37th Annual Midwest/Southeast Photosynthesis Meeting

November 11-13, 2011
Turkey Run State Park, Marshall, IN

PROGRAM AND ABSTRACTS

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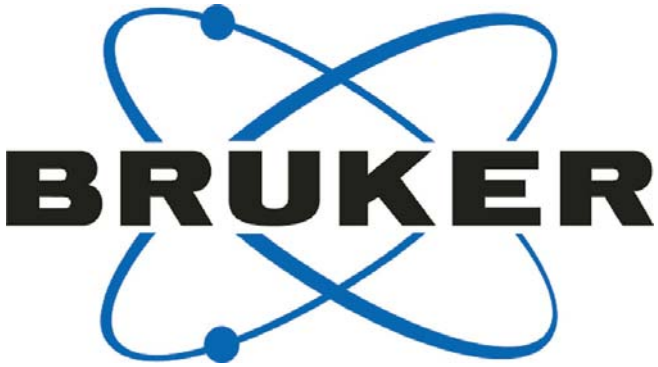

37th Annual Midwest/Southeast Photosynthesis Meeting

Turkey Run State Park
Marshall, Indiana

November 11-13, 2011

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Please note the dates of next year's MW/SE Photosynthesis Meeting: November 9-11, 2012

On the Cover:
Top: Leaves, Paolo Neo (public-domain-image.com)
Bottom: Grass, Petr Kratochvil (http://www.publicdomainpictures.net/view-image.php?image=6020)

Schedule of Events

All events will take place at Turkey Run Inn. Meeting sessions and poster presentations will take place in the Lusk Room. Meals will be served in the Narrows Dining Room.

Friday, November 11

4:00-6:00 PM	Arrival and Registration
6:00 PM	Dinner
7:30 PM	Session I: Keynote Lectures
9:20 PM	Mixer and Poster Viewing

Saturday, November 12

7:00 AM	Breakfast
8:30 AM	Session II: Contributed Papers
10:10 AM	Coffee Break
10:30 AM	Session III: Contributed Papers
12:00 PM	Lunch
1:00-4:00 PM	Leisure Time, Posters Viewing
4:00 PM	Poster Session, Refreshments
6:00 PM	Dinner
7:00 PM	Session IV: Contributed Papers
9:00 PM	Mixer and Poster Viewing

Sunday, November 13

7:00 AM	Breakfast and preliminary checkout
8:40 AM	Session V: Contributed Papers
10:00 AM	Coffee Break and Checkout
10:20 AM	Session VI: Contributed Papers
11:25 AM	Award Ceremony
11:30 AM	Best poster talks
11:50 AM	Closing Remarks
12:00 PM	Departure

CONTENTS:

Friday Evening, November 11

Session I. Keynote Lectures

7:30 PM Opening Remarks and Welcome: Alice Haddy and Sergei Savikhin

7:40 PM Keynote Speaker: Donald Ort, University of Illinois
IMPROVING PHOTOSYNTHETIC EFFICIENCY FOR GREATER YIELD

8:30 PM Keynote Speaker: David Kramer, Michigan State University
THE IMPORTANCE OF THE PROTON CIRCUIT IN BALANCING PHOTOSYNTHESIS

9:20 PM **Mixer and Poster Viewing**

Saturday Morning, November 12

Session II. CO_2 Metabolism, Chloroplast biogenesis

chair: Mautusi Mitra

8:30 AM EFFECTS OF CARBON DEPRIVATION ON CYCLIC ELECTRON TRANSPORT IN *SYNECHOCYSTIS* SP. PCC 6803
Steven C. Holland, Anthony D. Kappell, Robert L. Burnap

8:50 AM PROBING THE CONSEQUENCES OF THE CO_2 CONCENTRATING MECHANISM AND CO_2 RESPONSES ON THE PHOTOSYNTHETIC CIRCUIT
Ben F. Lucker and David M. Kramer

9:10 AM METABOLIC ATTRIBUTES OF *CYANOTHECE*, A GROUP OF UNICELLULAR NITROGEN FIXING CYANOBACTERIA
Anindita Bandyopadhyay, Thanura Elvitigala, Eric Welsh, Jana Stöckel, Michelle Liberton, Hongtao Min, Louis A. Sherman, and Himadri B. Pakrasi

9:30 AM THE PSBP DOMAIN 1 (PPD1) PROTEIN IS AN ESSENTIAL GENE INVOLVED IN CHLOROPLAST BIOGENESIS IN ARABIDOPSIS
Johnna L. Roose, Laurie K. Frankel, and Terry M. Bricker

9:50 AM FUNCTIONS OF THE DUPLICATED HIK31 OPERONS IN CENTRAL METABOLISM AND RESPONSES TO LIGHT, DARK AND CARBON SOURCES IN *SYNECHOCYSTIS* SP. STRAIN PCC 6803
Sowmya Nagarajan and Louis A. Sherman

10:10 AM Coffee/tea Break

Session III. Biofuels and Artificial Photosynthesis

chair: Harvey J.M. Hou

10:30 AM HETEROLOGOUS PRODUCTION OF PLANT SECONDARY METABOLITES CAFFEIC ACID AND RESVERATROL IN CYANOBACTERIA *SYNECHOCYSTIS* SP. PCC 6803
Yong Xue and Qingfang He

10:50 AM METABOLIC ENGINEERING OF ISOPRENE PRODUCTION IN A RAPIDLY GROWING, HIGH-LIGHT TOLERANT CYANOBACTERIUM
Matthew E. Nelson, Eric L. Singsaas & Toivo Kallas

11:10 AM SPECTROSCOPIC CHARACTERIZATION OF THE WATER OXIDATIONINTERMEDIATES IN THE RU-BASED CATALYSTS FOR ARTIFICIAL PHOTOSYNTHESIS
Dooshaye Moonshiram, Jonah W.Jurss, Javier Concepcion, Thomas Meyer, and Yulia Pushkar

11:30 AM A ROBUST PS II MIMIC IN PHOTO WATER SPLITTING: COMBINATION OF MANGANESE-OXO OLIGOMER COMPLEX AND TUNGSTEN OXIDE SEMICONDUCTOR
Harvey J.M. Hou

12:00 PM Lunch

Saturday Afternoon

1:00 PM Leisure Time, Poster Viewing

4:00 PM Poster Session, Refreshments

6:00 PM Dinner

Saturday Evening

Session IV. Photosystem II

chair: Robert Burnap

7:00 PM MULTIPLE RADICAL SCAVENGER PATHWAYS FOR THE ESSENTIAL TYROSYL RADICAL IN RIBONUCLEOTIDE REDUCTASE
Adam R. Offenbacher, Ellen Minnihan, JoAnne Stubbe, and Bridgette A. Barry

7:20 PM CONFORMATIONAL SWITCHING ACCOMPANYING PCET IN PHOTOSYSTEM II INSPIRED BIOMIMETIC PEPTIDES PROBED BY UVRR
Cynthia V. Pagba and Bridgette A. Barry

7:40 PM DETECTION OF WATER BINDING SITES IN THE OXYGEN-EVOLVING COMPLEX OF PHOTOSYSTEM II POISED IN THE S2 STATE USING HIGH FIELD (94 GHZ) 17O-ELDOR DETECTED NMR SPECTROSCOPY
Leonid Rapatskiy, Nicholas Cox, Anton Savitsky, William Ames, Julia Sander, Marc Nowacyzk, Matthias Rögner, Alain Boussac, Frank Neese, Johannes Messinger and Wolfgang Lubitz

8:00 PM SINGLE AMINO ACID SUBSTITUTIONS TO THE CP43 SUBUNIT OF PHOTOSYSTEM II INHIBIT OXYGEN EVOLUTION AND ALLOW THE OBSERVATION OF INTERMEDIATES IN THE FORMATION AND RELEASE OF O_2
Preston Dilbeck, Cindy Putnam Evans and Robert Burnap

8:20 PM *N*-FORMYLKYNURENINE, PHOTOINHIBITION, AND D1 POLYPEPTIDE TURNOVER IN PHOTOSYSTEM II
Tina M. Dreaden, Sascha Rexroth, and Bridgette A. Barry

8:40 PM EXPRESSION OF ALTERNATE FORMS OF *PSBA2* GENE FROM NATIVE AND ECTOPIC LOCATION TO STUDY THEIR EFFECTS ON THE D1 TURNOVER PROCESS IN *SYNECHOCYSTIS* SP. PCC 6803
Aparna Nagarajan and Robert Burnap

9:00 PM **Mixer and Poster Viewing**

Sunday Morning, November 13

Session V. Macromolecular complexes: cytochrome, antennae, PS I, and others. chair: Jason Cooley

8:40 AM NATIVE MASS SPECTROMETRY OF MEMBRANE-BOUND PROTEIN-PIGMENT COMPLEXES SUGGESTS INDUCED COFACTOR DISSOCIATION
Lucas Harrington, Hao Zhang, Michael Gross, Robert Blankenship

9:00 AM STRUCTURAL ANALYSIS OF A DEPSIPEPTIDE IN THE LIPID MEMBRANE
Christopher M. Halsey, Mia C. Brown, Derek A. Benham, Renee D. JiJi, Jason W. Cooley

9:20 AM REGULATION OF THE CHLOROPHYLL ANTENNA SIZE IN *CHLAMYDOMONAS REINHARDTII BY TLA1* GENE OVER-EXPRESSION AND RNA INTERFERENCE
Mautusi Mitra, Henning Kirst, David Dewez, Sam Ng and Anastasios Melis

9:40 AM EXPRESSION AND CHARACTERIZATION OF THE DIHEME CYTOCHROME *C* SUBUNIT OF THE CYTOCHROME *BC* COMPLEX IN *HELIOBACTERIUM MODESTICALDUM*
Hai Yue, Yisheng Kang, Hao Zhang, Xinliu Gao
and Robert E. Blankenship

