe, AZ 85287-4501, wim@asu.edu*

Cyanobacteria have been part of the biosphere for billions of years, and are thought to be a main contributor to the transition from an anoxic to an oxic atmosphere during the Great Oxidation Event that occurred over two billion years ago. They still are an important part of global photosynthetic productivity, particularly in the oceans.

Fast forward to about 50 years ago, when the idea arose to genetically modify photosynthetic organisms to learn more about them, with in the back of scientists' mind to perhaps improve agricultural production over time. The first study on transforming a cyanobacterium was published by Sergey Shestakov in Moscow in 1970. Around the same time the first plant transformations were performed by Rob Schilperoort in Leiden and other groups in Belgium and the US. It became clear that the genomes in some cyanobacteria and chloroplasts (derived from cyanobacteria long ago) could undergo double-homologous recombination, a process that allows for insertion of markers into genes, thus interrupting them, or for exact gene replacement. With this approach in the 1980s the donor to P680 in PSII was identified as a Tyr residue in the D1 protein, with the equivalent Tyr in D2 serving as Y<sub>D</sub>. Similarly important residues and subunits were identified in the photosystems of cyanobacteria and other photosynthetic bacteria. Guidance was provided by the crystal structure of the purple bacterial reaction center (earning Hartmut Michel, Johann Deisenhofer and Robert Huber a Nobel Prize), which was recognized to be homologous to PSII.

When the gene for the homodimeric heliobacterial reaction center was sequenced and analyzed, the idea arose of the two photosystems in plants and cyanobacteria to be homologous, with the PSI reaction center protein having been split in an antenna and reaction center component in PSII.

In the 1990s genomic sequencing of prokaryotic genomes became feasible, and the *Synechocystis* genome was sequenced by Satoshi Tabata and coworkers in Chiba (near Tokyo) even before that of *Escherichia coli*. This led to a flurry of activity to determine the function of open reading frames in the *Synechocystis* genome using double-homologous recombination knock-out and insertion strategies, and provided a way to understand not only the function of individual genes but also metabolic pathways, regulation of gene expression, and the role of photosynthesis in this all.

Now with the major functional components of the cyanobacterial "blueprint of a cell" known, it has become more feasible to start viewing the cyanobacterial cell as a chassis that can be modified to make products for human use. Indeed, strains producing and excreting biofuels (ethanol, fatty acid esters, alkanes) have been developed and grown at pilot plant scale; their productivity per acre far exceeds that of plants. However, fossil fuels are still too cheap for cyanobacterial biofuels to be fully price-competitive, and valuable co-products typically are needed to make the economics work. Phycocyanin is an example of a valuable co-product from cyanobacteria, and work has been ongoing to improve its thermostability in order to develop it as a natural food dye, replacing blue chemical dyes that have toxicity issues.

A 2022 Executive Order on the Bioeconomy from President Biden has invigorated the microbial biotechnology field. In the years and decades ahead, the use of cyanobacteria and other living systems for production of compounds currently made from fossil fuels or even by non-sustainable agriculture hopefully will become a lasting and sustainable part of the social fabric globally.

# A Photosynthetic Variant of *Synechocystis* sp. PCC 6803 Sacrifices a Stress Response Pathway to Outcompete its Peers under Optimal Growth Conditions

David J. Vinyard<sup>1</sup>, Brandon P. Russell<sup>1</sup>, Vasily Kurashov<sup>2</sup>, David F. Iwig<sup>3</sup>, Patrick Landry<sup>4</sup>, Wade Johnson<sup>5</sup>, Art van der Est<sup>6\*</sup>, John H. Golbeck<sup>2\*</sup>, and K. V. Lakshmi<sup>4\*</sup>

<sup>1</sup>Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, 70803, USA. dvinyard@lsu.edu

<sup>2</sup>Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, 16802, USA.

<sup>3</sup>Department of Chemistry and Howard Hughes Medical Institute, The Pennsylvania State University, University Park, PA, 16802, USA.

<sup>4</sup>Department of Chemistry and Chemical Biology and The Baruch '60 Center for Biochemical Solar Energy Research, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA.

<sup>5</sup>Department of Chemistry, Susquehanna University, Selinsgrove, PA, 17870, USA.

<sup>6</sup>Department of Chemistry, Brock University, 500 Glenridge Avenue, St. Catharines, ON Canada L2S 3A1.

Phylloquinone (PhQ) plays a unique role in photosynthesis as the A<sub>1A</sub> and A<sub>1B</sub> intermediates in light-driven electron transfer in Photosystem I (PSI). When PhQ biosynthesis is inhibited by deletion of the *menB* gene in the cyanobacterium *Synechocystis* sp. PCC 6803, previous studies have shown that plastoquinone-9 (PQ-9) occupies the A<sub>1A</sub> and A<sub>1B</sub> sites instead of PhQ.

However, a recent cryo-electron microscopy structure of a strain of  $\Delta menB$  from the year 2023 revealed an unusual quinone electron acceptor in the A<sub>1A</sub> and A<sub>1B</sub> sites with a benzoquinone head group similar to PQ-9 and a phytyl tail similar to PhQ (Gisriel, et al. 2024 *Science Advances*, in press). Here, we use mass spectrometry to identify the quinone molecule as 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ). In contrast, only PQ-9 was found in PSI from the original  $\Delta menB$  strain. Whole genome sequencing reveals that this difference is the result of a mutation in *slr1737* (tocopherol cyclase) that leads to the accumulation of DMPBQ, an intermediate in the tocopherol biosynthetic pathway. Transient optical and electron paramagnetic resonance spectroscopy studies show that when DMPBQ occupies the A<sub>1</sub> sites, it does not exchange with exogenously supplied PhQ in contrast to PQ-9 which exchanges readily. We propose that the  $\Delta menB$  strain with the *slr1737* mutation has sacrificed a stress response pathway under low stress laboratory growth conditions, resulting in a strain that incorporates DMPBQ instead of PhQ in the A<sub>1A</sub> and A<sub>1B</sub> sites. The better quinone binding and function of DMPBQ allow this  $\Delta menB$  strain to outcompete its peers under optimal growth conditions and dominate the population.

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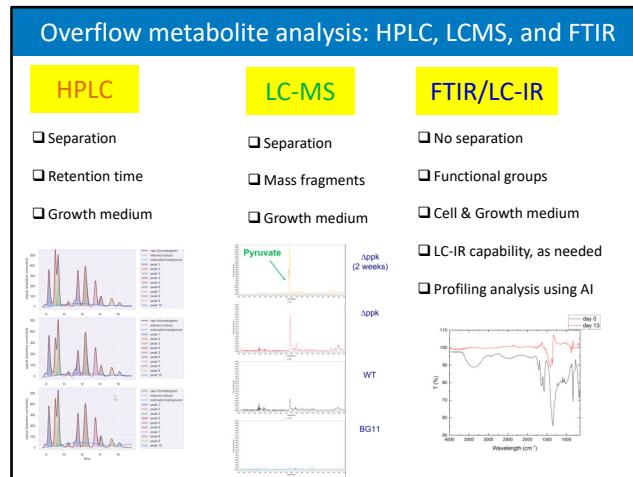
# HPLC, LCMS, and FTIR Analysis of Cyanobacterial Metabolite Overflow for Energy Management

Ibrahim Alamin<sup>1</sup>, Jacob Sebesta<sup>2</sup>, Arnav Deshpande<sup>2</sup>, Lieve Laurens<sup>2</sup>, Vida A Dennis<sup>1</sup>, Jianping Yu<sup>2,\*</sup>, and Harvey J.M. Hou<sup>1,3,\*</sup>

<sup>1</sup>Center for NanoBiotechnology Research, Ph.D. program in Microbiology, Alabama State University, Montgomery, Alabama 36104; <sup>2</sup>Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401. <sup>3</sup>Laboratory of Forensic Analysis and Photosynthesis, Department of Physical Sciences, Alabama State University, Montgomery, Alabama 36104.

\*Email: [hhou@alasu.edu](mailto:hhou@alasu.edu), [jianping.yu@nrel.gov](mailto:jianping.yu@nrel.gov)

Recent report indicated that overflow of alpha-ketoglutarate and pyruvate may be an alternative energy management mechanism in a mutant of cyanobacteria (Cano et al 2018). However, the molecular details of overflow metabolism are not completely known. We hypothesize that using analytical chemical methods, we can monitor the detailed changes of overflow metabolism in cyanobacteria and offering insights into their energy management strategies during growth. In this work, we use HPLC, LCMS and FTIR to analyze the growth medium of wild-type cyanobacterium *Synechocystis* sp. PCC 6803 at high light conditions (500 uE) as shown in Figure 1. LCMS results identified 56 targeted and 170 untargeted metabolite end-products of overflow metabolism in cyanobacteria. The 226 metabolites include organic acids, amino acids, organic bases, esters, alcohols, ketones, aldehydes, and sugars. HPLC data showed that pyruvate overflow is sensitive to the presence of nitrate and lower pH in the growth medium. FTIR analysis showed the changes in overflow metabolites end-products in cyanobacterium *Synechocystis* 6803 at high light condition between day 0 and day 13. FTIR results also showed the apparent different IR profiles of the cells of *Synechocystis* 6803 and *Synechococcus* 7942 from day 0 to day 4, which suggested the feasibility of FTIR analysis on the intact cells. The preliminary data provide new information of the cyanobacterial overflow metabolism. This work may open new route in assessing the energy management in cyanobacteria for bioproducts production. This work was supported by the U.S. Department of Energy, Office of Science Energy Earthshot Initiative, as part of the Science Foundations for Energy Earthshot (DOE SFE) Projects under grant number DE-SC0024702.



**Figure 1:** HPLC, LCMS and FTIR analysis of cyanobacteria at high light conditions.

Cano et al (2018) Manipulation of glycogen and sucrose synthesis increases photosynthetic productivity in cyanobacteria, Front Microbiol. 14: 1124274.

# Computational Mutagenesis: Investigating Single-point Mutation Effects on the Spectra of the Fenna-Matthews-Olson (FMO) complex.

Richmond Arhin<sup>1</sup>, Sergei Savikhin<sup>2</sup> and Lyudmila Slipchenko<sup>1</sup>

<sup>1</sup>Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA.

[rarhin@purdue.edu](mailto:rarhin@purdue.edu).

<sup>2</sup>Department of Physics and Astronomy, Purdue University, West Lafayette, Indiana 47907, USA.

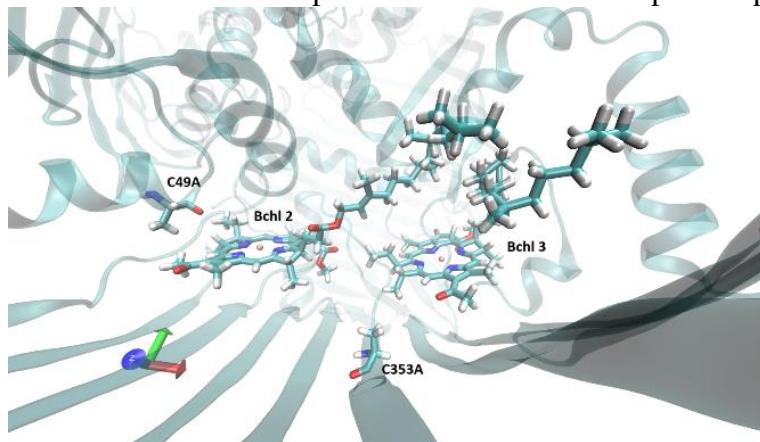
[sergei@purdue.edu](mailto:sergei@purdue.edu).

<sup>1</sup>Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA.

[lslipche@purdue.edu](mailto:lslipche@purdue.edu).

Pigment-protein complexes involved in light harvesting, such as the Fenna-Matthews-Olson (FMO) protein complex, are renowned for their near-perfect quantum efficiency in capturing and transferring excitation energy to the reaction center (RC). This remarkable occurrence in nature has been investigated by many curiosity-driven researchers and found to result from the intrinsic electronic properties of the individual pigments and the couplings between these pigments. On the other hand, the intricate overlapping of electronic absorptions from numerous pigments that make up the structure of these photosynthetic complexes often leads to ambiguous experimental or spectroscopic assignments of the interactions between the pigments.

Mutagenesis has been widely used to explore intrinsic interactions in proteins and to resolve some of the ambiguities of spectroscopic measurements of wild-type systems. Specifically, in photosynthetic complexes, point mutations in the protein's secondary structures surrounding the pigments revealed significant impacts of the protein environment on the dynamics of the excitation energy transfer process. To gain a deeper understanding of the energy transfer in photosynthetic systems, we are developing theoretical methods that allow for accurate correlations between structure and spectroscopy in these complexes. For example, we have shown that combining classical molecular dynamics, the quantum mechanics/molecular mechanics (QMMM) and quantum mechanics/effective fragment potentials (QM/EFP) methods are highly efficient in predicting the absorption and circular dichroism spectra of the FMO complex and its mutants. In this study, our goal is to create a computationally efficient approach to predict how single-point mutations affect the optical spectroscopy of photosynthetic pigment complexes, using the FMO complex as a test case. This will help establish a workflow for the targeted design of mutations to achieve specific effects on selected protein-pigment components.



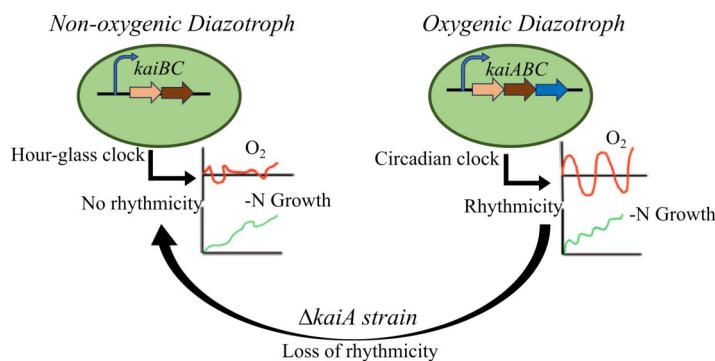
**Figure. 01.** A structure of the FMO mutant with PDB-ID, 5H8Z showing single point mutations of cysteine residues to alanine at bacteriochlorophyll sites 2 and 3.