10:00 AM Coffee/tea Break

Session V continued. Macromolecular complexes: cytochrome, antennae, PS I, and others.
chair: Toivo Kallas

10:20 AM CONSERVED LIPID FUNCTIONS IN CYTOCHROME *bc* COMPLEXES
S. Saif Hasan, Eiki Yamashita, Christopher M. Ryan, Julian P. Whitelegge and William A. Cramer

10:40 AM CYCLIC ELECTRON TRANSFER PATHWAYS IN SYNECHOCOCCUS SP. PCC 7002 DURING PHOTOSYNTHESIS AT HIGH LIGHT INTENSITY
Anuradha Marathe, Brandon Thomas, Stephanie Krueger & Toivo Kallas

11:00 AM SPECTRAL RESOLUTION OF THE FIRST ELECTRON ACCEPTOR IN PHOTOSYSTEM I
Adrien Chauvet, Naranbaatar Dashdorj, John H. Golbeck, T. Wade Johnson, and Sergei Savikhin

11:20 AM Technical Break

11:25 AM Awards

11:30 AM Talk: Best undergraduate student poster

11:40 AM Talk: Best graduate student poster

11:50 AM Closing notes and announcements

12:00 PM Departure

Oral Presentation Abstracts

(In order of presentation in the program)

KEYNOTE TALK

IMPROVING PHOTOSYNTHETIC EFFICIENCY FOR GREATER YIELD

Donald Ort

University of Illinois, Department of Plant Biology,
USDA/ARS Photosynthesis Research Unit, Urbana, IL 61801

Increasing the yield potential of the major food grain crops has contributed very significantly to a rising food supply over the past 50 years, which has until recently more than kept pace with rising global demand. Whereas improved photosynthetic efficiency has played only a minor role in the remarkable increases in productivity achieved in the last half century, further increases in yield potential will rely in large part on improved photosynthesis. Here we examine inefficiencies in photosynthetic energy transduction in crops from light interception to carbohydrate synthesis, and how classical breeding, systems biology, and synthetic biology are providing new opportunities to develop more productive germplasm. Near-term opportunities include improving the display of leaves in crop canopies to avoid light saturation of individual leaves and further investigation of a photorespiratory bypass that has already improved the productivity of model species. Longer-term opportunities include engineering into plants carboxylases that are better adapted to current and forthcoming CO_2 concentrations, and the use of modeling to guide molecular optimization of resource investment among the components of the photosynthetic apparatus, to maximize carbon gain without increasing crop inputs. Collectively, these changes have the potential to more than double the yield potential of our major crops.

KEYNOTE TALK

THE IMPORTANCE OF THE PROTON CIRCUIT IN BALANCING PHOTOSYNTHESIS

David M. Kramer

Biochemistry and Molecular Biology, DOE-Plant Research Laboratory
Michigan State University, East Lansing, MI 48824

Photosynthesis powers essentially all life on the planet. It also involves the highest energy reactions of life. If these reactions are not properly controlled they can produce reactive side products that can damage cellular components. To protect themselves from such 'photodamage', plants purposely dump a large fraction of photosynthetic energy. Essentially, plants regulate energy capture to balance their competing needs for efficient photochemistry and avoidance of photodamage. This balance determines the efficiency and survival of plants and algae to changing environmental conditions, and is thus critical for production of both food and biofuel. My research aims to understand how this balancing occurs, how it evolved, and how it might be manipulated to increased photosynthetic efficiency. We know a great deal about the primary reactions of photosynthesis. The major open questions concern how the parts of the machines operate together in the living plant, i.e. how they are co-regulated to supply the correct amount of energy without self-destruction from high energy intermediates. In many respects this view leads directly back to biophysical questions,

To answer these questions, my laboratory has developed tools, techniques and basic knowledge that allow us to observe the energy capture and storage reactions of photosynthesis as they occur *in vivo*. Using these tools, we have delineated many of the mechanisms by which the light and dark reactions of photosynthesis are co-regulated. In particular, we have focused on the regulation of the proton circuit of photosynthesis, on how the ATP synthase regulates proton efflux to co-regulate the light and dark reactions and how cyclic electron transfer balances the output of ATP and NADPH to meet metabolic demands. The next step is to understand how these regulatory mechanism themselves are integrated with the rest of the plant to finely tune photosynthesis. This strategy requires an understanding of how photosynthesis is integrated into the rest of the plant. We are thus developing high-throughput photosynthetic phenomics facilities for both plants and algae, giving us the ability to rapidly observe the key regulatory steps of photosynthesis in many strains or mutants under many conditions, with complementary techniques to probe plant physiology, biochemistry, genetics, genomics, metabolomics and transcriptomics. This work is supported by grants from National Research Initiative (2008-35318-04665) from the USDA National Institute of Food and Agriculture,and the U.S. Department of Energy, Office of Science, Basic Energy Sciences Program (DE-FG02-04ERl5559)

EFFECTS OF CARBON DEPRIVATION ON CYCLIC ELECTRON TRANSPORT IN SYNECHOCYSTIS SP. PCC 6803

Steven C. Holland, Anthony D. Kappell, Robert L. Burnap

Dept. of Microbiology and Molecular Genetics; Oklahoma State University, 307 LSE, Stillwater, OK 74078

The cyanobacterial carbon concentrating mechanism (CCM) is an evolutionary adaptation that functions to sequester CO_2 in low inorganic carbon environments. Comprising part of the CCM in *Synechocystis* sp. PCC 6803, are two constitutive and three inducible carbon sequestering protein complexes (1). The inducible CCM genes are controlled by two LysR-type transcriptional regulators: CmpR and CcmR (NdhR). CcmR represses the transcription of five genes/operons involved in the CCM, including a Na^+-dependent HCO_3^- transporter and a NDH-1 type CO_2 hydrator (2, 3).

Bioenergetic effects that result from carbon depletion in *Synechocystis* have been studied using P700 absorbance and chlorophyll a (Chl) fluorescence. Cells were grown in high-carbon conditions, and a sample transferred to low inorganic carbon media and then monitored by a Walz DUAL-PAM fluorometer outfitted for chlorophyll fluorescence and P700 absorbance measurements. Illumination at growth light intensities produced carbon depletion of the medium due to photosynthesic inorganic carbon uptake. The sample was exposed to growth light intensities overnight with intermittent measurement of Chl fluorescence and P700 kinetics.

Comparisons between wild-type and Δ*ccmR* P700 absorbance kinetics before and after carbon depletion have been made. The onset of carbon deprivation leads to a more reduced plastoquinone pool and a change in cyclic electron transport behavior. There was little change in P700 kinetics during saturating pulses in the dark, while P700 kinetics during the presence of an actinic light showed a distinct difference in absorbance patterns before and after depletion. After carbon depletion, P700 kinetics of wild-type cells taken in the presence of an actinic light were similar to those of the *ccmR* mutant. An interesting reduction event can be seen in kinetic traces of saturating pulses given in the dark (see figure), with a delay of rereduction in the mutant. It is likely that these changes are due to constitutive expression of the NdhD3/F3/CupA NDH-1 type CO_2 hydration protein complex in the mutant.

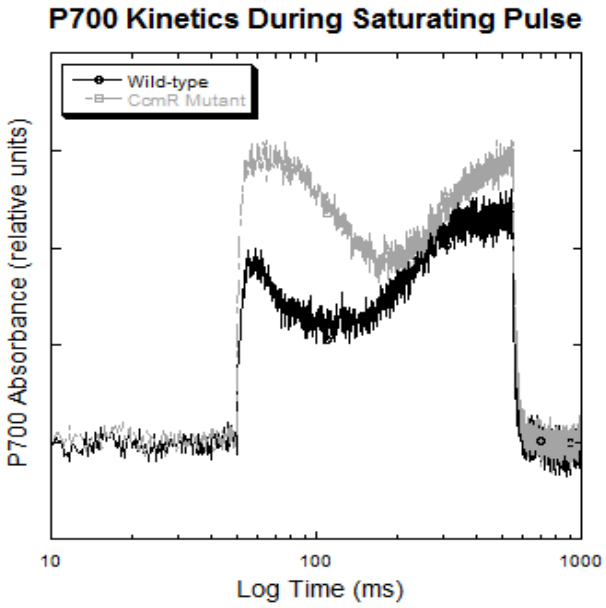


Figure: Kinetics traces of wild-type and mutant strains during a saturating pulse given in the dark. Five saturating pulses were averaged for each trace.

Refs:
(1) Reviewed in: Badger *et al.* (2006), *J. of Exp. Bot*; 57(2); 249-265
(2) Figge *et al.*, (2001) *Molecular Microbiology*; 39(2); 455-468
(3) Wang *et al.*,(2004) *J. of Biol. Chem*; 209(7); 5739-5751

Talk: Saturday 8:50 AM

Probing the Consequences of the CO_2 Concentrating Mechanism and CO_2 Responses on the Photosynthetic Circuit

Ben F. Lucker[1,2] and David M. Kramer[1,2]

[1]Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA.
[2]Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA.

Although CO_2 diffusion in an aqueous environment is 10,000 times slower than in air, algae are capable of biomass productivity rates higher than land plants. An important tool algae use to accomplish this high growth rate is through a CO_2 concentrating mechanism (CCM). Two important features of the CCM are carbonic anhydrases (CAs) and transporters. Algae actively harvest inorganic carbon (C_i) by conversion of CO_2 s to HCO_3^- using CAs and transport HCO_3^- into the chloroplast stroma prior to fixation. Once concentrated, stromal C_i is predicted to enter the acidified thylakoid lumen and utilize the protons for dehydration of HCO_3^- to CO_2. $CO_2$is subsequently shuttled into the pyrenoid for fixation by RUBISCO. We are investigating the effects of induction of the CCM and CO_2 responses in *Chlamydomonas* and *Chlorella*. We have tested the current CCM model that lumenal protons are used for dehydration of HCO_3^- to CO_2 in both *Chlorella sorokiniana* and *Chlamydomonas*. Currently we are investigating how the CCM and CO_2 responses affect the photosynthetic circuit, particularly, induction of cyclic electron flow and state transitions.

METABOLIC ATTRIBUTES OF *CYANOTHECE*, A GROUP OF UNICELLULAR NITROGEN FIXING CYANOBACTERIA

Anindita Bandyopadhyay[a], *Thanura Elvitigala*[a], *Eric Welsh*, *Jana Stöckel*[a], *Michelle Liberton*[a], *Hongtao Min*[c], *Louis A. Sherman*[c], *and Himadri B. Pakrasi*[a]

Department of Biology, Washington University, St. Louis, Missouri, USA[a]; Biomedical Informatics Core, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA[b]; and Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA[c]

Cyanothece is a genus of morphologically diverse and ecologically versatile unicellular cyanobacteria. Various studies have shown that members of this genus play important roles in the marine ecosystem, impacting the global nitrogen and carbon cycle. A systems-level analysis of *Cyanothece* 51142, a prototypic member of this group isolated from the Texas Gulf coast, revealed interesting metabolic attributes, which led to the establishment of an efficient hydrogen production system in this unicellular diazotroph. To explore the metabolic potentials of this class of cyanobacteria, five additional *Cyanothece* strains were sequenced to completion. Genome analysis uncovered the presence of a large and contiguous nitrogenase gene cluster in four of the five newly sequenced strains. These strains also exhibit aerobic nitrogen fixation, distinguishing *Cyanothece* as a genus of unicellular, aerobic nitrogen-fixing cyanobacteria. *Cyanothece* cells generate an anoxic intracellular environment at night, conducive for oxygen-sensitive processes like nitrogen fixation and hydrogen production. During the day, the cells photosynthesize to accumulate large carbohydrate reserves, thereby ensuring sufficient energy for the processes that require the anoxic phase of the cells. Our study indicates that this genus maintains a plastic genome, incorporating new metabolic capabilities while simultaneously retaining archaic metabolic traits, a unique combination which provides the flexibility to adapt to various ecological and environmental conditions. The contiguity of the nitrogenase gene cluster in *Cyanothece* sp. 7425 is interrupted by a 2.5Mbp insertion and this cluster exhibits a rearrangement of the *nif* genes as seen in some of the anaerobic nitrogen fixing *Synechococcus* strains. *Cyanothece* 7425 also appears to have lost its aerobic nitrogen-fixing ability, suggesting that an analogous rearrangement/disruption of the nitrogenase gene cluster might have been at play in cyanobacterial strains that eventually lost their nitrogen-fixing ability.

This work was supported by funding from DOE-BER (DE-FC02-07ER64694). The work conducted by the U.S. Department of Energy Joint Genome Institute was supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231.

THE PSBP DOMAIN 1 (PPD1) PROTEIN IS AN ESSENTIAL GENE INVOLVED IN CHLOROPLAST BIOGENESIS IN ARABIDOPSIS

Johnna L. Roose, Laurie K. Frankel, and Terry M. Bricker

Department of Biological Sciences, Biochemistry and Molecular Biology Section, Louisiana State University, Baton Rouge, LA 70803

Plants contain an extensive family of PsbP-related proteins termed PsbP-like (PPL) and PsbP domain (PPD) proteins, which are localized to the thylakoid lumen. The founding member of this family, PsbP, is an established component of the Photosystem II (PSII) enzyme, and the PPL proteins have also been functionally linked to photosynthetic processes. However, the functions of the PPD proteins remain poorly understood. To elucidate the function of the PPD1 protein in Arabidopsis (At4g15510), we have characterized two different T-DNA insertion lines of this gene. The *ppd1-1* allele (SALK_131704), whose insertion resides within the first exon of *PPD1,* is an embryonic lethal for homozygous individuals. Homozygotes for the *ppd1-2* allele (SALK_143493), whose insertion resides within an intron of *PPD1*, are variegated. While the green sectors of these individuals do not show significant photosynthetic defects, the yellow sectors have altered induction kinetics and significantly reduced variable fluorescence yield. Progeny of these variegated *ppd1-2* individuals yield extremely pale plants with very few green sectors. The photosynthetic phenotype of the *ppd1-2* individuals is correlated with a loss of normal chloroplast structure. We propose that the PPD1 protein is essential for normal chloroplast biogenesis in Arabidopsis.

FUNCTIONS OF THE DUPLICATED HIK31 OPERONS IN CENTRAL METABOLISM AND RESPONSES TO LIGHT, DARK AND CARBON SOURCES IN *SYNECHOCYSTIS* SP. STRAIN PCC 6803.

Sowmya Nagarajan and Louis. A. Sherman.

Department of Biological Sciences, Purdue University, West Lafayette, Indiana- 47907.

There are two closely related (>95 % identical) *hik31* operons involved in signal transduction on the chromosome and the pSYSX plasmid in the cyanobacterium *Synechocystis* sp. PCC 6803. We studied the growth, cell morphology and gene expression in operon and *hik* mutants for both copies, in different growth conditions, to examine whether the duplicated copies have the same or different functions and gene targets, and whether they are similarly regulated. Phenotype analysis suggested that both operons regulated common and separate targets in the light and the dark. The chromosomal operon (C3) was involved in negative control of autotrophic events including photosynthesis, whereas the plasmid operon (P3) was involved in positive control of heterotrophic events. Both the plasmid and double operon mutant cells were larger and had division defects. The growth data also showed a regulatory role for the chromosomal *hik* in high CO_2, and the plasmid operon in low O_2 conditions. Metal stress experiments indicated a role for both copies in mediating Ni, Zn and Cd tolerance. We conclude that both operons are differentially and temporally regulated. We suggest that the chromosomal operon is the primarily expressed copy and the plasmid operon acts as a backup to maintain appropriate gene dosages. Both operons share an integrated regulatory relationship, are induced in high light, glucose and in active cell growth. Additionally, the plasmid operon is induced in the dark with or without glucose. Microarray analysis of the wild type (WT) and the plasmid operon mutant (Δ*P3*) after addition of glucose in a 12 hour light-12 hour dark growth cycle revealed significant impact on transcription in the mutant. Gene categories that were up-regulated included several transporters and chaperones, whereas genes involved in photosynthesis, respiration, glycolysis, chemotaxis and cell division were down-regulated. We believe that the plasmid copy is an important regulator that controls metabolism at night and influences cell division and chemotaxis.

Fig.1

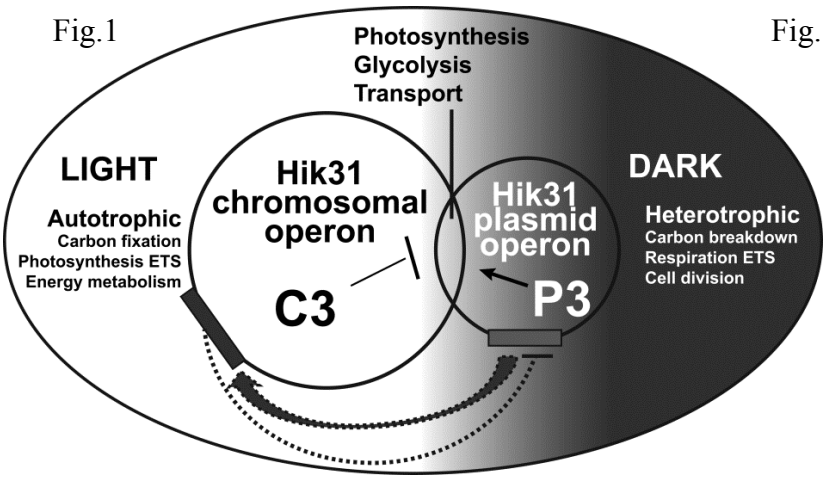


Fig. 2

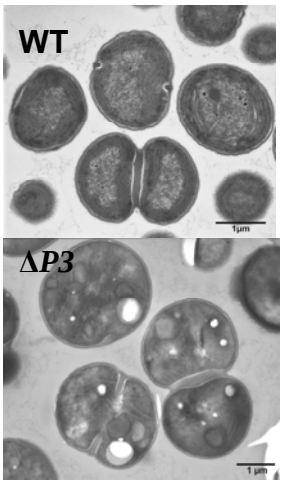


Figure 1. Regulatory relationship of the *hik31* operons representing the different and overlapping functions of C3 and P3 in the light and the dark. Figure 2. Transmission electron micrographs of *Synechocystis* sp. PCC 6803 WT and Δ*P3* grown under mixotrophic light dark conditions.

HETEROLOGOUS PRODUCTION OF PLANT SECONDARY METABOLITES CAFFEIC ACID AND RESVERATROL IN CYANOBACTERIA SYNECHOCYSTIS SP.PCC 6803

Yong Xue and Qingfang He

Department of Applied Science, University of Arkansas at Little Rock, Little Rock, AR

Caffeic Acid and Trans-Resveratrol are polyphenol compounds, produced by plant as secondary metabolites under stress conditions. They are known to exert beneficial effects on human health for their anticancer, anti-toxic and anti-inflammatory functions. Both Caffeic Acid and Resveratrol can be synthesized either from phenylalanine or from tyrosine by plants. Cyanobacteria *Synechocystis* is photoautotroph which can be used as a 'photo-bioreactor' for biosynthesis of nutriceuticals after genetic modifications.

C3H (p- coumarate 3- hydroxylase) is the enzyme converting p-coumarate into caffeic acid. *ref8* gene (coding for C3H) was cloned and integrated into the genome of *Synechocystis* sp. PCC 6803. PCR was done to verify the insertion of *ref8* gene. Expression of *ref8* in *Synechocystis* sp. PCC 6803 were confrimed by SDS-PAGE and Western Blot. Upon feeding of p-coumarate to the culture, production of caffeic acid was detacted by HPLC and LC/MS.