# Endogenous clock-mediated regulation of intracellular oxygen dynamics is essential for diazotrophic growth of unicellular cyanobacteria

Anindita Bandyopadhyay, Annesha Sengupta, Thanura Elvitigala, Michelle Liberton, Alexandra Siemer and Himadri B. Pakrasi

Washington University, 1 Brookings Drive, St. Louis, MO 63130  
anindita@wustl.edu

The ability of unicellular diazotrophic cyanobacteria to perform nitrogen fixation and photosynthesis in the same cellular platform is an enigma that continues to intrigue biologists. The observation made decades ago, that unicellular cyanobacteria can perform nitrogen fixation under continuous light, provided the first clues to the existence of a circadian clock in prokaryotes. However, owed to their recalcitrance to any genetic manipulation, the clock-mediated segregation of processes remained largely unexplored in this group of prokaryotes. To investigate its function in these diazotrophs, we disrupted the circadian clock defined by the *kaiABC* genes in the now well-established model strain *Cyanothece* ATCC 51142. Unlike non-diazotrophic cyanobacteria, *Cyanothece* 51142 exhibits conspicuous self-sustained rhythms in various discernable phenotypes, offering a platform to directly study the effects of the clock on the physiology of an organism. Disrupting the clock by deleting *kaiA* led to impairment in nitrogen fixation and growth under continuous light or long day length conditions. Under such conditions, the conspicuous endogenous rhythms in oxygen cycling observed in the WT was disrupted in the mutant, suggesting that a loss in the regulation of oxygen cycling is detrimental to nitrogenase function and growth. This work provides the first molecular evidence of the involvement of the circadian clock in segregating essential yet incompatible processes in unicellular diazotrophic cyanobacteria. Our findings suggest that the addition of KaiA to the KaiBC clock was likely an adaptation that ensured optimal nitrogen fixation as microbes evolved from an anaerobic to an aerobic atmosphere under nitrogen constraints.



**Figure 1.** Schematic showing the importance of KaiA in regulating cellular oxygen dynamics in unicellular diazotrophic cyanobacteria.

This work was supported by the National Science Foundation (MCB 1933660) and by the U.S. Department of Energy (DOE), Office of Science Energy Earthshot Initiative, as part of the CyaNofertilizer project (DE-SC0024702).

# Thermoregulation of the trophic modes of *Galdieria yellowstonensis*

**Kishan Bharwad<sup>1</sup>**, Julia Van Etten<sup>2</sup>, Debasish Bhattacharya<sup>2</sup>, Colin Gates<sup>1,3</sup>

<sup>1</sup>1068 W Sheridan Rd, Chicago, IL 60660, Loyola University Chicago. Department of Chemistry and Biochemistry. [Kbharwad@luc.edu](mailto:Kbharwad@luc.edu)

<sup>2</sup>2123 Bevier Rd, Piscataway, NJ 08854, Rutgers University Department of Biochemistry and Microbiology.

<sup>3</sup>1032 W Sheridan Rd, Chicago, IL 60660, Loyola University Chicago. Department of Bioinformatics.

*Galdieria yellowstonensis* (strain YNP 5572) is an extremophilic red alga capable of switching between autotrophic and heterotrophic modes of growth. This study aimed to investigate the regulation of trophic switching in response to thermal conditions. *Galdieria yellowstonensis* was exposed to temperatures of 30°C and 42°C under conditions with glucose (+G) and without glucose (-G) in a diurnal cycle. In the absence of glucose, notably, more PSI and PSII are present regardless of temperature. +G at 30°C produces a diurnal cycle of PSI and PSII activity, and under other conditions, this is relatively constant. In the absence of glucose, more phycobilin is expressed and more of it is associated with the photosystems, suggesting enhanced light capture and charge separation, whereas +G cultures at both temperatures expressed less phycobilin. Fast repetition rate fluorometry was used to assess PSII-WOC activity. The -G cultures exhibited prominent oscillations, whereas +G cultures at both temperatures displayed no oscillations and, thus, no activity. Oscillation quality was higher at the higher temperature. In -G cultures, at 30°C a lower Q-factor of 2.26 was observed, while at 42°C a higher Q-factor of 2.70 was seen. -G has similar PSI activity observed at both temperatures. In contrast, +G cultures at 42°C show more PSI oxidation than at 30°C, implying that PSI reoxidation depends on temperature when sugar is present.

# Investigation of Water Splitting Mechanisms in Photosystem II Using Near-IR Resonance Raman Spectroscopy

Sudip Bhowmick, Roman Ezhov and Yulia Pushkar

*Department of Physics & astronomy, Purdue University, 525 Northwestern Ave, West Lafayette, IN 47907, bhowmick@purdue.edu*

Photosynthesis, a process present in algae, green plants, and cyanobacteria, converts sunlight into energy that sustains all living beings on Earth. Photosystem II (PSII) catalyzes the light-driven water oxidation in the process of light to energy conversion. [1]. This reaction is driven by the Mn4Ca complex, which transitions through four stable states (S0 to S4) as described in Kok's cycle, and is embedded in the thylakoid membrane [2]. The exact mechanism of water splitting by the Mn4Ca cluster and the intermediate structural changes of the catalyst during this process are still not fully understood [3]. Previously, some studies using FTIR spectroscopy have attempted to explain this mechanism [4], but Near-IR resonance Raman spectroscopy shows potential for additional approach in understanding of this process. In this study, PS II-enriched thylakoid membranes were prepared from spinach [5] and Near-IR resonance Raman spectra (at 830 nm excitation) were recorded from these freshly prepared PS II samples at room temperature and on cryostage. The addition of hydroxylamine solution which reduces Mn4Ca cluster to Mn<sup>2+</sup> ions and results in its destruction resulted in changes of the Raman peaks of PS II sample indicating contribution of Mn4Ca cluster vibrations. Two laser pulses were used to transition PS II from the S1 to S3 state, and the corresponding resonance Raman spectra were recorded at nearly 77K temperature. Thereafter, a 50 mM PPBQ solution in DMSO was added as an artificial electron acceptor before excitation using laser pulses to enhance the conversion of the S3 state. The changes in the Raman peaks of Mn in the MnIV oxidation state were analyzed using resonance Raman signal. Additionally, we studied some manganese model compounds, such as [Mn<sub>2</sub>O<sub>2</sub>(tPy)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]<sup>3+</sup>, to identify Mn's Raman signal using the same Near-IR resonance Raman spectroscopy and laser..

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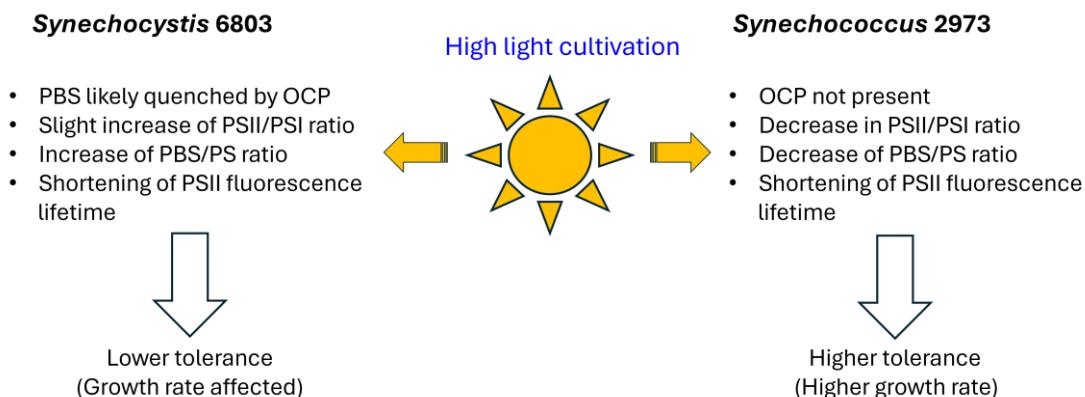
# Evaluating Changes To Excitation Energy Transfer Rates On High-Light Exposure In Two Model Cyanobacteria

Sandeep Biswas<sup>1</sup>, Dariusz M. Niedzwiedzki<sup>2,3</sup> and Himadri B. Pakrasi<sup>1</sup>

<sup>1</sup>Department of Biology, Washington University, One Brookings Drive, St. Louis MO 63130  
sandeeb.biswas@wustl.edu

<sup>2</sup>Center for Solar Energy and Energy Storage and <sup>3</sup>Department of Energy, Environmental & Chemical Engineering, Washington University, One Brookings Drive, St. Louis MO 63130

Light absorption by photochemically active pigments and proteins in photosynthetic organisms triggers reactions that fix light into usable chemical energy. Since the discovery of light reactions in photosynthetic organisms, many efforts have focused on understanding the excitation energy transfer (EET) mechanism through advanced spectroscopy. However, with the development of efforts to use cyanobacteria in synthetic biology, a critical approach has been taken to improve light tolerance. Increased light tolerance provides accelerated growth and excess energy that can be diverted for the sustainable production of chemicals without compromising growth. To better understand the changes in EET on high light exposure and what makes a strain more light-tolerant, we compared EET in the whole cells of *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973. Using whole cells allowed us to study the EET under native conditions. We preferentially excited chlorophylls. Elucidating the means to overcome photoinhibition employed by *Synechococcus* 2973 is imperative for helping design strategies for increasing the light tolerance of cyanobacteria used for carbon-neutral bioproduction. Our observations suggest that *Synechococcus* 2973 employs a three-pronged strategy to overcome photoinhibition on prolonged growth under high light. These include the possible involvement of an uncommon OCP, tighter stoichiometric regulation of PSII/I, and reduction of light-harvesting antenna.



**Figure. Schematic comparing the high light tolerance mechanism of *Synechococcus* 2973 with the response of *Synechocystis* 6803.** Under high light, *Synechococcus* 2973 deploys more than one strategy to increase its tolerance to high light intensity. Key factors include a decline in light absorption by regulating PBS, an increase in PSI, and a decrease in PSII.

This work is supported by funding from the U.S. Department of Energy, Office of Basic Energy Sciences (DE-FG02-99ER20350).

# Tackling the Many Ferredoxins Problem in *Chlamydomonas Reinhardtii* with Equilibrium and Enhanced Sampling Molecular Simulations

Daipayan Sarkar<sup>1</sup>, Duncan Boren<sup>1</sup>, Christoph W. A. Fischer<sup>2</sup>, Stefan Schmollinger<sup>1</sup>, Daniela Strenkert<sup>1</sup>, Josh V. Vermaas<sup>1</sup>

<sup>1</sup>MSU-DOE Plant Research Laboratory, East Lansing, Michigan, USA

<sup>2</sup>Institut für Biochemie, Heinrich Heine University, Düsseldorf, Germany

Ferredoxins (Fds) are found in all organisms from bacteria, archaea to higher eukaryotes and function to carry electrons between donor and acceptor pairs. Of the nine Fds identified in *Chlamydomonas Reinhardtii*, all feature an iron-sulfur cluster necessary for electron transport, though variations in amino acid sequence may signify unique donor or acceptor specificities. Through molecular simulation, our aim is to rank the different Fds based on their Photosystem I (PSI) binding affinities and binding stability. We use AlphaFold to build Fd models, fill in the structural gaps from the literature and dock these Fds to PSI combining high- and low-resolution information to create bound models for each Fd isoform to the thylakoid-embedded *Chlamydomonas Reinhardtii* PSI. By determining the non-equilibrium work associated with the bound pose, as well as equilibrium simulations, we can identify differences between individual Fd isoforms. Fd1 and Fd2 show smaller deviations from the bound pose during equilibrium simulations, and also had the smallest energetic cost to force the bound pose, suggesting that these isoforms preferentially bind to PSI. By comparison, other Fds have large deviations ( $> 3\text{\AA}$ ) from the bound pose in unrestrained simulation, and thus we predict are less favorable to bind to PSI. These qualitative findings from molecular simulations agree with ongoing in-vitro experiments and aid towards future experimental design. To gain insight into the thermodynamics of PSI-Fd binding, we calculate absolute binding free energies using Binding Free Energy Estimator 2 (BFEE2), an enhanced sampling technique. Overall, we demonstrate the reciprocal relationship between molecular simulations and in-vitro experiments, to map the genetic difference between different Fd isoforms to their phenotypic responses during photosynthesis.

# Physical and molecular sensing of single carboxysomes in an anti-Brownian electrokinetic trap

William B. Carpenter<sup>1</sup>, Abhijit A. Lavania,<sup>2</sup> Julia S. Borden,<sup>3</sup> Luke M. Oltrogge,<sup>3</sup> David F. Savage,<sup>3</sup> W. E. Moerner<sup>2,4</sup>

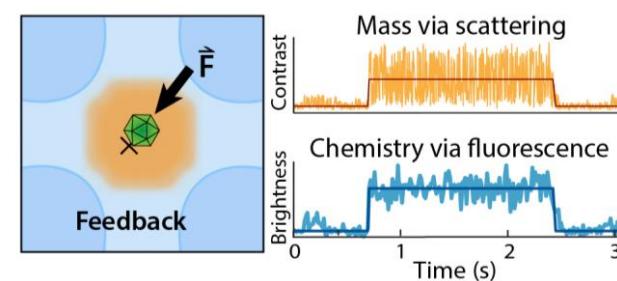
<sup>1</sup> Department of Chemistry and Biochemistry, The Ohio State University, Columbus, 100 W. 18<sup>th</sup> Ave. Newman and Wolfrom Hall Room 2102, OH, 43210, USA. Email: carpenter.1279@osu.edu

<sup>2</sup> Department of Applied Physics, Stanford University, Stanford, CA 94305

<sup>3</sup> Department of Molecular and Cell Biology, University of California Berkeley, Berkeley CA 94720

<sup>4</sup> Department of Chemistry, Stanford University, Stanford, CA 94305

In response to the slow catalytic rate and low specificity of the carbon fixation enzyme rubisco, autotrophic bacteria evolved the carboxysome, a bacterial microcompartment designed to locally concentrate CO<sub>2</sub> and enhance carbon fixation. The carboxysome encloses rubisco and other proteins with a proteinaceous shell permeable to small, metabolically important anions such as bicarbonate and ribulose-1,5-biphosphate. Direct observation of small-molecule transport into the carboxysome has proven challenging, and heterogeneity in carboxysome size and shape is expected to impact how small molecule species enter and exit the carboxysome. To address these challenges, we have used the ISABEL trap to observe molecular kinetics from single carboxysomes. The ISABEL trap applies electrokinetic feedback on a carboxysome's position using interferometric scattering signal in the near infrared. For molecular monitoring, we use fluorescent biosensors to study, for instance, the permeation of small redox molecules into single carboxysomes. We have also used a combination of scattering and fluorescence sensing to estimate the mass and cargo loading of single carboxysomes, as well as exploring the incorporation of shell protein hexamers on the carboxysome surface. These experiments benefit from multiplexed, correlated measurements utilizing both scattering and fluorescence, with many exciting future capabilities within reach.



**Figure 1.** The left panel illustrates that a single carboxysome is held in solution for extended times by applying electrokinetic feedback forces. The right panel illustrates simultaneous long-term measurements on a single carboxysome, highlighting the capability to extract physical parameters and chemical properties from single microcompartments.