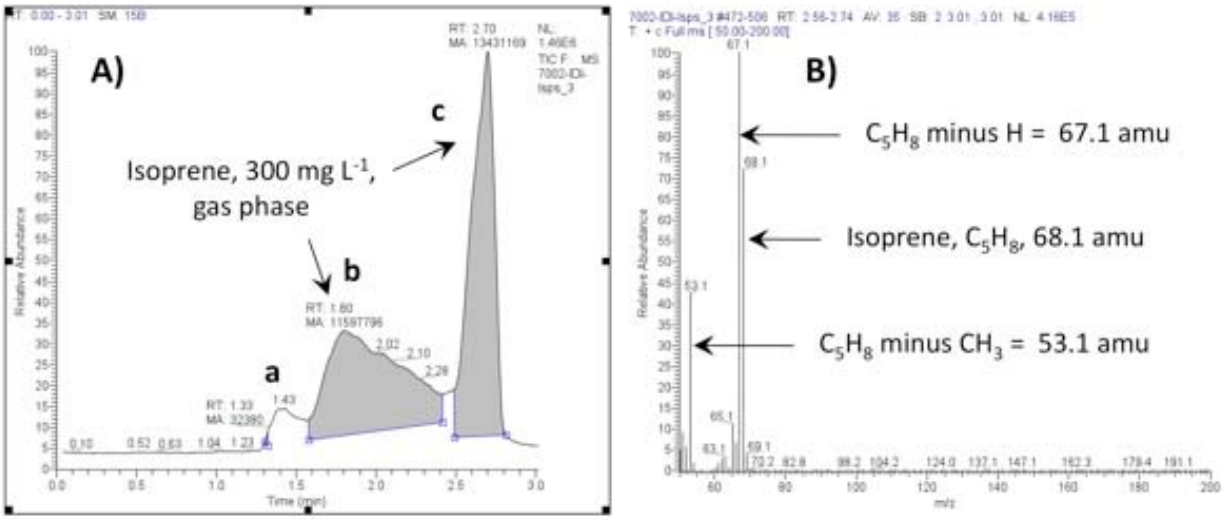
Three enzymes are involved in the pathway that converts tyrosine to resveratrol: TAL (Tyrosine ammonia-lyase), 4CL (Coumaroyl-CoA ligase), and STS (Stilbene synthase). Codons of these genes (*TAL* from *Saccharothrix espanaensis*, *4CL* from *Nicotiana tabacum* and *STS* from *Vitis vinifera*) were optimized to enhance their expression in *Synechocystis* sp. PCC 6803. Modified TAL, 4CL and STS genes were assembled together into an expression plasmid pACYCDuet-1, which was transformed into *E. coli* BL21 DE3 strain. Resveratrol was detected by HPLC and verified by LC/MS from culture medium of *E.coli* growing in 2xYT medium without adding any substrates. Formation of p-coumaric acid, the immediate precursor for resveratrol biosynthesis, was also found, and its amount decreased as biosnthesis of resveratrol proceeds. Construction of *Synechocystis* sp. PCC 6803 mutant expressing these three genes is in progress.

METABOLIC ENGINEERING OF ISOPRENE PRODUCTION IN A RAPIDLY GROWING, HIGH-LIGHT TOLERANT CYANOBACTERIUM

Matthew E. Nelson[1], Eric L. Singsaas[2] & Toivo Kallas[1]

[1]Department Biology-Microbiology, University of Wisconsin Oshkosh & [2]Wisconsin Institute for Sustainable Technology, University of Wisconsin Stevens Point

Because of escalating costs, climate change, and political uncertainty associated with fossil fuels, the development of carbon-neutral biofuels is a national-security imperative. Microalgae can help meet this need because they efficiently capture enormous amounts of solar energy and convert atmospheric carbon dioxide (CO_2) into carbon polymers. Modification of these pathways holds great potential for production of feedstock chemicals and carbon-neutral biofuels. One such feedstock is isoprene (C_5H_8, 2-methyl 1,3-butadiene), a precursor for synthetic rubber and high-density, liquid aviation fuels. Our goal is to produce isoprene in *Synechococcus* PCC 7002, a rapidly growing, high-light and halo-tolerant cyanobacterium. Isoprene can be made via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway whose products are isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Cyanobacteria possess the MEP pathway but lack the key isoprene synthase (IspS) enzyme for converting DMAPP into isoprene. We have introduced and expressed 'codon-optimized' *ispS* and MEP pathway genes in *Synechococcus* to generate strains that produce isoprene by solar energy conversion of CO_2 sources at rates that are promising for commercial development.



Gas chromatography-mass spectrometry (GC-MS) analysis of isoprene production by *Synechococcus* sp. PCC 7002. Panel A: GC-MS chromatogram showing isoprene in the head-space of a *Synechococcus* culture expressing a codon-optimized *IspS* gene. Panel B: Fragment ion spectrum from GC peaks a and b showing that both contain isoprene (58.1 amu).

SPECTROSCOPIC CHARACTERIZATION OF THE WATER OXIDATION INTERMEDIATES IN THE RU-BASED CATALYSTS FOR ARTIFICIAL PHOTOSYNTHESIS

Dooshaye Moonshiram[1], *Jonah W.Jurss*[2#], *Javier Concepcion*[2], *Thomas Meyer*[2], *and Yulia Pushkar*[1]

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Utilization of sunlight requires solar capture, light-to-energy conversion and storage. One effective way to store energy is to convert it into chemical energy by fuel-forming reactions, such as water splitting into hydrogen and oxygen. Ruthenium complexes are among few molecular-defined catalysts capable of water splitting. Insight into the mechanism of their action will help to design future robust and economically feasible catalysts for light-to-energy conversion. Mechanistic insights about the design of such catalysts can be acquired by spectroscopic analysis of short-lived intermediates of catalytic water oxidation. Use of techniques sensitive to the electronic states of molecules such as EPR and X-ray absorption spectroscopy (XAS) is crucial to determine the electronic requirements of catalytic water oxidation.

About 30 years ago Meyer and coworkers reported the first ruthenium-based catalyst for water oxidation, known as the "blue dimer"[1,] *cis*,*cis*-$[(bpy)_2(H_2O)Ru^{III}ORu^{III}(OH_2)(bpy)_2]^4$. We performed EPR studies and characterized structures and electronic configurations of intermediates of water oxidation by the "blue dimer". The intermediates of water oxidation were prepared chemically by oxidation of Ru-complexes with defined number of Ce (IV) equivalents and freeze-quenched at controlled times. Changes in the oxidation state of the Ru atom were detected by XANES at Ru K-edges. We demonstrated that K-edges are very sensitive to changes in Ru oxidation state for Blue Dimer $[3,3]^{4+}$, $[3,4]^{4+}$,$[3,4]'^{4+}$ and $[4,5]^{3+}$ thus allowing a clear assignment of Ru oxidation state in intermediates. EXAFS at Ru K-edge demonstrated clear structural changes in the oxidation states. EXAFS analysis also showed a considerably modified ligand environment in [3,4]' suggesting formation of a peroxidic ligand. Under the conditions of the experiment, the [3,4]' intermediate is the catalytic steady state form of the blue dimer catalyst, suggesting that it is either the active form of the catalyst or that oxidation of this intermediate is the rate limiting step.

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A Robust PS II Mimic in Photo Water Splitting: Combination of Manganese-oxo Oligomer Complex and Tungsten Oxide Semiconductor

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Photosynthesis provides an excellent example for utilizing solar energy to meet the global need on the large scale. The decomposition of a synthetic Mn-oxo mix-valence dimeric complex, $[OH_2(terpy)Mn(O)_2Mn(terpy)OH_2](NO_3)_3.6H_2O$, generated a highly active catalytic material, Mn-oxo oligomer. In this work we fabricated a novel Mn-oxo compound/tungsten oxide (WO_3) catalytic system for efficient photo water splitting (Figure 1). The Mn-oxo compound/WO_3 system was able to directly generate oxygen and hydrogen for solar energy harness. The hydrogen to oxygen ratio, O-18 water experiment, and photocurrent calculation demonstrated that the oxygen and hydrogen molecules are caused by water splitting. The experimental results also demonstrated that the water splitting reaction requires the cooperation of Mn-oxo catalytic material and WO_3 semiconductor. In PS II, sunlight energy is absorbed and induced charge separation through P_{680} and Pheo. The positive charge at P_{680}^+ is neutralized by receiving electrons from water via Mn_4Ca cluster as a water oxidant. In the Mn-oxo oligomer/WO_3 system, WO_3 absorbs light photon and generate electron and hole in a similar way as P_{680} in PS II. The electron is transferred to the cathode for H_2 production. The design described here takes advantage of highly catalytic activity of Mn-oxo compound and exceedingly efficient holes production by n-type semiconductor such as WO_3. Our design will not only produce new materials for efficient water splitting, but also establish new material design methodologies that can be extended to a wide range of combinations.

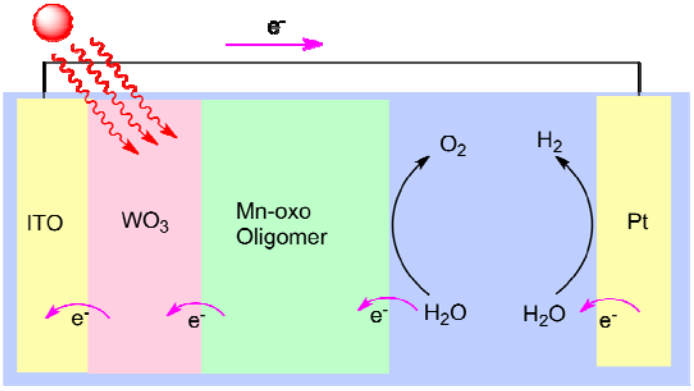

Figure 1. A Schematic of Mn-oxo Oligomer/Tunsgen Oxide Catalytic System Mimicking the Photosynthetic Water Splitting in PS II OEC.

MULTIPLE RADICAL SCAVENGER PATHWAYS FOR THE ESSENTIAL TYROSYL RADICAL IN RIBONUCLEOTIDE REDUCTASE

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Ribonucleotide reductase (RNR) catalyzes the rate limiting step for the conversion of ribonucleotides to deoxynucleotides in all organisms. Class Ia RNR is found in bacteria and mammals and contains a catalytically-essential tyrosyl radical, Y122•, in the β2 subunit. Y122• is the radical initiator in substrate reduction and is generated by a nearby diiron cluster. Since RNR plays a pivotal role in DNA biosynthesis, RNR inhibitors are important as anti-cancer agents. Radical scavengers inhibit RNR by reduction of Y122•. However, the mechanism of this process, which involves a proton coupled electron transfer (PCET) reaction, is not yet understood. We provide evidence for distinct radical scavenger PCET pathways in the class Ia β2 subunit from *E. coli*. Three different inhibitors, hydroxyurea (HU), N-methylhydroxylamine (NMHA), and 4-methylphenol (4MP), were employed. The dependence of the reaction rate on solvent isotope exchange and temperature was investigated by UV-Vis spectroscopy, which monitors the decay of Y122•. The solvent isotope effects were significantly different for the three inhibitors, with values of 15 ± 0.7 (HU), 5.2 ± 0.3 (NMHA) and 1.5 ± 0.2 (4MP). The activation energies were also significantly different when HU (79 ± 2 kJ/mol) and NMHA (83 ± 2 kJ/mol) were compared to 4MP (108 ± 2 kJ/mol). Reaction-induced Fourier transform infrared spectroscopy was then used to define the protein dynamics, which are coupled with Y122• reduction. While the amide I (1650 cm^{-1}) regions of the data were similar, the 1600-1300 cm^{-1} spectral regions were distinct for the three inhibitors. The vibrational spectra were consistent with shifts in carboxylate ligation to the metal cluster upon Y122• reduction. This work supports the conclusion that there are distinct PCET pathways, which involve different conformational interactions between Y122• and carboxylate ligands to the diferric cluster.

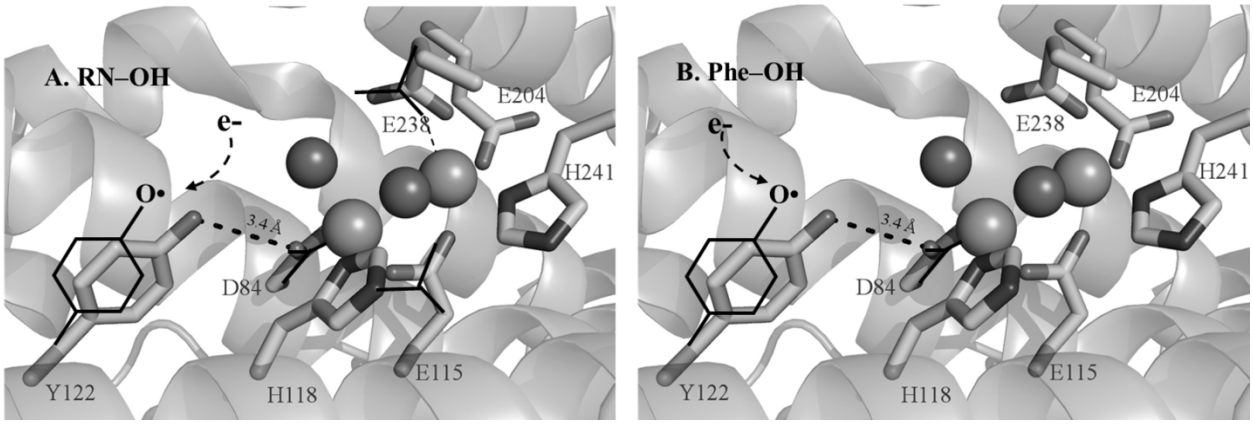

Figure 1. Hypothetical pathway and proposed protein structural changes for Y122• reduction with (A) hydroxyurea/NMHA or (B) 4-methoxyphenol. The diagrams of the crystal structure are adopted from PDB# 1MXR. Black sticks represent putative conformational relaxation events from the Y122• state. The different directions of the electron (e^-) represent alternative pathways during tyrosyl radical reduction for the three inhibitors.

CONFORMATIONAL SWITCHING ACCOMPANYING PCET IN PHOTOSYSTEM II INSPIRED BIOMIMETIC PEPTIDES PROBED BY UVRR

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Proton coupled electron transfer (PCET) reaction plays a critical role in many important biological processes including photosynthetic oxygen evolution, DNA synthesis and respiration. PCET reactions involving redox-active tyrosine residues are important not only in photosytsem II (PS II) but also in ribonucleotide reductase (RNR), galactose oxidase, cytochrome $P450_{cam}$, cytochrome *c* oxidase and amine oxidase. In the present study, PCET of tyrosine-containing peptide maquettes designed to mimic the D1 and D2 polypeptides in PS II are probed using UV Resonance Raman Spectroscopy (UVRR). Interstrand PCET between a pi-stacked histidine and tyrosine is initiated by ultra-violet photolysis of the tyrosine residue. The spectrum associated with the tyrosyl radical, generated in the process, is obtained using difference spectroscopy. The spectra of the radical from tyrosine and tyrosine-histidine dipeptide are also obtained for comparison. The spectra of the model peptides obtained at pD 11 have features that are similar to those of the control (tyrosine and tyrosine-histidine dipeptide) while those obtained at pD 8.5 exhibit new intense bands at 1441 cm^{-1} and 1472 cm^{-1}. We believe that these bands arise as a consequence of a conformational change accompanying the PCET reaction. Evidence leading to the assignment of these bands will be presented. This project is supported by NIH GM43273.

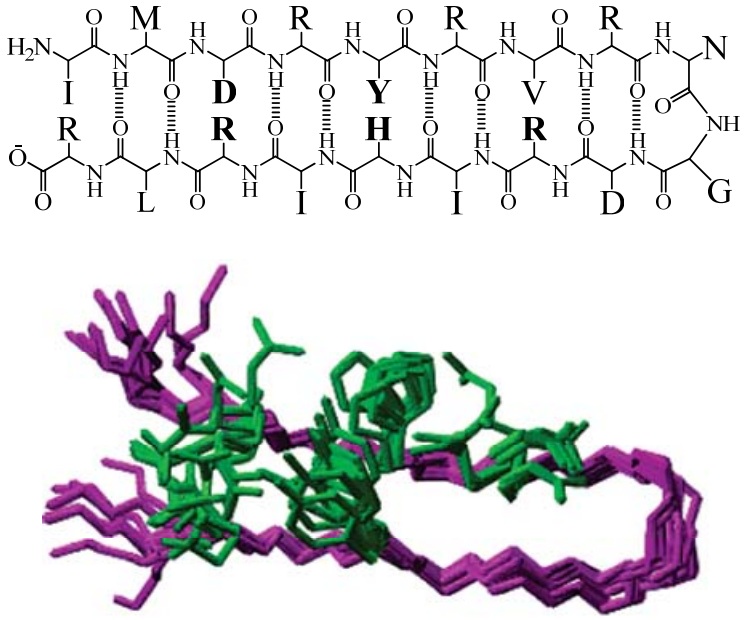

Figure 1. (Top) Primary sequence of one of the peptides (Peptide A) used in the study showing the cross-strand interactions (**bold letters**) of Tyr 5 (Y5) with His 14 (H14), Arg 12 (R12) and Arg 16 (R16). (Bottom) Overlap of the 20 lowest energy structures derived from 2D NMR Spectroscopy showing the residues involved in the cross-strand interaction with tyrosine. The structure also shows interaction between Asp 3 (D3) and Arg 16 (R16) (Sibert *et al*., *J. Am. Chem. Soc*. **2007**, 129, 4393-4400.

Detection of water binding sites in the oxygen-evolving complex of photosystem II poised in the S2 state using high field (94 GHz) 17O-ELDOR detected NMR spectroscopy

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The first definitive assignment of water-exchangeable oxygen's magnetically coupled to the Mn_4O_5Ca cluster of the photosystem II complex (PSII) poised in the S_2 state are reported. PSII samples were suspended in $^1H_2{}^{17}O$ and $^2H_2{}^{16}O$. Hyperfine couplings of coordinating ^{17}O (I = *5/2*) nuclei were detected using 94 GHz (W-band) ELDOR detected NMR and Davies/Mims ENDOR spectroscopies. Three classes of nuclei are identified: i) μ-oxo bridge(s); ii) terminal Mn-OH/OH_2; and iii) Ca/2nd shell-–OH/H_2O ligand(s). These assignments are based on ^{17}O model complex data and on comparison to the recent 1.9 Å resolution PS II crystal structure [Umena, Y. et al 2011, Nature, 473:55-60]. Universal ^{15}N (I = *1/2*) labeling was employed to uniquely identify the 17O couplings which overlap with background ^{14}N (I = *1*) signals. The relative orientation of the putative ^{17}O μ-oxo bridge hyperfine tensor to the $^{14}N/^{15}N$ hyperfine tensor of the background signal, the Histidine 332 ligand of Mn_{D1} [Stich, T. et al. Biochemistry 2011 50 (34), 7390-7404], suggests the exchangeable μ-oxo bridge links the outer Mn to the Mn_3O_3Ca open-cubodial unit. $^1H/^2H$ (I = *1/2, 1*) ENDOR data performed at 34 GHz and 94 GHz (Q and W-band) complement the above findings. The relatively small $^1H/^2H$ couplings observed requires that all the μ-oxo bridges of the Mn_4O_5Ca cluster are deprotonated in the S_2 state. Together, these results further refine the reaction pathway of O-O bond formation, supporting an oxo/oxyl coupling mechanism in S_4.