# Chloride Influence on Electron Transfer in Photosystem II of *Limnospira maxima*

Leslie Castillo<sup>1</sup>, Stavroula Nicolaou<sup>2</sup>, Nidhi Patel<sup>1</sup> and Colin Gates<sup>1,3</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry, Loyola University Chicago, 1068 w Sheridan Rd.  
60660, [lcastillo7@luc.edu](mailto:lcastillo7@luc.edu)*

<sup>2</sup>*Department of Biology, Loyola University Chicago*

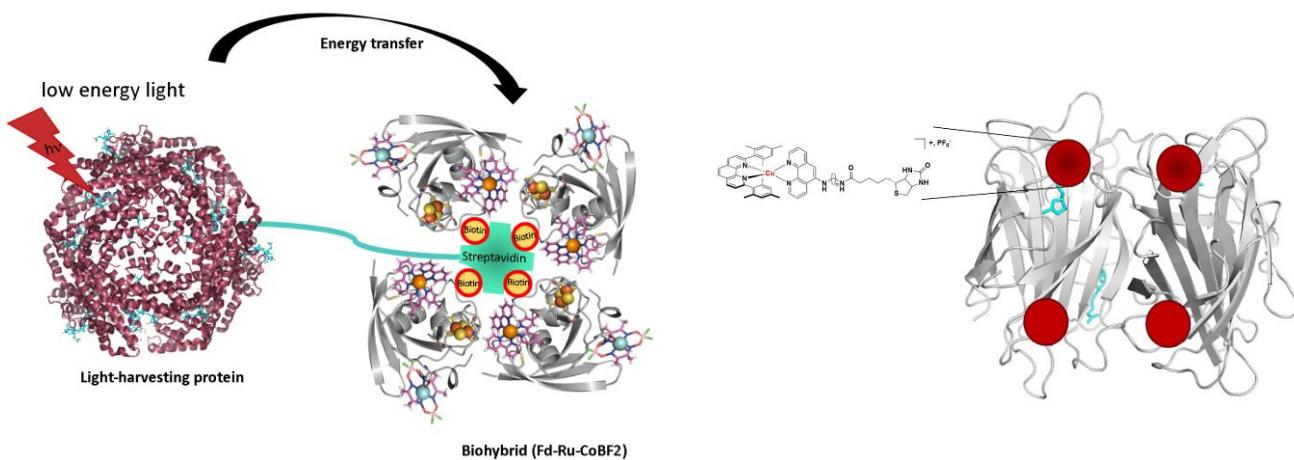
<sup>3</sup>*Department of Bioinformatics, Loyola University Chicago*

The role of dissolved inorganic carbon (DIC) on the donor side of Photosystem II (PSII) is postulated to be associated with proton removal, a process facilitated by chloride ions. Chloride ions are posited to facilitate proton removal from the water-oxidizing complex (WOC) through specific “water channels” to the lumen. Structural studies have confirmed the presence of chloride at consistent sites near the WOC, where they are essential for proper PSII function and proton release. The mechanism by which chloride ions influence PSII function is not fully resolved, though studies using anion substitution, such as replacing chloride with bromide, provide insight into their roles in proton transfer and PSII stability. The hypercarbonate-requiring filamentous cyanobacterium *Limnospira maxima* was used to investigate the broader effects of chloride's role in PSII. 77K spectrofluorometry suggests substitution of chloride results in less exciton transfer from the phycobilisome to PSII due to proportional increase of fluorescence emission from allophycocyanin. Chlorophyll fast repetition rate fluorometry revealed less efficient PSII operation under bromide compared to chloride.  $Q_A^-$ - reoxidation kinetics suggest that substitution results in fewer  $Q_B$  sites remaining in the semiquinone state, with more sites either empty or fully reduced. This effect may be due to bromide's influence and proton removal from the WOC. Overall, electron transfer from  $Q_A^-$  to  $Q_B$  and  $Q_A^-$  to  $Q_B^-$  is faster compared to native *L. maxima*. P700 redox kinetics reveal that under bromide conditions, there is an increase in electron on plastocyanin (PC) and PSI, while under chloride conditions PC experiences less oxidation.

# Biohybrids for studying photosynthetic mechanisms and solar fuel production

Claire W. Chang, Hala Atallah, Tyler N. Haddock, Karen L. Mulfort, Lisa M. Utschig  
9700 S. Cass Ave. Lemont, IL. Chemical Sciences and Engineering Division, Argonne National  
Laboratory. [craig.chang@anl.gov](mailto:craig.chang@anl.gov).

Natural photosynthetic energy research is aimed at resolving fundamental mechanisms of photochemical energy conversion in photosynthetic proteins. These basic studies provide us with insight into how to use nature's optimized photochemistry to drive non-native chemical reactions. Currently, we are designing new bio-inspired systems that capture and convert the sun's energy and store it in the energy-rich bond of hydrogen, a clean, carbon-neutral and renewable energy source. Specifically, we are creating a new class of small protein-based photocatalytic complexes that replicate essential design features of photosynthetic reaction centers (RCs) and enable the spectroscopic discernment of the structure and processes crucial to solar-driven proton reduction. In previous work we developed two systems for photocatalytic hydrogen production from water that directly link both a proton reduction catalyst ( $[\text{Co}(\text{dmgBF}_2)_2]$  or  $[\text{Co}(\text{dmgH})_2\text{pyCl}]$ ) and a photosensitizer molecule  $[\text{Ru}(\text{bpy})_3]^{2+}$  with the small electron transfer proteins, either ferredoxin or flavodoxin. Placement of redox active electron transfer moieties at designed locations in the protein scaffolds enables rapid forward electron transfer and prohibits charge recombination, resulting in strong photocatalytic H<sub>2</sub> generation. Currently, we are expanding these designs using Streptavidin (SA)-biotin interactions to (1) incorporate the light-harvesting protein phycoerythrin from red algae to capitalize on nature's optimized light-capture and energy transfer mechanism and probe photocatalysis using low-energy light (Figure 1A), (2) study protein microenvironmental control of the excited state dynamics of synthetic Cu(I) photosensitizer molecules (Figure 1B).



**Figure 1A.** Biohybrid comprised of a light-harvesting protein and photosensitizer-protein-catalyst complex.

**Figure 1B.** Cu-C<sub>n</sub>-biotin SA hybrid assembly.

# Computational Prediction and Experimental Validation of pKa Shifts in the Q57D Mutant of *Lepidium virginicum*WSCP: Implications for Tailored Chlorophyll Protein Design

Nicolas de Cordoba<sup>1</sup>, Mohamed A. Elrefaiy<sup>2</sup>, Andres S Urbina<sup>1</sup>, Gehan A. Ranepura<sup>3</sup>, M. R. Gunner<sup>3</sup>, Lyudmila Slipchenko<sup>1</sup>, Doran Raccah<sup>2</sup> and Mike Reppert<sup>1</sup>

<sup>1</sup> Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.

<sup>2</sup> Department of Chemistry, The University of Texas at Austin, Austin, TX 78712, USA.

<sup>3</sup> Department of Physics, City College of New York, New York, NY 10031, USA.

Engineering chlorophyll-binding proteins with customized optical properties is essential for advancing photobiology and renewable energy applications. Achieving this goal requires a thorough understanding of how the chlorophyll's local environment within protein complexes influences its spectral characteristics. A previous study demonstrated that a simple electrostatic model accurately predicted mutation-induced frequency shifts for eight out of nine single-point mutants of the Water-Soluble Chlorophyll Protein from *Lepidium virginicum* (LvWSCP). However, the Q57D mutant exhibited a small red shift typical of neutral amino acids instead of the anticipated large blue shift expected from negatively charged residues.

To elucidate the anomalous behavior of the Q57D mutant, we integrated computational predictions with experimental validations. Using Multi-Conformation Continuum Electrostatics (MCCE), we predicted the pKa values of ionizable residues and employed a simple electrostatic model using the Charge Density Coupling (CDC) method to calculate the expected absorption peak shifts. Our computational models predicted that all four Q57D residues in the LvWSCP tetramer have shifted pKa values, leading to their protonation at physiological pH (~7). We experimentally confirmed the protonation of all four Q57D residues at physiological pH through site-directed mutagenesis and spectroscopic analyses, including absorption and fluorescence spectroscopy.

Our findings underscore the critical importance of considering local pH environments and pKa shifts in engineering light harvesting proteins. By demonstrating the effectiveness of a combined computational and experimental approach, our study paves the way for the rational design of chlorophyll-binding proteins with tailored optical properties.

# Decoding the Interplay Between FMO and PscB: Unveiling Their Role in Excitation Energy Transfer in *Chlorobaculum tepidum*, a Green Sulfur Bacterium

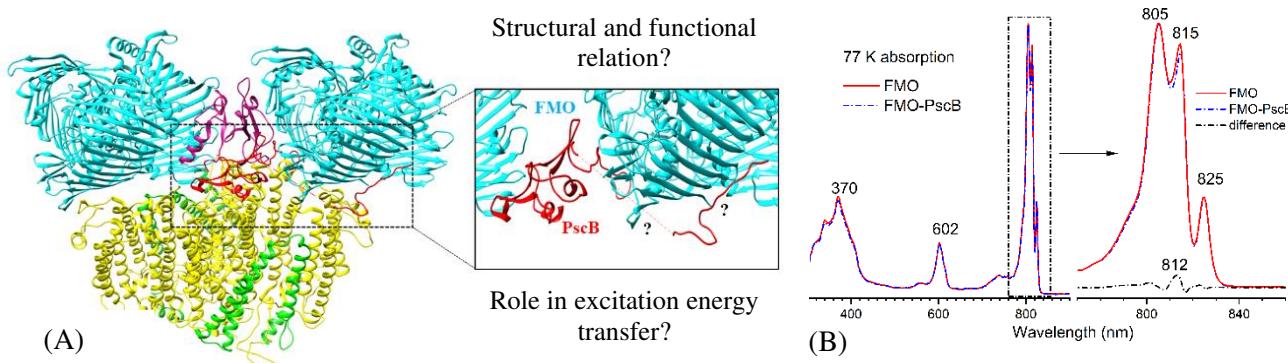
Anica Dadwal<sup>1</sup>, Dariusz M. Niedzwiedzki<sup>2,3</sup>, Ryan Puskar<sup>4</sup>, Po-Lin Chiu<sup>4</sup>, Haijun Liu<sup>1</sup>

<sup>1</sup>Department of Biology, Saint Louis University, St. Louis, MO 63103, USA,  
[anica.dadwal@slu.edu](mailto:anica.dadwal@slu.edu)

<sup>2</sup>Center for Solar Energy and Energy Storage and <sup>3</sup>Department of Energy Environmental and Chemical Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA

<sup>4</sup>School of Molecular Sciences, Arizona State University, Tempe, AZ 85281, USA

*Chlorobaculum tepidum*, a Green Sulfur Bacteria (GSB), has three major pigment protein complexes involved in early events of light-harvesting and photochemical conversion: Chlorosome, Fenna Matthews Olson (FMO) and Reaction Centre (RC). Chlorosome captures the light energy which is transferred to RC through FMO trimer. On the other hand, PscB, a pigment-less iron-sulfur cluster protein and subunit of the reaction center (RC), terminally binds to FMO (Figure 1A) and contains intrinsically disordered regions, the structure and function of which remain unclear, leaving its precise role yet to be elucidated. This study aims to elucidate FMO-PscB interactions using biochemical and optical molecular spectroscopic methods as steady-state absorption (Figure 1B) and fs-time-resolved transient absorption. Our preliminary studies suggest a potential interaction between PscB and FMO, which could offer new insights into the photosynthetic pigment protein complex assembly in the GSB.



**Figure 1:** (A) Cryo-EM structure of the whole photosynthetic complex from the green sulfur bacteria (cyan-FMO, yellow/green-RC) (PDB: 7Z6Q, [1]) reveals unresolved N-terminal domain of PscB and highlights missing information. (B) 77 K absorption spectra of FMO and FMO-PscB complexes with highlight of BChl *a* excitonic bands where some spectral differences are seen.

## Reference

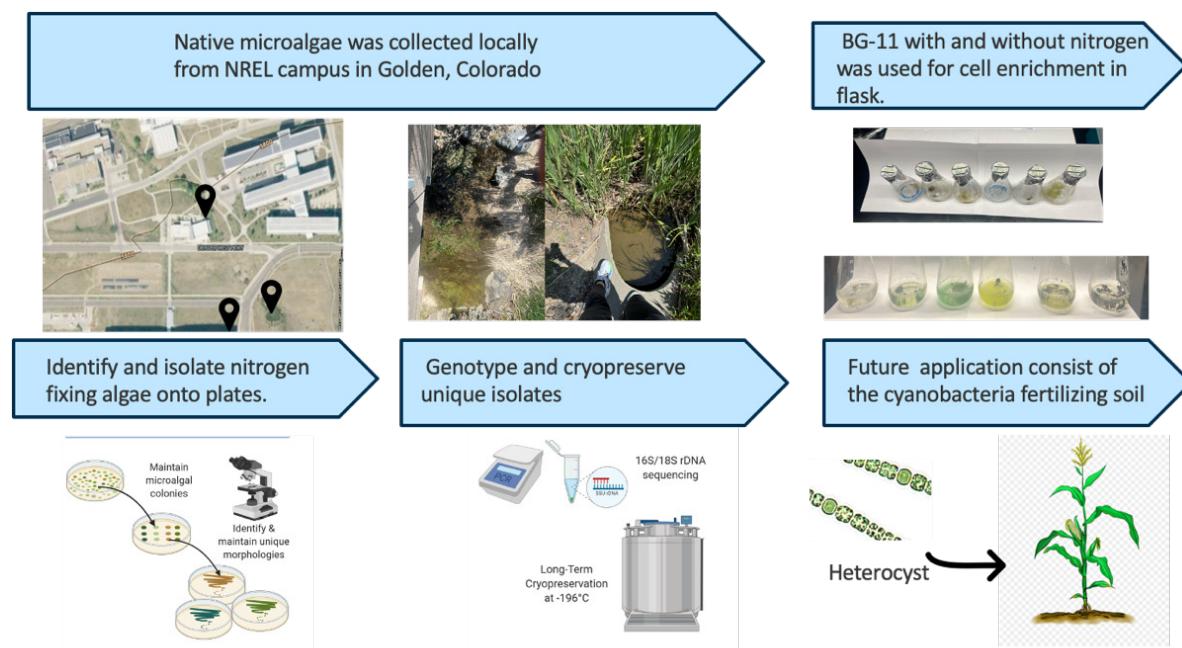
- [1] H. Xie, A. Lyratzakis, R. Khera, M. Koutantou, S. Welsch, H. Michel, G. Tsiotis, Cryo-Em Structure of the Whole Photosynthetic Reaction Center Apparatus from the Green Sulfur Bacterium *Chlorobaculum tepidum*, Proceedings of the National Academy of Sciences, 120 (2023) e2216734120

# Bioprospecting of Microalgae in Colorado for N-based Fertilizer Production

Lydia DavieBalogun<sup>1,2</sup>, Eric Schaedig<sup>2</sup>, Jacob Sebesta<sup>2</sup>, Harvey J.M. Hou<sup>1,\*</sup>, and Jianping Yu<sup>2,\*</sup>

<sup>1</sup>Department of Physical and Forensic Sciences, Alabama State University, Montgomery, Alabama 36104; <sup>2</sup>Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401. \*Email: [jianping.yu@nrel.gov](mailto:jianping.yu@nrel.gov), [hhou@alasu.edu](mailto:hhou@alasu.edu)

Alabama State University (ASU), Washington University in St Louis and National Renewable Energy Laboratory (NREL) have joined forces to develop nitrogen-fixing cyanobacteria as a source of fertilizer. Recently NREL used bioprospecting to discover natural strains of microalgae with desired traits (Schaedig et al 2024). The hypothesis of this work is that there is a diversity of cyanobacteria on the NREL campus and that by using nitrogen-deficient media we will be able to isolate nitrogen-fixing cyanobacteria. In this work, we isolated nitrogen-fixing cyanobacteria from water and soil samples collected on NREL campus. The growth of nitrogen-fixing cyanobacteria as well as the isolation and purification process of the algae were explored (Figure 1). DNA sequencing was used to identify the isolated strains. Lastly the future plan for this project, including the measurements of the N-fixing and chemical analysis of overflow metabolism in the strains at Alabama State University, will be discussed. This work was supported by the U.S. Department of Energy, Office of Science Energy Earthshot Initiative, as part of the Science Foundations for Energy Earthshot (DOE SFEE) Projects under grant number DE-SC0024702.



**Figure 1.** Scheme for isolating the N-fixing microalgae and cyanobacteria in Golden, Colorado.

Eric Schaedig, Michael Cantrell, Chris Urban, Jacob Sebesta, Katherine J. Chou, and Jianping Yu\* (2024) Isolation & bioprospecting of wild microalgae for biotechnology applications, *Front. Sci. Technol. Eng. Math.*, 8: 17-18

# **Elucidating the role of PGR5 and NDH in modulating cyclic electron flow under high temperature stress in *Arabidopsis thaliana***

**Madhushree Dutta<sup>1,2,3</sup>, Steven Mckenzie<sup>3</sup>, Gaurav Zinta<sup>1,2</sup>, and Sujith Pujithaveetil<sup>3</sup>**

<sup>1</sup>*Biotechnology Division, CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh 176061, India. Email: dutta86@purdue.edu*

<sup>2</sup>*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

<sup>3</sup>*Department of Biochemistry and Center for Plant Biology, West Lafayette, IN 47907, USA*

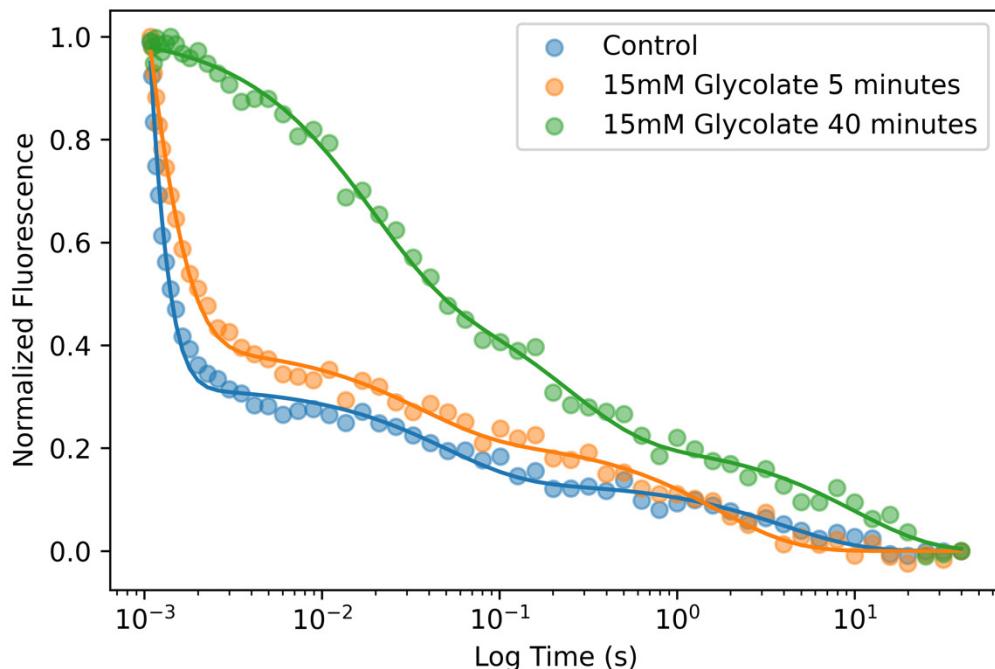
High temperature is a serious threat to sustainable crop production as it damages the photosynthetic machinery of plants. A cyclic electron transport (CET) around photosystem I (PSI) minimizes PSI photodamage under various abiotic stress conditions. In *Arabidopsis*, CET pathways mediated by Proton Gradient Regulation 5 (*pgr5*) and type I NAD(P)H dehydrogenase (NDH) have recently been shown to be important for combating thermal stress by protecting PSI. However, the underlying molecular mechanisms remain poorly characterized due to technical constraints in measuring CET in C3 plants. Here, we evaluate the thermal stress responses of *Arabidopsis thaliana* (*Col-0*), *pgr5*, and *ndh* mutants under lab growth conditions (22°C day/night) and high temperature stress (24 hours 35°C day/night). We will measure chlorophyll fluorescence of PSI and PSII, together with histochemical staining of reactive oxygen species (ROS) in control and heat-treated samples. We will also perform biochemical assays of stress markers and blue native gel analysis to study how cyt *b6f*, NDH, and PSI supercomplexes are altered by heat stress. Overall, our studies aim to delineate the role of CET pathways under heat stress and to understand how PSI-NDH-cyt *b6f* supercomplex assemblages contribute to photoprotection of PSI.

# Glycolate Reduces Photosystem II Electron Transport

Nicholas Ferrari<sup>1</sup>, Brandon Russell<sup>1</sup> and David J. Vinyard<sup>1</sup>

<sup>1</sup>Louisiana State University. 202 Life Science Building, Baton Rouge, LA 70803.  
nferr11@lsu.edu.

Glycolate is a major metabolite produced in oxygenic photosynthetic organisms lacking a carbon-concentrating mechanism. The accumulation of glycolate prevents growth under atmospheric carbon dioxide concentrations. Metabolic pathways such as photorespiration have evolved to transport and convert glycolate into 3-phosphoglyceric acid. Glycolate has previously been shown *in vitro* to compete with bicarbonate as a ligand for the non-heme iron (NHI) of photosystem II. Here we show evidence *in vivo* that glycolate binds at the NHI and disrupts electron transport to Q<sub>A</sub>.



**Fig. 1.** Fluorescent relaxation of PSII after a single turnover flash in *C. reinhardtii* grown at 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in TAP media. The cultures were treated with 15mM glycolate for 5 and 40 minutes before measurement. The data was modeled with a triexponential decay function.

# **Chromatic Adaptation of the Red Alga *Galdieria yellowstonensis***

Vidya Gundlapalli<sup>1</sup>, Evan Smolen<sup>1</sup> and Colin Gates<sup>1</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry. 1068 West Sheridan Rd, Chicago, IL 60660. Loyola University Chicago [vgundlapalli@luc.edu](mailto:vgundlapalli@luc.edu)*

*Galdieria yellowstonensis* is an extremophilic red alga which has a broad range of pigments of both cyanobacterial (phycobilins and phycobiliproteins) and eukaryotic (chlorophyll-based) origin. This alga is thus capable of adjusting its light-harvesting apparatus to the wavelengths available through chromatic adaptation and modulation of pigment usage and expression. Chromatic adaptation was induced in *G. yellowstonensis* to determine differences in exciton and electron usage driving the light reactions under specific light regimes. *G. yellowstonensis* cultures were grown under white, red, and blue light at 30°C and investigated for their pigment expression and distribution and electron transfer kinetics. Under blue light, an electrochromic shift three times stronger than in either of the other cultures was observed, as well as sixfold stronger oxidation at Photosystem I than under red light. While all three cultures showed similar redox poise of plastocyanin, cytochromes b and f of the b<sub>6</sub>f complex were about half as oxidized in response to light when adapted to either red or blue light. Red light prompted a threefold decrease in active Q<sub>B</sub> sites of photosystem II but despite this limitation, electron flux to cytochrome b<sub>6</sub>f and photosystem I remained high and appeared to be primarily linear. Under blue light, *G. yellowstonensis* appears to undergo strong cyclic electron flow around photosystem I which dominates the activity of the electron transport chain and generates a strong trans-thylakoid proton gradient. Despite this, the remaining, diminished, photosystem II population remains highly efficient in electron removal.

# Uncapping Carbon Capture – Elucidating and Engineering the “Ceiling” of Photosynthesis

Maxwell J. Harman<sup>1,2</sup>, Deserah D. Strand<sup>2</sup> and Berkley J. Walker<sup>2,3</sup>

<sup>1</sup>*Genetics and Genome Sciences & Molecular Plant Science Programs, harmanm4@msu.edu.*

<sup>2</sup>*MSU-DOE Plant Research Laboratory*

<sup>3</sup>*Department of Plant Biology*

*Michigan State University. 612 Wilson Road East Lansing, MI 48824*

Photosynthetic CO<sub>2</sub> assimilation sits at the nexus of two of the 21st century's greatest challenges: feeding a growing population with shrinking resources and combating greenhouse gas-driven climate change. While the limiting factors governing CO<sub>2</sub> uptake are well-characterized at low-to-ambient CO<sub>2</sub> concentrations, higher carbon atmospheres induce sink limitations – termed triose phosphate utilization limitation – that remain much less understood. This limitation represents the upper “ceiling” of photosynthesis and is therefore a necessary and productive target for increasing carbon capture and crop yields, especially as atmospheric CO<sub>2</sub> levels continue to increase. To better understand and engineer this limitation, we employ a novel combination of reverse genetics, synthetic biology, and leaf-level physiology to unravel the mechanisms governing triose phosphate utilization and plant responses to high-flux carbon metabolism. A novel high-throughput chlorophyll fluorescence imaging approach screened over 1000 EMS-mutagenized *Arabidopsis thaliana* lines and identified several promising mutant lines with aberrative TPU-associated phenotypes. Transient transformation was also paired with leaf-level gas exchange to test metabolic engineering hypotheses in a rapid, modular fashion. As CO<sub>2</sub> levels continue to rise, understanding and engineering triose phosphate utilization will be critical for contextualizing the impacts of climate change and informing rational-design crop improvement strategies.

# **Characterization of Diatom Chloroplast Response Regulator 1 (CRR1)**

Laura N. Hiotaky and Sujith Puthiyaveetil

*Department of Biochemistry and Center for Plant Biology, Purdue University, West Lafayette IN 47907, lhiotaky@purdue.edu*

*Phaeodactylum tricornutum* is a unicellular marine alga belonging to a family of photosynthetic eukaryotes called diatoms. Diatoms inhabit marine environments and are subjected to many environmental fluctuations, including the quantity of light. Nevertheless, diatoms display high photosynthetic productivity. Thus, it is crucial to understand the biochemical mechanisms of this photosynthetic acclimation, as diatoms are a major target for biofuel production and agricultural engineering. Diatoms trace their evolutionary origin to red algae through a secondary endosymbiotic event. Diatoms thus carry protist, red algal, and prokaryotic genetic material. One such conserved prokaryotic genetic material is the two-component system (TCS). The TCS consists of a sensor histidine kinase and a response regulator. The sensor histidine kinase senses an environmental stimulus and autophosphorylates on a conserved histidine residue. The phosphoryl group is then transferred from the kinase to a response regulator, eliciting a gene regulatory response. In diatoms, one of the sensor histidine kinases takes the form of chloroplast sensor kinase (CSK). There are two cognate response regulators of CSK in the form of chloroplast response regulator 1 (CRR1) and chloroplast response regulator 2 (CRR2) that are believed to have arisen from a gene duplication event. We hypothesize that CRR1 and CRR2 regulate plastid genes for heat shock proteins, and that CRR1 and CRR2 are activated during abrupt high temperature and light fluctuations. Here we show correlation between the expression of CRR1 and the expression of the heat shock plastid gene *dnaK* in *Phaeodactylum*. Also, we show preliminary data in the optimization of an anti-CRR1 antibody, which will be used in identification of the environmental conditions that activate CRR1 and gene targets of CRR1.

# Characterizing an Unexpected Peak in the UV-Vis Spectrum of T52K

Jacob H. Wat, Savannah R. Holt<sup>1</sup>, Michael E. Reppert

<sup>1</sup>Purdue University, 560 Oval Drive, West Lafayette, IN 47907. porte228@purdue.edu

The water-soluble chlorophyll protein (WSCP) from *Lepidium virginicum* is an ideal model system to study chlorophyll-protein interactions because it binds only four pigments in a symmetric "dimer of dimers" geometry. This makes it easier to isolate and study individual protein-pigment interactions through single-point mutations. Here we report on an unexpectedly large mutation-induced frequency shift — from 670 nm to 645 nm — in the T52K mutant of WSCP, discovered through small-volume screening of a large number of single-point mutants. Unlike the WT protein, the T52K mutant exhibits different absorption peak frequencies depending on reconstitution conditions in a somewhat unpredictable fashion. This poster focuses on identifying the cause of this large frequency shift and reproducibly inducing it.

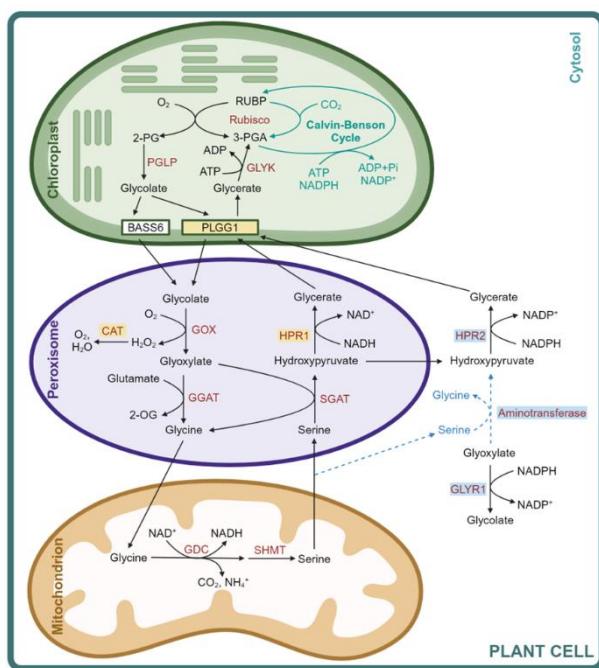
# A photorespiratory glyoxylate shunt in the cytosol supports photosynthesis and plant growth under high light conditions in *Arabidopsis*

Xiaotong Jiang<sup>1,2</sup>, Amanda M. Koenig<sup>1</sup>, Berkley J. Walker<sup>1,2</sup> & Jianping Hu<sup>1,2</sup>

<sup>1</sup>*Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI, USA.*

<sup>2</sup>*Department of Plant Biology, Michigan State University, East Lansing, MI, USA.*

Photorespiration is a central aspect of plant metabolism that is tightly connected to photosynthesis, and functions in part to support photosynthetic performance, especially under stress conditions such as high light. However, our understanding of the mechanisms underlying the role and regulation of photorespiration in plant response to high light is limited. To identify modulators of photorespiration under high light, we isolated genetic suppressors of the photorespiratory mutant *hpr1*, which is defective in the peroxisomal hydroxypyruvate reductase 1. A suppressor that partially rescued *hpr1* under high light was mapped to *GLYR1*, which encodes the cytosolic glyoxylate reductase 1 enzyme that converts glyoxylate to glycolate. Independent *GLYR1* loss-of-function mutants also partially rescued *hpr1* and another photorespiratory mutant, *catalase 2*. Our genetic, transcriptomic and metabolic profiling analyses together suggested a novel connection between cytosolic glyoxylate and a non-canonical photorespiratory route mediated by the cytosolic HPR2 enzyme, which we named the photorespiratory glyoxylate shunt. This shunt is especially critical under high light intensities when a high rate of photorespiratory flux is required and in the absence of a properly functional major photorespiratory pathway. Our findings support the metabolic flexibility of photorespiration and may help future efforts to improve crop performance under stress conditions.