SINGLE AMINO ACID SUBSTITUTIONS TO THE CP43 SUBUNIT OF PHOTOSYSTEM II INHIBIT OXYGEN EVOLUTION AND ALLOW THE OBSERVATION OF INTERMEDIATES IN THE FORMATION AND RELEASE OF O2

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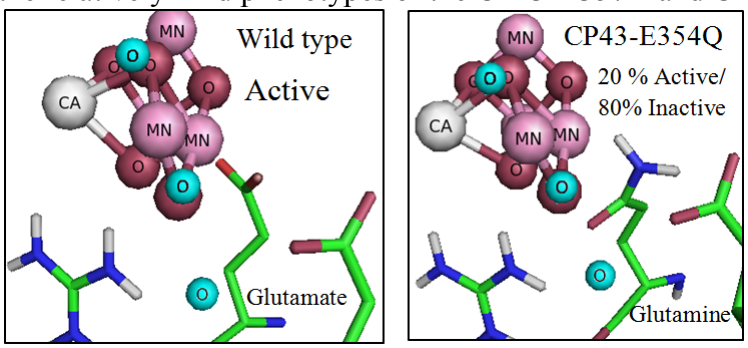
The active site water oxidation in PSII is an oxidized manganese calcium cluster (Mn_4CaO_5) that accumulates oxidizing equivalents as it undergoes a series of four redox state transitions. To prevent the accumulation of a positive charge on the Mn_4CaO_5, which would inhibit oxygen evolution, the release of protons from the water oxidation complex (WOC) is facilitated by a catalytic base that is formed by the deprotonation of the residue CP43-Arg357(1, 2), which would in turn, feed protons into a proton exit pathway beginning with the residue D1-Asp61. Mutant strains of *Synechocystis* sp. PCC 6803 where CP43-Arg357 had been substituted with serine or lysine have shown weaker S-state cycling and slower O_2 release kinetics (3, 4). Here the O_2 release kinetics of the CP43-R357K mutant have been further characterized using a high time resolution O_2 sensing bare platinum electrode with an improved technique that allows for the observation of a lag in the onset of O_2 release. A similar lag phase has been previously observed in the D1-D61N mutant (5) and may be attributable to the slowed release of the first of two protons during the S_3-S_0 transition. The E354Q mutant has an especially odd phenotype, in that while it possesses roughly the same number of fully assembled Mn_4CaO_5 clusters, it produces only 10-20% of the total amount of O_2 that wild type PSII produces and unlike other PSII mutants the oxygen evolving activity of E354Q is identical to wild type PSII in terms of O_2 release kinetics and S-state cycling efficiency (6). Here the O_2 release kinetics and S-state cycling of the CP43-E354Q mutant have been measured at various pH values, showing that the minority population of CP43-E354Q PSII centers that are capable of evolving oxygen have decreased S-state cycling and slowed O_2 release kinetics (including an observable lag phase) compared to wild type PSII centers in low pH buffers. These results will also be compared with the relatively mild phenotypes of the CP43-R357H and CP43-E354R mutations.



Substitution of the Mn_4CaO_5 ligand CP43-E354 with glutamine (right) results a loss of 80-90% of O_2 producing activity compared to wild type PSII (left), without significantly disturbing formation of the Mn_4CaO_5, O_2 release kinetics or S-state cycling.

1. L. I. Krishtalik, *Biochimica Et Biophysica Acta* **849**, 162 (APR 2, 1986).
2. J. P. McEvoy, G. W. Brudvig, *Chemical Reviews* **106**, 4455 (NOV, 2006).
3. G. Ananyev, T. Nguyen, C. Putnam-Evans, G. C. Dismukes, *Photochemical & Photobiological Sciences* **4**, 991 (DEC, 2005).
4. H. J. Hwang, P. Dilbeck, R. J. Debus, R. L. Burnap, *Biochemistry* **46**, 11987 (Oct 30, 2007).
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6. M. A. Strickler *et al.*, *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**, 1179 (Mar 27, 2008).

Talk: Saturday 8:20 PM

N-formylkynurenine, photoinhibition, and D1 polypeptide turnover in photosystem II

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Photosystem II (PSII) catalyzes photo-induced water oxidation at a Mn_4Ca cluster in plants, algae, and cyanobacteria. The D1, D2, CP43, CP47, and extrinsic polypeptides form the core of the reaction center. In our previous work, a post-translational modification of Trp-365 to doubly oxidized N-formylkynurenine (NFK) (Fig. 1A) was identified in the CP43 subunit from spinach PSII membranes (1). An approximate two-fold increase in the yield of NFK occurred under photoinhibitory, high light stress conditions, as determined by a quantitative HPLC assay (Fig. 1A) (1). During photoinhibition, D1 is known to exhibit a high rate of turnover. In our current studies, we show that NFK accumulation is associated with D1 degradation, as assessed by Western blot analysis. We report that inactivation of water oxidation inhibits the light-induced NFK increase, which suggests involvement of donor-side reactive oxygen species in NFK formation. We find that NFK is present in spinach thylakoid membranes, as well as detergent-isolated PSII membranes. Trp-365 is conserved in plants and cyanobacteria (3) and is located ~18 Å from the Mn_4Ca in the 1.9 Å PSII structure (Fig. 1B). We report that site-directed mutants at Trp-365 in *Synechocystis* 6803 exhibit increased rates of photoinhibition, and we present data concerning the light intensity dependence of this inhibitory process. These data support the conclusion that NFK acts as a signaling molecule during high light stress and photoinhibition.

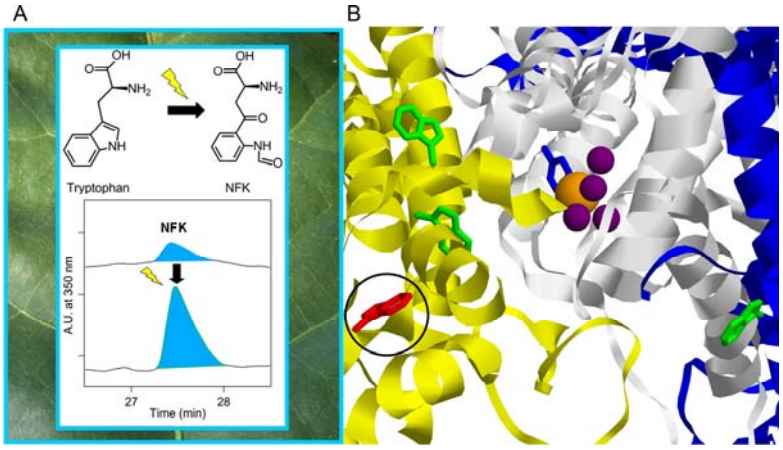

Figure 1. Trp-365 oxidation to N-formylkynurnine (NFK). The top portion of *A* shows the light-induced oxidation reaction of Trp to NFK. The bottom of *A* demonstrates the quantitative two-fold increase in NFK yield assessed by the HPLC chromatogram at 350 nm. *B* shows the location of Trp-365 (circled) in the 1.9 Å resolution structure from *T. vulcanus* (2).

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2. Umena Y, Kawakami K, Shen J-R, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473:55-60
3. Anderson LB, Maderia M, Ouellette AJA, Putnam-Evans C, Higgins L, Krick T, MacCoss MJ, Lim H, Yates JR III, Barry BA (2002) Posttranslational modifications in the CP43 subunit of photosystem II. *Proc Natl Acad Sci U S A* 99:14676-14681

Talk: Saturday 8:40 PM

EXPRESSION OF ALTERNATE FORMS OF *PSBA2* GENE FROM NATIVE AND ECTOPIC LOCATION TO STUDY THEIR EFFECTS ON THE D1 TURNOVER PROCESS IN *SYNECHOCYSTIS* SP. PCC 6803

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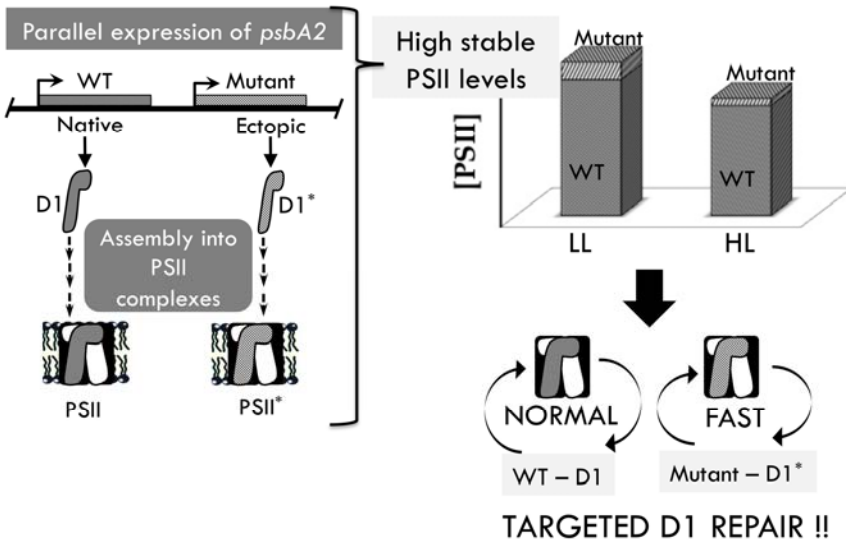
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078.

Photosystem II (PSII) is recognized as the main site for high light induced damage during the water oxidation process causing the impairment of PSII activity. One of the core subunits of PSII, D1 protein encoded by the *psbA* gene, is identified as a high turnover protein that undergoes degradation and replacement as a part of the repair process in PSII. Studies on D1 repair have shown the synchronous nature of D1 degradation and synthesis. Therefore, it is believed that damaged D1 is replaced by newly synthesized D1[1]. Although, it has not been shown if photodamage triggers the repair of only damaged D1 subunits in a targeted fashion excluding the pool of undamaged D1 subunits. Alternatively, it could trigger the replacement of all D1 subunits in a random and more generalized manner.