**Figure. The established photorespiration pathway and the cytosolic glyoxylate shunt proposed in this study.** Photorespiration involves a series of reactions in the chloroplast, peroxisome, mitochondrion, and cytosol. We propose that defective *GLYR1* allows the accumulated free glyoxylate in the cytosol to react with serine, catalyzed by an unknown aminotransferase. The hydroxypyruvate produced can be further converted by HPR2 to glycerate, which re-enters the chloroplast.

# Characterization of Structural Dynamics of Thylakoid Membrane Transporter Protein Hcf106

Nimarsha Kamaradiwela Arachchige<sup>1</sup> and Carole Dabney-Smith<sup>2</sup>

*<sup>1</sup>Department of Chemistry and Biochemistry, Miami University, 651 E High St, Oxford, OH.*

*kamarans@miamioh.edu.*

Most chloroplast proteins are encoded by nuclear genes and synthesized in the cytosol as higher molecular weight precursors possessing N-terminal transit peptides which direct them to cross membranes through specific protein translocation systems to reach their final destinations. The chloroplast twin-arginine transport (cpTAT) complex consisting of three transmembrane proteins, Tha4 (cpTatA), Hcf106 (cpTatB), and cpTatC, is one such protein translocation system that transports fully folded proteins across ion-tight membranes. Hcf106, a single transmembrane protein, plays an important role in the process. However, it has been challenging to unravel structural and dynamic information for Hcf106 using conventional biophysical techniques. EPR spectroscopy is a rapidly growing technique to gain pertinent structural and dynamic properties of biological systems. Here, we use EPR spectroscopy to probe the biophysical properties of Hcf106 to gain insight into its role in transport.

Five single Cys-Hcf106 variants from the N-terminal transmembrane domain, amphipathic  $\alpha$ -helix, and loosely structured C-terminus were generated using site-directed mutagenesis PCR. Purified Hcf106 variants were labeled with methyl methanesulfonothioate (MTSL) spin-label. The labeled Hcf106 was incorporated into 0.5% DPC detergent micelles, phosphatidylcholine (PC) liposomes, and thylakoid mimetic (ThML) liposomes. CW-EPR studies were conducted to report on the environment of the spin label. The CW-EPR spectral line shape analysis data suggested restricted motion of Hcf106 in lipid bilayer vesicles compared to detergent micelles. Moreover, spin label mobility data suggests that F5C, K37C, and A128C are solvent-exposed residues and V14C and L60C are transmembrane residues. In the future CW-EPR power saturation will be used to determine the relative membrane depth of regions of Hcf106 in proteoliposomes by calculating the depth parameter. This will allow us to identify the amino acid residues of Hcf106 that delineate the membrane boundaries as well as gain a more accurate understanding of Hcf106 conformation in the membrane.

# Investigating Photosystem II Cyclic Electron Flow in *Chlorella*

Arivu Kapoor<sup>1</sup>, Grant Steiner<sup>1</sup>, and Dr. Colin Gates<sup>1,2</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry and <sup>2</sup>Department of Bioinformatics, Loyola University Chicago, 1068 W Sheridan Rd, Chicago, IL 60660.* akapoor3@luc.edu.

The desert-native, extreme light (2,000  $\mu$ Ein)-adapted green alga *Chlorella ohadii* expresses exceptional photosystem II photoprotection, due in part to high levels of PSII-cyclic electron flow. This facilitates *C. ohadii* possessing the fastest recorded doubling time of any known phototroph, 1.4 hours, in high light and 2% CO<sub>2</sub>. When *Chlorella* NIES 642, a temperate, low light (20  $\mu$ Ein)-adapted alga is subjected to the same growth conditions as *C. ohadii*, we have observed various changes to the PETC indicative of high PSII-CEF and recorded a minimum doubling time of 1.6 hours. In extreme light grown NIES 642, PSII reaction centers do not fully saturate under conditions that can induce single turnovers in organisms as recalcitrant as *C. ohadii*. 77K fluorometry and chlorophyll extraction suggest this may result from extensive minimization of chlorophyll pigments; in extreme light conditions the *C. ohadii* to *C. NIES 642* chlorophyll *a* ratio is  $1.82 \pm 0.18$ . Once acclimated, NIES 642 also shows various characteristics of high PSII-CEF on the acceptor side of PSII, including near-constant utilization of the plastoquinone pool ( $4.2 \pm 2.5\%$  performing no electron transfer) and a majority of centers with complexed to oxidized Q<sub>B</sub> ( $59.7 \pm 2.9\%$ ), opposed to semiquinone ( $35.1 \pm 2.6\%$ ). P700 utilization monitored via  $\Delta\lambda_{810\text{nm}}$  absorbance illustrates that extreme light conditions greatly diminish charge separation at PSI, and *C. ohadii* expresses more PSI, comparatively. Electrochromic shift measurements of the thylakoid membrane show a diminished trans-thylakoid proton gradient in the high PSII-CEF systems, strongly suggesting a large role of PSII-CEF in optimizing ATP production.

# Localization of Deg Proteases in *Chlamydomonas reinhardtii*

**Nidhi Kulkarni<sup>1</sup>, Lexi Cheramie<sup>1</sup> and David Vinyard<sup>1</sup>**

<sup>1</sup>*Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803,  
USA*

Photosystem II (PSII) is frequently subjected to damage by reactive oxygen species. In the PSII repair cycle, the D1 subunit is selectively removed and degraded using the FtsH protease system. In plants, Deg proteases facilitate this process by clipping D1 loops that extend outside the thylakoid membrane. In algae, D1 fragments accumulate in FtsH mutants of *Chlamydomonas reinhardtii*. However, it is unknown if Deg proteases also play a role in algal PSII repair. The *Chlamydomonas* genome encodes 14 predicted Deg proteases and redundancy among this group poses a major challenge towards elucidating the exact roles and functions. We reasoned that any Deg proteases that localize to the chloroplast may play a role in Photosystem II (PSII) repair. We fused a yellow fluorescent protein variant to the C-termini of Deg open reading frames and imaged the resulting strains using confocal microscopy. We have localized Deg1A, Deg2, and Deg5. Interestingly, the localization patterns change as a function of light/dark phase of the light cycle. After completing our localization study, we will use CRISPR-Cas9 method to knock out chloroplast-localized Deg proteases to study their role (if any) on the PSII repair cycle.

# Designing a stable, functional consortium between a phototroph and a heterotroph

Matthew Lima<sup>1</sup>, Alexandria Wilcox<sup>1</sup>, Gaurav Bingi<sup>2</sup>, Anindita Bandyopadhyay<sup>1</sup>, Yinjie J. Tang<sup>1</sup>, Pramod P. Wangikar<sup>2</sup>, Maitrayee Bhattacharyya-Pakrasi<sup>1</sup>, and Himadri B. Pakrasi<sup>1</sup>

<sup>1</sup>Washington University in St. Louis, 1 Brookings Drive, St. Louis, MO 63130, USA.  
mattlima@wustl.edu

<sup>2</sup>Indian Institute of Technology Bombay, Powai, Mumbai 400 076, India.

Natural biological systems often exist in a consortium, containing multiple organisms that coexist and interact with each other<sup>1</sup>. These systems are complex and interesting, though the mechanisms of interaction between members and the influence(s) the partners have on each other are poorly understood. In this context, the combination of a heterotroph with a phototroph within a coculture is particularly interesting. Although most heterotrophs are susceptible to oxidative stress generated by the phototroph in a coculture environment, previous work has shown phototroph-heterotroph cocultures to be functional, and resistant to perturbations and stable over time. Synthetic consortia have been shown to improve growth of cyanobacteria as compared to pure culture<sup>2</sup>. *Vibrio natriegens* is an exceptional heterotrophic bacterium, known for its rapid growth, versatility in genetic engineering, and resilience to various environmental conditions. In their natural aquatic habitats, *Vibrio* species often coexist with phototrophic organisms, particularly cyanobacteria<sup>3</sup>. *Cyanothece* sp. ATCC 51142, a unicellular marine cyanobacterium, thrives under carbon and nitrogen-fixing conditions, demonstrating strong growth in these environments. Both these organisms are well studied at the systems level and are genetically amenable. We aim to establish a stable, sustainable and functional coculture system involving *V. natriegens* and *Cyanothece* 51142, with the goal of understanding the mechanism of interaction between a photoautotroph and a heterotroph.

In our initial coculture experiments using a transgenic *V. natriegens* strain expressing a fluorescent reporter and *Cyanothece* 51142, we observed that both strains could coexist while promoting mutual growth. We tested various parameters, such as salt concentrations and inoculum ratios, to identify the optimal growth conditions necessary for coculture. We hypothesize that, unlike many heterotrophs, *V. natriegens* is tolerant to oxidative stress, and the elevated oxygen levels produced by the cyanobacterium in the coculture may enhance its growth and metabolism. RNA-seq analysis, <sup>13</sup>C metabolic analysis, and coculture modeling are currently underway to investigate differential gene expression and metabolic regulation in both organisms, including oxidative stress responses under coculture conditions compared to pure cultures. The insights gained will be applied to manipulate interactions between the two organisms, enabling the use of photosynthetically fixed carbon and nitrogen as feedstocks for nitrogen-rich product formation by the heterotroph.

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# CSK as a regulator of chloroplast gene expression under changing light intensities

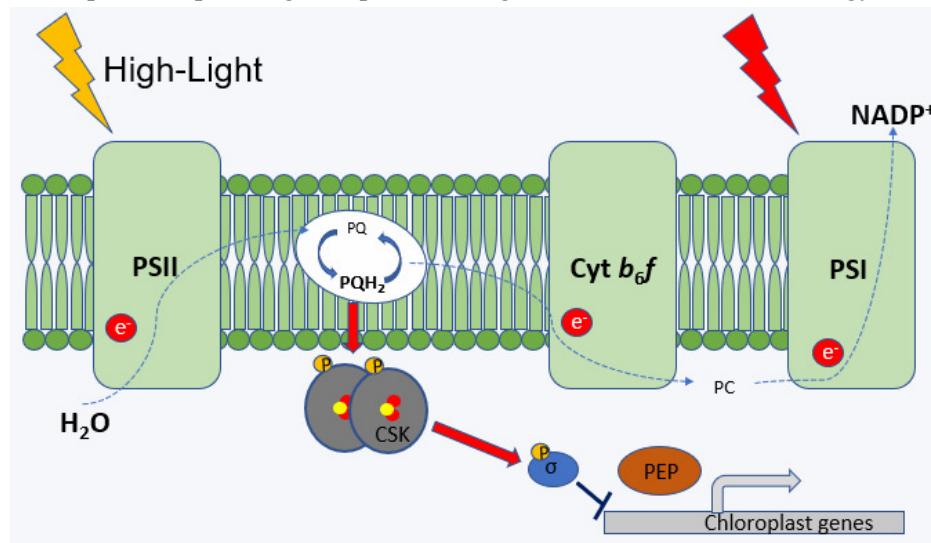
Matthew Martin<sup>1</sup>, Emily Fannin<sup>1</sup>, and Sujith Puthiyaveetil<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Center for Plant Biology, Purdue University, West Lafayette IN 47907

email: mart2361@purdue.edu

Two-component signaling systems involving a sensor histidine kinase and its cognate response regulator are wide spread in most prokaryotes, including cyanobacteria. In this regulatory system, an environmental stimulus provokes a trans-autophosphorylation event in which one sensor kinase monomer phosphorylates a conserved histidine residue in the second sensor kinase monomer. This phosphorylated sensor kinase then transfers the phosphoryl group to its cognate response regulator, altering the conformation of its effector domain, thus provoking a physiological response. A single modified version of this two-component system has been reported in the chloroplasts of *Arabidopsis thaliana*. In this system, Chloroplast Sensor Kinase (CSK) no longer contains the conserved histidine residue and does not appear to have a true cognate response regulator partner. CSK has been shown to regulate chloroplast gene expression by reporting on the redox state of the plastoquinone pool to chloroplast gene expression machinery, utilizing an iron-sulfur cluster within its sensor domain. Preliminary fluorescence data taken with a MultispeQ v1 show CSK mutants have altered photosynthetic efficiency compared to wildtype. Additionally, a CSK knockout mutant appears to have increased transcript abundance of certain chloroplast genes whose transcription is initiated by sigma factor (SIG5) specifically under high light. This effect appears to be most notable later in the day, suggesting that CSK is necessary for proper down regulation of transcription to maintain balance of cellular resources in preparation for darkness. Establishing a role for CSK in stress response and energy homeostasis represents a significant advancement in understanding the function of this elusive sensor kinase.

**Figure 1.** Hypothesis: When the plastoquinone pool (PQ) is reduced by high-light or other abiotic stresses, CSK is activated. Phosphorylation of chloroplast sigma factor(s) by activated CSK decreases initiation of transcription at specific gene operons to regulate the use of cellular energy and resources.



# Diversity of acclimatory strategies to Polar Night across Antarctic phytoplankton

Conall McGinness<sup>1</sup>, Devon Popson<sup>1</sup>, Zev Cariani<sup>1</sup>, Cristina Takacs-Vesbach<sup>2</sup>, Peter Doran<sup>3</sup>, John Priscu<sup>4</sup>, and Rachael Morgan-Kiss<sup>1</sup>

<sup>1</sup>*Miami University. 700 E. High St. Oxford, OH 45056. mcginncp@miamioh.edu, popsond@miamioh.edu, morganr2@miamioh.edu*

<sup>2</sup>*University of New Mexico. 219 Yale Blvd NE, Albuquerque, NM 87131. cvesbach@unm.edu.*

<sup>3</sup>*Louisiana State University. E235 Howe Russell Kniffen, Baton Rouge, LA 70803. pdoran@lsu.edu.*

<sup>4</sup>*Montana State University. 334 Leon Johnson Hall, Bozeman, MT 59717. jpriscu@montana.edu.*

Phytoplankton residing in high latitude environments experience extended periods of complete darkness during the winter, known as the ‘polar night’. Historically, the polar night represents a logistical challenge for field science and has also been considered a quiescent period of minimal biological activity. However, recent advancements in autonomous sensors/sampling have revealed new insight into seasonal dynamics of microbial communities. Photoautotrophic and mixotrophic algal species, which represent the primary producers of most polar aquatic habitats, are expected to be particularly sensitive to the lack of light. While past research has proposed a few physiological mechanisms underlying winter survival, there remains a knowledge gap connecting findings based on lab-controlled experiments and responses of native algal communities. Here we combined field- and lab-based studies to evaluate how several key phytoplankton species acclimate to polar night within the permanently ice-covered Lake Bonney (McMurdo Dry Valleys, Antarctica). The phytoplankton communities in this lake are composed predominantly of chlorophytes (*Chlamydomonas* spp.), haptophytes (*Isochrysis* sp.), and cryptophytes (*Geminigera cryophila*). In the field, seasonal dynamics of these three algal groups were monitored by year-round autonomous profiling using a spectral Chlorophyll-a fluorometer. Additionally, four Lake Bonney isolates, *C. priscui*, *C. sp. ICE-MDV* (Chlorophytes), *Isochrysis* sp. MDV, and *G. cryophila* were subjected to mimicked polar night under lab-controlled conditions in a series of experiments to compare acclimation strategies among photoautotrophic vs. mixotrophic algae. In native Lake Bonney communities, green algae abundance declined during the polar night, whereas mixotrophic haptophytes and cryptophytes increase in abundance during early winter. In the lab, chlorophytes species decreased in culture density, functionally downregulated photosynthesis, but retained their photosystems. Conversely, the haptophyte, *Isochrysis* sp. MDV and cryptophyte *G. cryophila* exhibited minor reductions in cell density and photosynthetic capacity during mimicked polar night, and showed an increase in B-glucosaminidase, a key enzyme involved in complex carbon breakdown. A higher resolution study conducted in continuous cultures of *C. priscui* revealed that the alga increased photosynthetic activity during the transition from summer to winter. Photosynthetic activity rapidly recovered during the onset of summer in continuous cultures. On the other hand, recovery of growth lagged behind photosynthetic recovery by 10 days. Collectively, the results of this study will provide a more comprehensive understanding of how Antarctic phytoplankton communities prepare for and recover from several months of darkness.

# Diversity of acclimatory strategies to Polar Night across Antarctic phytoplankton

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<sup>1</sup>*Miami University. 700 E. High St. Oxford, OH 45056. mcginncp@miamioh.edu, popsond@miamioh.edu, morganr2@miamioh.edu*

<sup>2</sup>*University of New Mexico. 219 Yale Blvd NE, Albuquerque, NM 87131. cvesbach@unm.edu.*

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# **Phosphorylation and oxidative damage mediates disassembly of Photosystem II in *Arabidopsis thaliana***

Steven McKenzie<sup>1</sup> and Sujith Puthiyaveetil<sup>1</sup>

<sup>1</sup>*Department of Biochemistry, Purdue University, 175 South University Street,  
smckenz@purdue.edu*

The photosynthetic electron transport chain of oxygenic photoautotrophs is composed of several large hetero-oligomeric pigment protein complexes. Photosystem II (PSII) utilizes radiant light energy to oxidize water into dioxygen and protons, reducing the mobile electron carrier plastoquinone and contributing to the generation of a transmembrane  $\Delta\text{pH}$  gradient. In doing so, the electron transport chain creates usable forms of chemical energy necessary for carbon fixation. Although PSII is necessary for functional electron transport, PSII also forms various reactive oxygen species (ROS) as a byproduct of its reactions. The direct result of these ROS manifests as irreversible protein damage to subunits of PSII, particularly the D1 subunit, leading to a loss of its catalytic activity. To ensure functional electron transport, the damaged PSII supercomplex undergoes a rapid disassembly repair cycle that involves the degradation of the damaged D1 subunit, followed by its *de novo* synthesis and reassembly to form the PSII holocomplex. Disassembly of the PSII supercomplex, specifically removal of the CP43 subunit, ensures access of the D1 subunit by the integral membrane protease FTSH. Although many aspects of the PSII repair cycle have been investigated, the mechanisms governing disassembly of PSII are not well understood. More specifically, it is unclear if PSII disassembly is controlled through a strictly controlled mechanism. Here, we utilize BN-PAGE to show the disassembly of PSII through removal of CP43 is induced through the addition of exogenous ROS. Additionally, we show through the use of *Arabidopsis* phosphorylation mutants that phosphorylation of PSII is important for the disassembly of large dimeric PSII supercomplexes. Together, these results demonstrate that both controlled and damage-mediated disassembly mediate the PSII repair cycle.

# The Role of Bicarbonate in *Limnospira maxima* Electron Transport Chain

Stavroula Nicolaou<sup>1</sup>, Leslie Castillo<sup>1</sup> and Colin Gates<sup>1,2</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, Loyola University Chicago, 1068 W. Sheridan Rd., Chicago, IL 60660. [snicolaou@luc.edu](mailto:snicolaou@luc.edu)

<sup>2</sup>Department of Bioinformatics, Loyola University Chicago, 1032 W. Sheridan Rd., Chicago, IL 60660. [cgates4@luc.edu](mailto:cgates4@luc.edu)

The cyanobacterium *Limnospira maxima* is unique in its ability to thrive under high concentrations of dissolved inorganic carbon (bicarbonate) because it uses bicarbonate as its major inorganic carbon source. Bicarbonate plays a major regulatory role in PSII, with the best-characterized site coordinated to the non-heme iron, which sits between acceptor plastoquinones Q<sub>A</sub> and Q<sub>B</sub>. To investigate the regulatory roles of bicarbonate in PSII, bicarbonate was depleted from a functional site of unknown location with sodium formate. Connectivity of antenna pigments to photosystems was observed via 77K spectrofluorometry in response to bicarbonate depletion, which showed loss of chlorophyll connectivity in PSII and dissociation of the phycobilisome. Chlorophyll fast repetition rate fluorometry revealed that bicarbonate depletion resulted in one population of PSII showing normal oscillations in water oxidation and another that stopped after two charge-separating events. This suggested that the water oxidizing complex remained active in the first fraction of centers. Q<sub>A</sub><sup>-</sup> reoxidation kinetics showed that depletion causes the electron transfer time from Q<sub>A</sub> to Q<sub>B</sub> to have comparable time scales as the control, suggesting electron transfer to Q<sub>B</sub>, forming semiquinone Q<sub>B</sub><sup>-</sup>. As DCMU inhibits the second electron transfer, this suggests that an electron is being transferred to Q<sub>B</sub> in bicarbonate-depleted *L. maxima* and must be affecting proton delivery that would yield plastoquinol PQH<sub>2</sub>. Cytochrome b<sub>6</sub>f redox kinetics revealed that under depletion, *L. maxima* experiences intense oxidation, which suggests a powerful PSI. P700 kinetics displayed a predicted delayed transfer of electrons to PSI, suggesting an effect of bicarbonate depletion there as well.

# Light-Induced Charge Separation in Photosystem I from Different Biological Species Characterized by Multifrequency Electron Paramagnetic Resonance Spectroscopy

Jens Niklas<sup>1</sup>, Jasleen K. Bindra<sup>1</sup>, Tirupathi Malavath<sup>1</sup>, Mandefro Y. Teferi<sup>1</sup>, Moritz Kretzschmar<sup>2</sup>, Jan Kern<sup>2</sup>, Lisa M. Utschig<sup>1</sup>, Oleg G. Poluektov<sup>1</sup>

<sup>1</sup>Argonne National Laboratory, Lemont, IL 60439. [jniklas@anl.gov](mailto:jniklas@anl.gov).

<sup>2</sup>Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

Photosystem I (PSI) serves as a model system for studying fundamental processes such as electron transfer (ET) and energy conversion, which are not only central to photosynthesis but also have broader implications for bioenergy production and biomimetic device design. In this study, we employed electron paramagnetic resonance (EPR) spectroscopy to investigate key light-induced charge separation steps in PSI isolated from several green algal and cyanobacterial species. Following photoexcitation, rapid sequential ET occurs through either of two quasi-symmetric branches of donor/acceptor cofactors embedded within the protein core, termed the A and B branches. Using high-frequency (130 GHz) time-resolved EPR (TR-EPR) and deuteration techniques to enhance spectral resolution, we observed that at low temperatures prokaryotic PSI exhibits reversible ET in the A branch and irreversible ET in the B branch, while PSI from eukaryotic counterparts displays either reversible ET in both branches or exclusively in the B branch. Furthermore, we observed a notable correlation between low-temperature charge separation to the terminal [4Fe-4S] clusters of PSI, termed F<sub>A</sub> and F<sub>B</sub>, as reflected in the measured F<sub>A</sub>/F<sub>B</sub> ratio. These findings enhance our understanding of the mechanistic diversity of PSI's ET across different species and underscore the importance of experimental design in resolving these differences. Though further research is necessary to elucidate the underlying mechanisms and the evolutionary significance of these variations in PSI charge separation, this study sets the stage for future investigations into the complex interplay between protein structure, ET pathways, and the environmental adaptations of photosynthetic organisms.

# Bioinformatics Investigation of the Twin-Arginine Translocation pathway in Cyanobacteria

Victory Olagunju<sup>1</sup>, Carole Dabney-Smith<sup>1,2</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry, Miami University, Oxford Ohio*

*Olagunva@miamioh.edu*

<sup>2</sup>*Cell, Molecular and Structural Biology, Miami University, Oxford Ohio*

*Smithac5@miamioh.edu*

Protein translocation systems are essential in living organisms to transport proteins to specific locations for required cellular functions, as correct localization is essential for different cellular activities. One of the significant translocation systems is the twin-arginine translocation (TAT) system, which transports fully folded proteins in plants and bacteria, thus making it important in biotechnology and pathogenic studies. The *Synechocystis sp. PCC 6803*, a model photosynthetic organism, possesses two distinct membranes with single genes that code for the TAT transport system components. Evidence has shown the presence of the activity of twin-arginine transporters in both the thylakoid and the plasma membrane. However, the difference in the protein sorting process between the two membranes and what guides protein translocation to the thylakoid or plasma membranes using the same TAT system is still unknown. Using predictive algorithms and bioinformatic tools, we have identified *Synechocystis* TAT proteins that are predicted to be localized to the plasma and thylakoid membranes, respectively. Across several cyanobacteria species, we also observed the occurrence of one of the TAT components (TatA) almost invariably in the plasma membrane. At the same time, the other component (extended length version we dub TatAL) was present in both the plasma and thylakoid membranes. There was also a major difference in the amino acids in the transmembrane region in the two of the three components that make up the TAT system in cyanobacteria species. Other results also show a close relationship between plants (e.g., *Pisum sativum* or *Arabidopsis thaliana*), *Bacillus subtilis*, and the *Synechocystis* TAT systems. The *B. subtilis* TAT is interesting because it is composed of only two components (TatA and a cognate TatC); whereas, the other TAT systems have three distinct components (e.g., TatA, TatB, and TatC). These results suggest that *Synechocystis* uses a unique process by which specific proteins are directed and transported across the thylakoid and plasma membranes and involves a dual TAT system that combines elements of the plants and *Bacillus subtilis* TAT system. This dual system could be represented by the plasma membrane primarily utilizing a plant-type 3-membered TAT system, while the thylakoid membrane employs a gram-positive 2-membered TAT system. The unique TAT system allow *Synechocystis* to transport different precursors across the thylakoid or plasma membranes, respectively, with the individual TAT components playing a significant role in distinguishing translocation to the two distinct membranes.

# Shedding Light on Phytoplankton in Antarctic Lakes

Halie M. Seifert, Lehua D. Hoops, Gejla Toromani, Tareen R. Haque, Rochelle Pereira, Kaylie Wheeless, Devon Popson, and Rachael M. Morgan-Kiss

*Department of Microbiology, Miami University. 700 E. High Street Oxford, OH 45056. Email: seiferhm@miamioh.edu, hoopsld@miamioh.edu, toromag@miamioh.edu, haquetr@miamioh.edu, morganr2@miamioh.edu*

Like all aquatic ecosystems, phytoplankton are essential to polar ecosystems as foundational primary producers and as vital contributors to nutrient cycling. Polar phytoplankton conduct photosynthesis under extreme conditions (e.g., low temperatures, hypersalinity, nutrient limitation). These polar phytoplankton are also adapted to seasonal extremes in light availability, from 24-hour daylight (summer) to total darkness (winter). The McMurdo Dry Valleys (MDV), located within the valleys of the Transantarctic mountain range (Victorialand, East Antarctica) harbor numerous ice-covered lakes and ponds, each supporting unique phytoplankton communities. The MDV lakes are genomic reservoirs for novel extremophilic microorganisms and pristine models of simplified food webs. The McMurdo Long Term Ecological Research program ([mcmlter.org](http://mcmlter.org)) has monitored several MCM lakes for more than 3 decades. Two MDV lakes, Lakes Bonney and Fryxell, are representative of the extremes in water column chemistry: Bonney is highly oligotrophic and hypersaline, while Fryxell is mesotrophic and relatively freshwater. Both lakes exhibit permanent chemical stratification through the water columns.

The Morgan-Kiss laboratory is undertaking a large effort to monitor temporal and spatial trends in MDV lake phytoplankton communities over decadal and seasonal time scales. A diving spectral fluorometer, the bbe FluoroProbe (Moldaenke, Germany), represents a major tool for this project. Since 2005, depth profiles of four MDV lakes have been collected throughout the short austral summer. Additionally, moored instruments have been autonomously deployed year-round to collect annual trends in phytoplankton. The FluoroProbe utilizes several excitation wavelengths to convert chlorophyll a fluorescence to determination of four spectral algal groups (chlorophytes, cyanobacteria, cryptophytes, and ‘mixed’). Here we present four research questions utilizing the long-term FluoroProbe dataset to explore questions about drivers of phytoplankton dynamics: 1) How will different algal classes respond to drastic environmental changes such as temperature and nutrient levels over time? This project could provide an understanding of which phytoplankton will be most impacted by climate change within an Antarctic ecosystem. 2) Does metabolic flexibility allow for greater survivability of cryptophytes over chlorophytes in a changing environment? This will increase understanding of how diverse lifestyles can impact survivability in response to environmental shifts. 3) How do changes in temperature, and as a result, ice thickness, influence the distribution of green algae within Lake Bonney? The findings are expected to elucidate the ecological impacts of climate change on a key group of primary producers. 4) How do seasonal changes in phytoplankton biomass in Antarctic Lakes Bonney, Fryxell, and Hoare correlate with environmental factors such as temperature and photosynthetically active radiation (PAR) during the transition from polar night to austral summer? The analysis aims to clarify the interactions between phytoplankton dynamics and climatic variables, providing insights into the potential impacts of climate change on these remote ecosystems. Students representing all four projects will present a joint poster to highlight this robust long-term dataset representing phytoplankton dynamics from this isolated and unique environment.