An attempt is being made to address this question of targeted versus generalized D1 repair using a dual D1 expression system. A strain expressing two *psbA2* genes from native and ectopic location was constructed using chemical synthesis, fusion PCR and traditional cloning. The D1 ectopic strain was constructed at first by transforming a triple *psbA* deletion strain with a chimeric *psbA2* gene[2]. Transformation of the D1 ectopic strain with the plasmid bearing *psbA2* gene resulted in a strain consisting of two copies of *psbA2* genes. Additionally, strains have been constructed with mutant *psbA2* having higher rates of D1 turnover expressing from native in parallel with WT *psbA2* expressing from the ectopic location. Therefore, these dual D1 strains (2D1 strains) have alternate forms of *psbA2* genes with different rates of D1 turnover. Evaluation of the PSII activity in these strains using fluorescence and oxygen evolution show similar levels of PSII in all the 2D1 strains. Similar rates of photoinhibition and recovery were also observed for these strains. These results and others collectively imply that mutant D1 (high turnover form) does not form a part of the PSII holocomplex providing early indications that D1 degradation process might be targeted towards damaged D1 proteins only. This could be important to understand the D1 signaling process occurring during repair.



Schematic representation of dual D1 expression system developed to address targeted versus generalized D1 repair

Ref 1) Reviewed in Adir et al. Photosynthesis Research (2003), 76: 343–370
Ref 2) Nagarajan et al. J. Photochem Photobiol B. (2011), 104(1-2): 212-9

NATIVE MASS SPECTROMETRY OF MEMBRANE-BOUND PROTEIN-PIGMENT COMPLEXES SUGGESTS INDUCED COFACTOR DISSOCIATION

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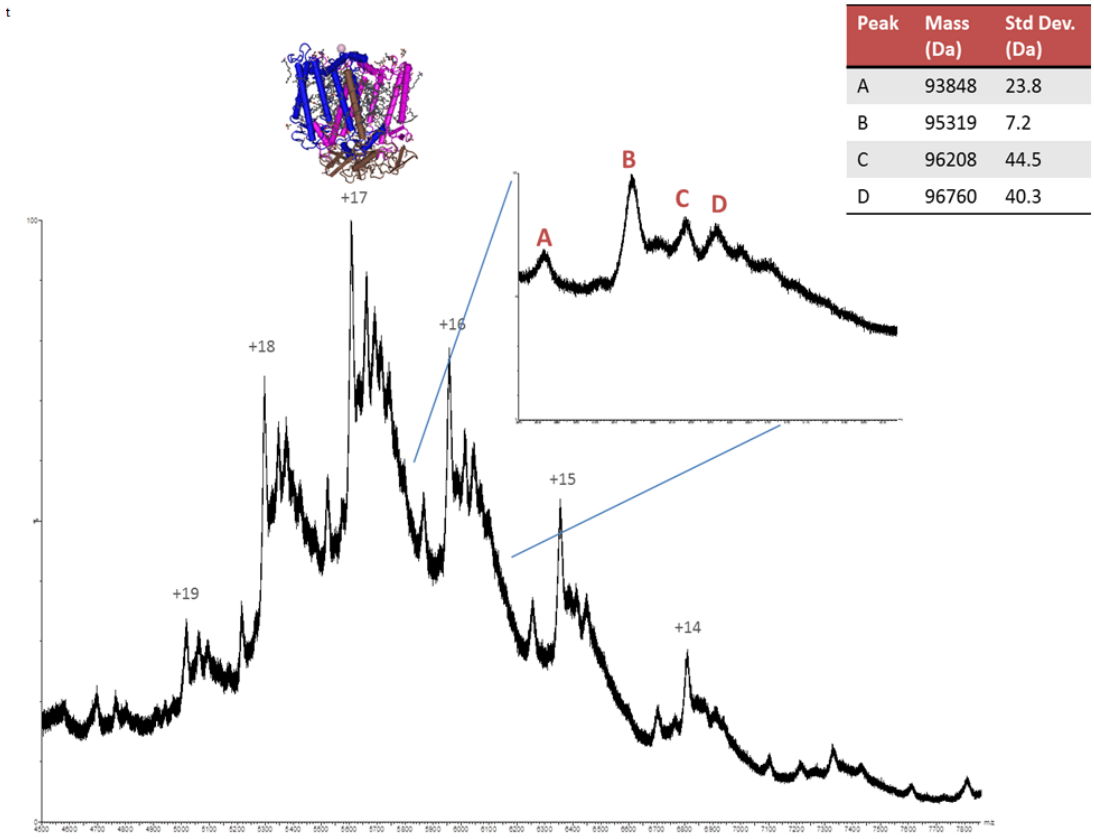
Mass spectrometry of large protein complexes in their native form is a developing technology that holds enormous promise as a high-throughput tool for both structural biology and proteomics. Direct analysis of protein complexes in their near native form can provide stoichiometric as well as structural information. However, with a few notable exceptions, its application has been restricted to soluble complexes, due to the challenges posed by the requirement for detergent to stabilize membrane-bound complexes in solution. Here, we demonstrate the potential of this technique to characterize membrane-bound protein-pigment complexes, using purified reaction centers from the purple photosynthetic bacterium *Rhodobacter sphaeroides* as a model. Reaction centers were purified and solution conditions (including the concentration of the protein, ammonium acetate and detergent, as well as detergent type) were optimized. Following introduction of the complex into the gas phase via electrospray ionization, high energy was applied at both the source and collision cells in order to strip away bound detergent molecules. When total energy applied exceeded the optimal desolvation conditions, significant loss of pigment and non-pigment cofactors was observed, suggesting partial unfolding of the complex. These developments open up native mass spectrometry to other membrane-bound photosynthetic complexes.



Peak	Mass (Da)	Std Dev. (Da)
A	93848	23.8
B	95319	7.2
C	96208	44.5
D	96760	40.3

STRUCTURAL ANALYSIS OF A DEPSIPEPTIDE IN THE LIPID MEMBRANE

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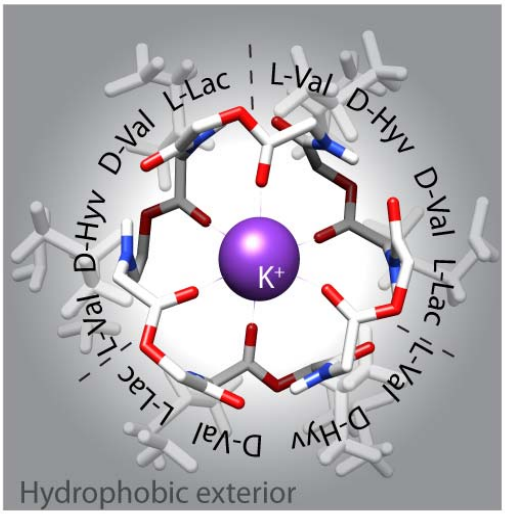
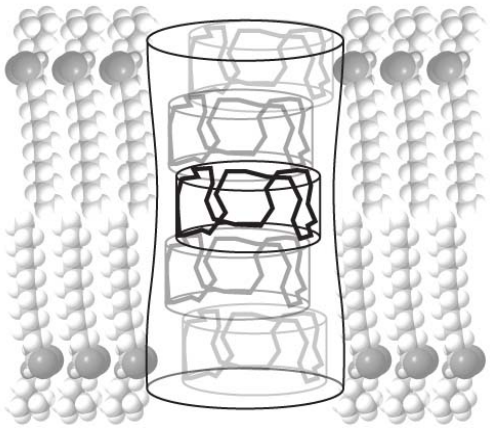
Characterization of protein solution structure in the membrane has enjoyed much recent success with X-ray diffraction and NMR techniques, but still suffers limitations with respect to protein size, conformational flexibility, and need for sample modification. Recent investigations using deep-UV resonance Raman (DUVRR) spectroscopy have made headway into probing secondary structure in the membrane without the need for sample modification or high concentrations. Already highly accurate for quantifying protein secondary structure of globular proteins, DUVRR is selective and sensitive to the peptide backbone without significant interference from water or lipid. Here we investigate the solution conformation of valinomycin, a depsipeptide used to collapse the $\Delta\psi$ across a membrane. Previous characterization of valinomycin has primarily relied on solution studies in organic solvent. In the presence of both micelles and small unilamellar vesicles, and also upon complexation with potassium, valinomycin undergoes subtle differences in conformation as revealed by DUVRR spectroscopy. Insights into the ionophoric mechanism, supported by CD, DLS and SAXS measurements, are discussed.





Left: Top view of potassium-complexed structure of valinomycin, with repeating individual residues L-valine (L-Val), D-hydroxyvaleric acid (D-Hyv), D-valine (D-Val), and L-lactic acid (L-Lac).
Right: The carrier mechanism of valinomycin across the lipid bilayer.