# ***Engineering S. elongatus* PCC 7942 chassis for interrogating plant-like photorespiration**

Luke Sharpe<sup>1,2</sup>, Rees Rillema<sup>1,2</sup>, María Santos-Merino<sup>2</sup>, Berkley Walker<sup>2,3</sup>, Danny Ducat<sup>1,2</sup>  
[sharpelu@msu.edu](mailto:sharpelu@msu.edu)

<sup>1</sup>*Department of Biochemistry & Molecular Biology, Michigan State University. 603 Wilson Rd  
Rm 212, East Lansing, MI 48823.*

<sup>2</sup>*MSU-DOE Plant Research Laboratory, Michigan State University. Plant Biology Laboratories,  
612 Wilson Rd, East Lansing, MI 48824.*

<sup>3</sup>*Department of Plant Biology, Michigan State University. Plant Biology Laboratories, 612  
Wilson Rd #166, East Lansing, MI 48824.*

Photorespiration occurs when Rubisco fixes an O<sub>2</sub> molecule instead of a CO<sub>2</sub>, producing the toxic intermediate 2-phosphoglycolate (2-PG), which must be recycled into 3-phosphoglycerate (3-PGA) for reintegration into the Calvin-Benson-Bassham (CBB) cycle. In most plants, the oxygenation reaction occurs once for every four to five carboxylation reactions, making it the second-highest flux pathway on Earth, right after the CBB cycle itself. Photorespiration is thought to have originated in ancient cyanobacteria and was subsequently passed onto plants and algae via endosymbiosis. While certain enzymes in the pathway are highly conserved throughout the photosynthetic lineage, others have been adapted to meet the specific needs of different organisms. Nonetheless, the essential function of photorespiration remains consistent: recycling the inhibitory 2-PG into a useful carbon skeleton. In cyanobacteria, photorespiration seems to be less impactful than in plants, primarily due to the efficiency of the carboxysome carbon concentrating mechanism (CCM), which elevates CO<sub>2</sub> levels around rubisco and minimizes oxygenation reactions. Therefore, the role of photorespiration in cyanobacteria is relatively understudied. However, with recent advancements in understanding carboxysome dynamics and permeability, there is growing interest in elucidating the contributions of photorespiration to cyanobacterial metabolism and photosynthesis. This study seeks to characterize cyanobacterial photorespiration and its impact on photosynthetic performance in parallel with the construction of plant-like photorespiratory strains, which will serve as platforms for protein engineering by providing a fast-growing, robust system to test enzymes identified in our engineering pipeline. The pipeline entails using molecular dynamics to identify enzymatic domains that can be stitched into plant photorespiratory enzymes to improve performance under a range of stress conditions. To achieve this, peripheral pathways in cyanobacterial photorespiration will be modified to mimic a more plant-like pathway, allowing for the optimization of engineered enzymes. The performance of strains and photorespiration generally will be assessed using several core techniques such as membrane inlet mass spectrometry (MIMS), chlorophyll fluorescence, and gas exchange.

# Hierarchical Structural Differences Between Green Algae and Cyanobacteria May Lead to Inefficiencies in H<sub>2</sub> and NADPH Production

Audrey H. Short<sup>1</sup> and Lisa M. Utschig<sup>1</sup>

<sup>1</sup>9700 S. Cass Ave, Lemont, IL. Chemical Sciences and Engineering Division, Argonne National Laboratory; [ashort@anl.gov](mailto:ashort@anl.gov)

Photosynthetic organisms harness energy from the sun to drive photosynthesis, which converts solar energy to chemical energy, storing it in high-energy bonds. In this highly sustainable reaction, photosystems (PS) II and I utilize energy from photons to efficiently separate electrons from the PSs, driving water oxidation and the formation of NADPH (and ATP). Using the native machinery in photosynthetic organisms in conjunction with synthetic catalysts allows us to produce hydrogen gas, a clean and renewable energy source, with only water and sunlight as the starting reagents.<sup>1</sup> Previous work has shown that platinum nanoparticles (PtNP) as well as molecular catalysts can self-assemble with PSI in spinach and cyanobacteria membranes, which allows for solar overall water splitting to generate hydrogen gas.<sup>2</sup> In cyanobacteria, PSI is an abundant trimer protein, ubiquitously found throughout the thylakoid membrane. However, the morphology of thylakoids differs greatly between prokaryotes and eukaryotes. In green algae and plants, thylakoid membranes have stacked regions that are connected by stroma lamella. PSI is typically found in the stroma lamella while PSII is within the stacked region. Additionally, eukaryotic PSI is a monomer and present in much lower concentrations. In this work, we explore how these morphological differences between the thylakoid membranes and PSI may affect hydrogen production efficiencies as well as higher order complex formation between PSI and ferredoxin and ferredoxin-NADP<sup>+</sup> reductase. Utilizing the green algae *Scenedesmus obliquus* and *Chlorella vulgaris*, we isolated PSI and examined H<sub>2</sub> and NADPH formation in comparison to isolated PSI from the cyanobacteria, *Synechococcus leopoliensis*. Understanding the fundamental mechanisms of electron transfer between PSII and PSI as well as between PSI and electron acceptors will inform design strategies for sustainable photosynthetic-inspired systems with efficient solar energy conversion and solar fuel synthesis capabilities.

<sup>1</sup>L. M. Utschig and K. L. Mulfort, “Photosynthetic biohybrid systems for solar fuels catalysis” *Chem. Commun.*, **2024**, 60, 10642. DOI: 10.1039/d4cc00774c

<sup>2</sup>L. M. Utschig, S. R. Soltau, K. L. Mulfort, J. Niklas, O. G. Poluektov, “Z-scheme solar water splitting via self-assembly of photosystem I-catalyst hybrids in thylakoid membranes”, *Chem. Sci.*, **2018**, 9, 8504. DOI: 10.1039/c8sc02841a

# Chromatic Adaptation of *Galdieria yellowstonensis*

Evan Smolen<sup>1</sup>, Vidya Gundlapalli<sup>1</sup> and Colin Gates<sup>1</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, 1068 West Sheridan Rd, Chicago, IL 60660.  
Loyola University Chicago. esmolen@luc.edu

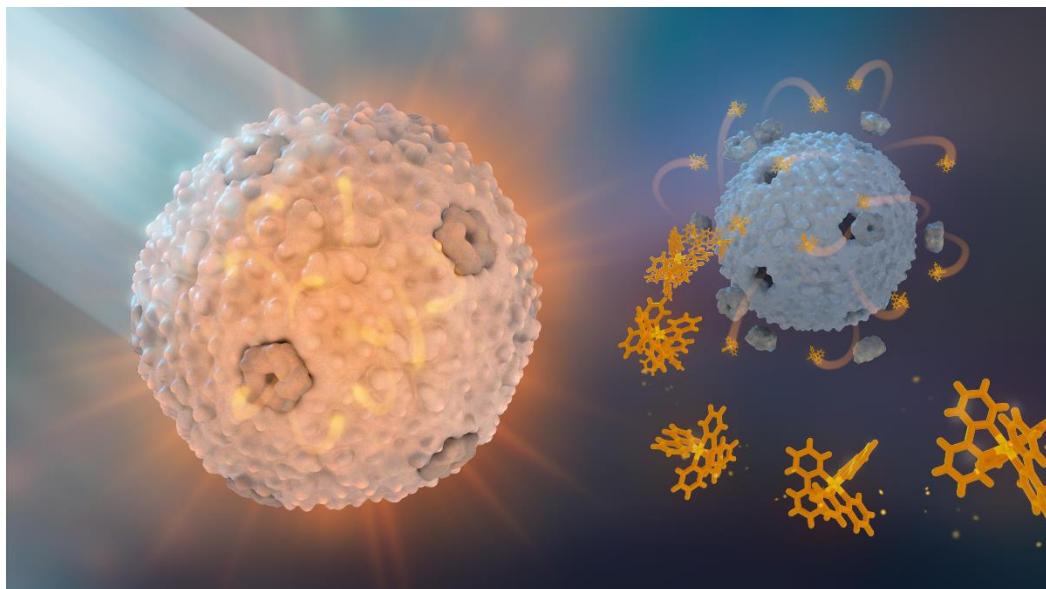
*Galdieria yellowstonensis* is an extremophilic red alga which has a broad range of pigments of both cyanobacterial (phycobilins and phycobiliproteins) and eukaryotic (chlorophyll-based) origin. This alga is thus capable of adjusting its light-harvesting apparatus to the wavelengths available through chromatic adaptation and modulation of pigment usage and expression. Chromatic adaptation was induced in *G. yellowstonensis* to determine differences in exciton and electron usage driving the light reactions under specific light regimes. *G. yellowstonensis* cultures were grown under white, red, and blue light at 30°C and investigated for their pigment expression and distribution and electron transfer kinetics. Under blue light, an electrochromic shift three times stronger than in either of the other cultures was observed, as well as sixfold stronger oxidation at Photosystem I than under red light. While all three cultures showed similar redox poise of plastocyanin, cytochromes b and f of the b6f complex were about half as oxidized in response to light when adapted to either red or blue light. Red light prompted a threefold decrease in active QB sites of photosystem II but despite this limitation, electron flux to cytochrome b6f and photosystem I remained high and appeared to be primarily linear. Under blue light, *G. yellowstonensis* appears to undergo strong cyclic electron flow around photosystem I which dominates the activity of the electron transport chain and generates a strong trans-thylakoid proton gradient. Despite this, the remaining, diminished, photosystem II population remains highly efficient in electron removal.

# Bacterial Microcompartment Shell Biohybrids: Photophysics and Light-Driven Electron Transfer of Ruthenium Photosensitizers in Confinement

Samuel N. Snyder<sup>1</sup>, Karen L. Mulfort<sup>1</sup> and Lisa M. Utschig<sup>1</sup>

<sup>1</sup>Argonne National Laboratory, 9700 S Cass Ave, Lemont, IL 60439. samuel.snyder@anl.gov.

Bacterial microcompartments (BMCs) are self-assembling, selectively permeable protein shells that encapsulate enzymes to enhance the catalytic efficiency of segments of metabolic pathways through means of confinement. The modular nature of BMC shells' structure and assembly enables programming of shell permeability and underscores their promise in biotechnology engineering efforts for applications in industry, medicine, and clean energy. Realizing this potential requires methods for encapsulation of abiotic molecules, which have been developed here for the first time. We report *in vitro* cargo loading of BMC shells with ruthenium photosensitizers (RuPS) by two approaches—one involving site-specific covalent labelling and the other driven by diffusion, requiring no specific interactions between cargo molecules and shell proteins. The shells retain the encapsulated RuPS cargo over one week without egress, denoting the impressive structural stability of these biohybrid constructs. The photophysical properties of the RuPS(II)\* excited state are responsive to confinement within BMC shell's unique interior microenvironment, but overall activity as photosensitive electron donor is maintained. This study is an important foundation for further work that will converge biological BMC architecture with synthetic chemistry to facilitate biohybrid photocatalysis.



**Figure 1.** Scheme for abiotic cargo loading of a BMC shell with ruthenium photosensitizers.

# Binding Interface of PrxQ to the Membrane Probed by Molecular Simulation

Andrew Strong, T.J. Nicodemus, John Froehlich, Christoph Benning, Josh Vermaas

*Andrew Strong, Michigan State University. strong41@msu.edu*

*T.J. Nicodemus, nicodem8@msu.edu*

*John Froehlich, froehli5@msu.edu*

*Christoph Benning, benning@msu.edu*

*Josh Vermaas, vermaasj@msu.edu*

During photosynthesis, hydrogen peroxide is frequently produced as a byproduct in chloroplasts of photosynthetic organisms and must be detoxified. The Peroxiredoxin family of proteins is involved in the cellular defense of reactive oxygen and nitrogen species such as hydrogen peroxide. Through its catalytic process in which it binds to a phospholipid bilayer, proteins in the Peroxiredoxin family can reduce phospholipid hydroperoxides produced by oxidative stress from elevated levels of hydrogen peroxide. However, the binding site in Peroxiredoxin proteins found in *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* is unknown. Using molecular simulations, we found that the binding position of AtPrxQ and CrPrx6 involves an important pair of cystine residues in close contact to the lipid bilayer. This binding position was the only consistent pose for both proteins across all replicates of the simulations. These results help us understand how exactly proteins in the Peroxiredoxin family bind to membranes and potentially reveal residues involved in reducing phospholipid hydroperoxides. We expect these early simulations to be the beginning of further molecular simulations investigating how this family of proteins interact with phospholipid hydroperoxides to maintain homeostasis. In the future, simulations could be run with different concentrations of these phospholipid hydroperoxides to investigate whether they aggregate near the protein binding position to investigate how the mechanism of these proteins work as well as how differing levels of oxidative stress impact their function.

# The structural cross-linking mass spectrometry justification of an AlphaFold2-generated PsbS structure of the elongated stromal loop region

Blake Trembath<sup>1</sup>, Yanchun Lin<sup>2</sup>, Masakazu Iwai<sup>3,4</sup>, Rupal S. Tomar<sup>1</sup>, Krishna K. Niyogi<sup>3,4</sup>, Michael L. Gross<sup>2</sup>, Haijun Liu<sup>1</sup>

<sup>1</sup>*Department of Biology, Saint Louis University in St. Louis, St. Louis, MO 63103, USA*

<sup>2</sup>*Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130, USA*

<sup>3</sup>*Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA*

<sup>4</sup>*Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*

PsbS is a protein in Photosystem II (PSII) that is involved in non-photochemical quenching (NPQ), a process involved in shielding plants from excess light and preventing photoinhibition. Understanding the structure of PsbS more deeply could aid in understanding plants' mechanisms of adapting to excess light and varying environmental conditions which could enable new, yield-improving developments in agriculture. The structural location and function of PsbS in PSII, however, remain not fully understood, plausibly due to intrinsically disordered regions (IDRs) in the N-terminal domain and stromal loop that prove difficult to characterize via X-ray crystallography or cryo-EM. This study used cross-linking mass spectrometry (XL-MS), a methodology that allows for protein residues that lie in proximity to be chemically linked and subject to interrogation via mass spectrometry. Isotopically encoded intrachain cross-links of PsbS were used as spatial constraints to critically justify AlphaFold2. This research highlights the combinatorial use of structural mass spectrometry and *in silico* protein structure prediction to quickly produce a whole proteome protein structure of an organism.

# Evaluation of TR-XRD and DFT based models of the Oxygen-Evolving Complex of Photosystem II versus EXAFS data

Hasan Tunçer<sup>1</sup>, Gabriel Bury<sup>1</sup> and Yulia Pushkar<sup>1</sup>

<sup>1</sup>*Department of Physics and Astronomy, Purdue University, IN, USA  
htuncer@purdue.edu*

Photosystem II (PSII) is a crucial component of oxygenic photosynthesis, responsible for splitting water molecules to produce molecular oxygen. At the core of this process is the oxygen-evolving complex (OEC), containing a Mn4Ca cluster, which catalyzes the water-splitting reaction. Understanding the precise structure of this Mn4Ca cluster under functional conditions is key to gaining insights into the mechanism of water oxidation in natural photosynthesis.

In this study, we aim to refine the structure of the OEC of PSII via comparison of structural models obtained in recent time-resolved X-ray diffraction experiments [Bhowmick et al. 2023; Li et al. 2024] and via DFT analysis. Several controversies still exist such as proposal of low oxidation state OEC where instead of more widely accepted 2xMnIII2xMnIV OEC composition in the S1 state, a two-electron reduced form of 4xMnIII is hypothesized. We compared EXAFS spectra derived from DFT models of the low oxidation state paradigm [Chen et al. 2018] with high quality room temperature EXAFS of the S1 state. Poor agreement with the experiment indicates that these models are unlikely to represent the actual S1-state.

One more open question is the discrepancy of the O5-O6 distance in the S3 state between XRD results indicating ~2.0 Å and majority of DFT models of this state indicating ~2.5-2.6 Å distance. Researchers conducting XRD consider this discrepancy to be above the uncertainty of the XRD method, thus, explanation is needed. One approach is to propose a dynamic interconversion, or low barrier O-O coordinate in this state. [Corry and O'Malley. 2020] Others implicated X-ray induced damage.[ Chrysina et al. 2023] Our modeling of the S3-state EXAFS has shown that this state can be satisfactorily modeled with a combination of two states with protonated Mn-O6-H and deprotonated Mn=O6 both featuring the O5-O6 distances of 2.5-2.6 Å.[Bury and Pushkar 2024] Studying the Mn4Ca cluster at room temperature poses significant challenges due to its sensitivity to X-ray radiation which will also be discussed.

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# **Investigation of Tha4 oligomerization based on the hydrophobicity of transmembrane helix during the active cpTAT translocation.**

Vidusha Weesinghe<sup>1</sup>, Carole Dabney-Smith<sup>2</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry, Miami University. 701 E. High St., Oxford, OH 45056. [weesinvs@miamioh.edu](mailto:weesinvs@miamioh.edu).*

Most of the proteins required by chloroplasts for proper metabolic and photosynthetic function are encoded by genes in the nucleus and translated in the cytosol of plant cells. These newly synthesized proteins contain an N-terminal amino acid extension that serve to direct or target the protein, called a precursor, to the chloroplast envelope and thylakoids. Protein transport complexes in membranes provide the conduit through which precursors are transported across the membrane. One such transport complex is the Twin Arginine Transport (cpTAT) pathway. The cpTAT pathway transports fully folded proteins by using the proton motive force (PMF) as the only source of energy.<sup>1</sup>

The cpTAT pathway consists of three membrane-bound proteins namely, Tha4, Hcf106, and cpTatC. From previous studies, it was found that the conserved glutamate residue (E10) in the transmembrane domain (TMD) of Tha4 is essential for function in the translocation of precursor proteins and for Tha4 assembly.<sup>2</sup> It was found that the substitution of alanine for the glutamate prevents transport while an aspartate substitution partially recovers the transport. We predict that the E10 of Tha4 TMD acts as a sensor of the formation of PMF to assemble into large oligomers, which aid the transport of the precursor. First, oligomer formation of each Tha4 E10/A/D variant, in the presence and absence of PMF was investigated by substituting cystines to the lumen proximate positions in the transmembrane helix of Tha4 by employing radiolabeled crosslinking assays. Tha4 oligomer formation increases when alanine is substituted for the glutamate in the Tha4 TMD, while an aspartate substitution showed a lower degree of oligomerization. From these studies, we show that modulations to helix hydrophobicity of Tha4 impact monomer and oligomer stability and their packing, thereby altering precursor transport via cpTAT pathway.

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# Acetate Consumption Confers Resistance of *Chlamydomonas reinhardtii* to Abiotic Stress

Kaylie Wheeless<sup>1</sup>, Bill Gibbons<sup>2</sup>, Andrew Jones<sup>2</sup>, and Rachael Morgan-Kiss<sup>1</sup>

<sup>1</sup>Department of Microbiology, Miami University, 700 E. High St. Oxford, OH,  
Wheele34@miamioh.edu.

<sup>2</sup>Department of Chemical, Paper, and Biomedical Engineering, Miami University, 650 E. High St. Oxford, OH.

*C. reinhardtii* is a model alga commonly used in the study of photosynthesis. One quality which led to the establishment of *C. reinhardtii* as a model organism was its capacity to grow autotrophically, mixotrophically, or heterotrophically depending on the availability of light and acetate. In the natural environment, this capacity allows for interactions between *C. reinhardtii* and organisms within the soil community. In the laboratory, mixotrophic growth has attracted the interest of many as it allows for production of photosynthetic mutants, exhibits fast growth rates, accumulates high biomass, and has high stress resistance. The popularity of the mixotrophic model has led to many studies of stress acclimation in mixotrophically grown *C. reinhardtii*, but few studies have focused on the photosynthetic response to stress. This project aims to expand upon knowledge of the differences in the photosynthetic response of mixotrophically and autotrophically grown *C. reinhardtii* to abiotic stress. To test this, *C. reinhardtii* was grown in the presence and absence of acetate under control and stress (high salt, high light, and combined high salt/high light) conditions. Upon entry into logarithmic growth, samples were collected to evaluate differences in photochemistry, energy distribution, and expression of key photosynthetic proteins between the two growth methods during control and stress. In general, it was found that mixotrophically grown *C. reinhardtii* exhibited faster growth rates and achieved higher densities than autotrophically grown *C. reinhardtii*. Photosynthetic capacity and rates of cyclic electron flow were higher in mixotrophic cells during stress. In addition, both induction of non-photochemical quenching (NPQ) and shifts in expression of major photosynthetic proteins was reduced in mixotrophic cells during all stressors. These findings suggest that acetate consumption may protect the photosynthetic electron transport chain (PETC) during abiotic stress, allowing mixotrophic cells to continue robust growth in conditions which stunt autotrophic *C. reinhardtii* cultures. While further study of protein and metabolite production is necessary to support differences in downstream activity, we have concluded that growth method should be considered when using *C. reinhardtii* to study the photosynthetic stress response.

# The free protein pool: quantitative insights into the 3 major photosynthetic complexes

Anna Williams<sup>a</sup>, Philip J. Jackson<sup>b</sup>, Steven M. Theg<sup>c</sup>, Diana Kirilovsky<sup>d</sup>, Terry M. Bricker<sup>e</sup>, Niel C. Hunter<sup>b</sup>, Michael Gross<sup>f</sup>, Haijun Liu<sup>a</sup>

<sup>a</sup>Department of Biology, Saint Louis University, St. Louis, MO, 63130, USA

<sup>b</sup>Plants, Photosynthesis and Soil, School of Biosciences, University of Sheffield, Sheffield, UK

<sup>c</sup>Department of Plant Biology, University of California, Davis, CA, 95616, USA

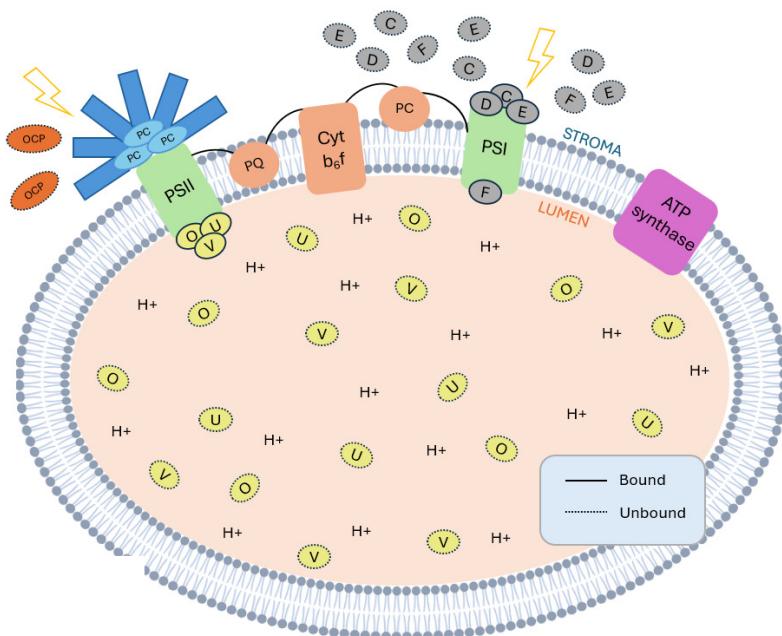
<sup>d</sup>Institute for Integrative Biology of the Cell (I2BC), CNRS, CEA, Université Paris-Sud,

Université Paris-Saclay, 91198 Gif sur Yvette, France

<sup>e</sup>Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, 70803, USA

<sup>f</sup>Department of Chemistry, Washington University in St. Louis, St. Louis, MO, 63130, USA

For decades, the thylakoid lumen of photosynthesizing organisms has been thought to harbor proteins associated with only a limited number of photosynthetic complexes. The question of what and how many proteins exist within the thylakoid lumen has remained largely unanswered. In this study, we utilized quantitative mass spectrometry to determine the abundance of photosystem II (PSII) luminal proteins and the stromal subunits of photosystem I (PSI), that are presumably linked to two major pigment protein complexes. Phycobilisome (PBS) components were also quantified, including the physiologically relevant orange carotenoid protein (OCP) and ApcG. A substantial free pool of PSII components on the thylakoid luminal side exists while an additional free pool of PSI components is present on the stromal side. This model challenges the current structural stoichiometry of these two reaction centers previously revealed by X-ray crystallography and cryo-EM through propositions of natively unfolded PsbO. The stoichiometry of OCP and PBS offers a better understanding of the photoprotective mechanisms in cyanobacteria.



**Figure 1.** A schematic representation of the free-floating pool of proteins for PSII (lumen), PSI (stroma), and OCP. Dashed perimeters show unbound proteins while solid perimeters demonstrate protein binding to a complex. Proteins are annotated by their last letter (i.e. PsaF is "F").

# **POPG vs SQDG: The tale of two lipids in LH1RC**

Neetu Singh Yadav<sup>1</sup>, Josh V. Vermaas<sup>1</sup>

<sup>1</sup>Plant Research Laboratory, Michigan State University, East Lansing, MI, USA.

From cyanobacteria to higher plants photosynthetic membranes contain galactolipids such as mono- and digalactosyldiacylglycerol (MGDG and DGDG), as well as negatively charged lipids, such as sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). Often, plants and algae struggle to survive without nutrients, leading to the development of nutrient-saving mechanisms. Under phosphate starvation, PG is replaced by SQDG in some photosynthetic membranes, while under sulfur deprivation, the opposite will occur. Biological data strongly support a complementarity between SQDG and PG and indicate the importance of maintaining the total amount of anionic lipids in photosynthetic membranes. Photosynthetic purple bacteria can capture and convert sunlight with a remarkable nearly 100% quantum efficiency. A key component of this process is the light-harvesting complex1 - reaction center (LH1-RC), which is responsible for light harvesting and charge separation. The LH1-RC membrane complex has a highly conserved local lipid composition that is dominated by anionic lipids, which may establish specific interactions with the protein. Characterization of these functionally important lipid–protein interactions with experimental techniques is however still prohibitively challenging. With the Martini force field, we present coarse-grained molecular dynamics simulations of monomeric and dimeric LH1RC, in PG and SQDG rich membranes. Physical properties of SQDG and PG membrane are similar despite their different chemical headgroups. Results show that LH1-RC prefers anionic over neutral lipids. Additionally, anionic lipids in the inner leaflet of the membrane promote energy transfer from LH1 to RC by interacting with basic amino acids. The results presented here establish that SQDG and PG are good substitutes for each other in nutrient starvation conditions to maintain the LH1RC functional organization and its photosynthesis activity.

# Useful Information

The conference will be held at the Turkey Run Inn, just inside Turkey Run State Park in Marshall, Indiana October 25-27. **Meals** will be in the main dining area, near the check-in desk. **Talks, coffee breaks, and posters** will be in the lower level, past the cozy lobby and down the stairs in the meeting area. Forwarned is forarmed, the Wi-Fi can be spotty at Turkey Run, so please be prepared with flash drives to transfer presentations as needed.

## Organizing committee

Sujith Puthiyaveetil (chair)	Josh Vermaas (co-chair)
Department of Biochemistry	Plant Research Laboratory
Purdue University	Department of Biochemistry
West Lafayette, IN 47907	Michigan State University
spveetil@purdue.edu	vermaasj@msu.edu

## Code of Conduct

The Midwest/Southeast Photosynthesis Conference is committed to providing a welcoming, inclusive, and harassment-free environment in all interactions regardless of race, age, ethnicity, national origin, language, gender, gender identity, sexual orientation, disability, physical appearance, political views, military service, health status or religion. We welcome the opportunity to bring photosynthesis research to all people regardless of their identity or background.

As a program that aims to share ideas and freedom of thought and expression, it is essential that the interaction between participants take place in an environment that recognizes the inherent worth of every person by being respectful of all. All participants strive to be empathetic, respectful, welcoming, friendly, and patient. We strive to be collaborative and use language that reflects our values.

The conference does not tolerate harassment in any form. Harassment is any form of behavior intended to exclude, intimidate or cause discomfort. Harassment includes, but is not limited to, the use of abusive or degrading language, intimidation, stalking, harassing photography or recording, inappropriate physical contact, and unwelcome sexual attention.

## Examples of unacceptable behavior

All participants are committed to making participation in this community a harassment-free experience.

We will not accept harassment or other exclusionary behaviors or actions that are illegal, such as:

- The use of sexualized language or imagery
- Excessive profanity (please avoid curse words; people differ greatly in their sensitivity to swearing)
- Posting sexually explicit or violent material
- Violent or intimidating threats or language directed against another person or group
- Inappropriate physical contact and/or unwelcome sexual attention or sexual comments
- Sexist, racist, or otherwise discriminatory jokes and language
- Trolling or insulting and derogatory comments
- Written or verbal comments which have the effect of excluding people on the basis of membership in a specific group, including level of experience, gender, gender identity and expression, sexual orientation, disability, neurotype, personal appearance, body size, race, ethnicity, age, religion, or nationality
- Public or private harassment
- Continuing to initiate interaction (such as photography, recording, messaging, or conversation) with someone after being asked to stop
- Sustained disruption of talks, events, or communications, such as heckling of a speaker
- Publishing (or threatening to post) other people's personally identifying information ("doxing"), such as physical or electronic addresses, without explicit permission
- Other unethical or unprofessional conduct
- Advocating for, or encouraging, any of the above behaviors All participants are governed by local laws, including Turkey Run State Park rules and their organization's code of conduct and policies.

## How to Submit a Report

If you feel your safety is in jeopardy or the situation is an emergency, contact local law enforcement before making a report to the conference organizers. (In the U.S., dial 911.)

Anyone who experiences, observes or has knowledge of threatening behavior is expected to immediately report the incident to a member of the event organizing committee, state park staff, or a trusted friend or colleague. The Midwest/Southeast photosynthesis conference reserves the right to take appropriate action.

Take care of each other. Alert the organizers if you notice a dangerous situation, someone in distress, or violations of this code of conduct, even if they seem inconsequential. For possibly unintentional breaches of the code of conduct, you may want to respond to the person and point out this code of conduct (either in public or in private, whatever is most appropriate). If you would prefer not to do that, please report the issue to the conference chair or co-chair.

### **What Happens Next**

All complaints will be reviewed and investigated and will result in a response that is deemed necessary and appropriate to the circumstances. All reports will be kept confidential, with the exception of cases where the organizers or state park staff determines the report should be shared with law enforcement. In those cases, the report will be shared with the proper legal authorities.

In some cases the conference organizers may determine that a public statement will need to be made. If that's the case, the identities of all involved parties and reporters will remain confidential unless those individuals instruct us otherwise.