REGULATION OF THE CHLOROPHYLL ANTENNA SIZE IN *CHLAMYDOMONAS REINHARDTII BY TLA1* GENE OVER-EXPRESSION AND RNA INTERFERENCE

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TLA1 (Truncated Light harvesting Antenna mutant 1) is the first gene to be identified that has been shown to play a role in the signal transduction pathway involved in the Chl antenna size modulation in the green alga *Chlamydomonas reinhardtii*. In this study the *TLA1* gene was specifically genetically manipulated for over-expression and RNAi down-regulation to study its effect on the regulation of the Chl antenna size and chloroplast structure. Chlorophyll parameter analyses showed that *TLA1* RNAi mutants have smaller chlorophyll antenna sizes in both photosystems and lower levels of Chl *b* per cell relative to the wild type while the *TLA1* over-expressors have larger chlorophyll antenna sizes in both photosystems and higher levels of Chl *b* per cell relative to the wild type. Western analyses of the *TLA1* RNAi mutants and *TLA1* over-expressors show a significant reduction and over-expression of TLA1 and other photosynthetic proteins like Lhcb, D1, D2 and VIPP1, respectively. The level of NAB1, another cytosolic protein that has been identified previously to play a role in the regulation of the Chl antenna size, did not change significantly in the *TLA1* RNAi mutants and *TLA1* over-expressors. Bioinformatic analyses of the TLA1 protein sequence reveals a high degree of identity in the secondary structure organization of the TLA1 with that of the MOV34 domain containing proteins which are found in proteasome regulatory subunits, eukaryotic initiation factor 3 subunits and regulators of transcription factors. Immunolocalization and transmission electron microscopy studies show that the TLA1 protein is located in the chloroplast and plays a role in the structural organization of the thylakoid membranes in the chloroplast. (Supported by grants from US-DOE)

Expression and Characterization of the Diheme Cytochrome *c* Subunit of the Cytochrome *bc* Complex in *Heliobacterium modesticaldum*

Hai Yue[a], *Yisheng Kang*[b], *Hao Zhang*[a], *Xinliu Gao*[a] *and Robert E. Blankenship**[a,b]

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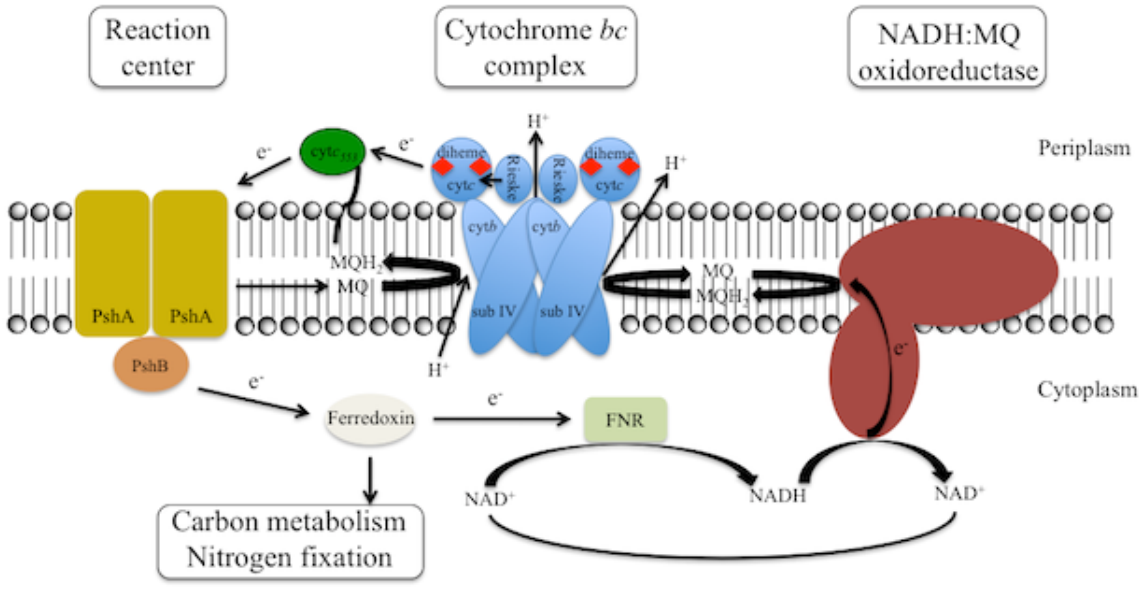
Heliobacterium modesticaldum is a gram positive, anaerobic, anoxygenic photoheterotrophic bacterium. Its cytochrome *bc* complex (Rieske/cyt *b* complex) has some similarities to cytochrome b_6f complexes from cyanobacteria and chloroplasts, and also shares some characteristics of typical bacterial cytochrome bc_1 complexes. One of the unique factors of the heliobacterial cytochrome *bc* complex is the presence of a diheme cytochrome *c* instead of the monoheme cytochrome *f* in the cytochrome b_6f complex or the monoheme cytochrome c_1 in the bc_1 complex. To understand the structure and function of this diheme cytochrome *c* protein, we expressed the N-terminal transmembrane-helix-truncated soluble *H. modesticaldum* diheme cytochrome *c* in *Escherichia coli*. This 25 kDa recombinant protein possesses two *c*-type hemes, confirmed by mass spectrometry and a variety of biochemical techniques. Sequence analysis of the *H. modesticaldum* diheme cytochrome *c* indicates that it may have originated from gene duplication and subsequent gene fusion, as in cytochrome c_4 proteins. The recombinant protein exhibits a single redox midpoint potential of + 71 mV vs NHE, which indicates that the two hemes have very similar protein environments.



CONSERVED LIPID FUNCTIONS IN CYTOCHROME *bc* COMPLEXES[1]

S. Saif Hasan[a], *Eiki Yamashita*[b], *Christopher M. Ryan*[c], *Julian P. Whitelegge*[c] *and William A. Cramer*[a]

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An understanding of structure properties of lipids internal to membrane proteins is essential to structure-function analysis of these proteins. Lipid binding sites and properties are compared using crystal structure information in cytochrome *bc* complexes-cytochrome b_6f of photosynthesis vs. cytochrome bc_1 of the yeast respiratory chain.[1] Comparison of lipid and detergent binding sites shows significant conservation of lipid positions. Significant overlap is observed between seven lipid binding sites in the cyanobacterial b_6f complex, three natural sites in the *Chlamydomonas reinhardtii* algal complex and four sites in the yeast mitochondrial bc_1 complex. The identity of the lipids is different: b_6f contains sulfoquinovosyl-diacylglycerol, phosphatidylcholine, phosphatidylglycerol, monogalactosyldiacylglycerol, and digalactosyldiacylglycerol, whereas cardiolipin, phosphatidylethanolamine, and phosphatidic acid are present in the yeast bc_1 complex. The lipidic photosynthetic pigments, chlorophyll-*a* and *β*-carotene and eicosane, are unique to b_6f. The following lipid functions can be inferred in the b_6f complex on the basis of sequence conservation, interatomic distances, and the *B*-factors of interacting lipid groups and coordinating amino acid residues: (**i**) substitution in b_6f of a lipid and chlorophyll chlorin ring for the 8th trans-membrane helix of the cytochrome *b* subunit in the bc_1 complex; (**ii**) lipid and *β*-carotene connection of peripheral and core trans-membrane domains; (**iii**) stabilization of Rieske iron-sulfur protein trans-membrane helix; (**iv**) n-side charge and polarity compensation in the trans-membrane domain; and (**v**) *β*-carotene mediated super-complex with photosystem I complex.

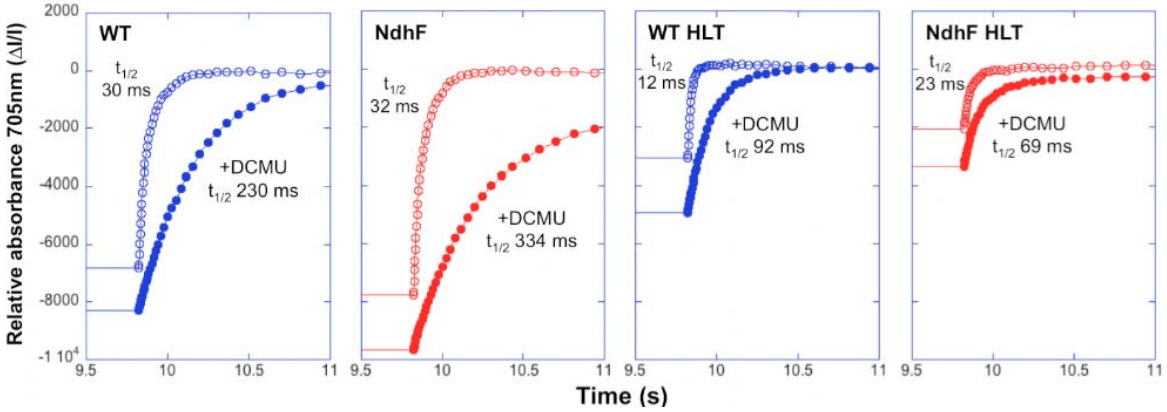
[1]J. Mol. Biol., 2011, *in press*; supported by NIH GM-38323.

CYCLIC ELECTRON TRANSFER PATHWAYS IN *SYNECHOCOCCUS* SP. PCC 7002 DURING PHOTOSYNTHESIS AT HIGH LIGHT INTENSITY

Anuradha Marathe, Brandon Thomas, Stephanie Krueger & Toivo Kallas

Department of Biology-Microbiology, University of Wisconsin, Oshkosh.

Linear electron flow from photosystem II (PSII) via the plastoquinone (PQ) pool, cytochrome (Cyt) *bf* complex, and photosystem I (PSI) generates ATP and NAD(P)H. Cyclic electron flow around PSI and the Cyt *bf* complex generates ATP only, provides the 'extra' ATP for efficient CO_2 fixation, and is implicated in defenses against photodamage. Cyclic electron flow mediated by the NAD(P)H dehydrogenase (NDH-1) complex is the major, known cyclic pathway in cyanobacteria. In plant chloroplasts, a PSI – Cyt *bf* supercomplex catalyzes cyclic flow (Iwai et al., 2010 *Nature*). Such a supercomplex has not been identified in cyanobacteria and the relative contributions of linear and cyclic electron flow under different environmental conditions remain poorly understood. We have used a Joliot-type, pump-probe kinetics spectrophotometer (BioLogic JTS-10) to re-examine cyclic flow in native *Synechococcus* sp. PCC 7002 and an NdhF (NDH-I) mutant. The NDH-I route accounted for most of the cyclic flow under optimal conditions (~200 µmol photons m^{-2} s^{-1}, 3% CO_2) as observed previously. At high, full-sunlight, light intensity (2000 µmol m^{-2} s^{-1}), cyclic electron flow increased markedly in both the wild type and NdhF mutant, and accounted for 30% or more of linear flow in the mutant. Active, cyclic flow at high-light intensity in the NdhF mutant indicates the activation of an NDH-independent cyclic electron pathway. We hypothesize that this efficient cyclic flow is catalyzed by the formation of a Cyt *bf* – PSI supercomplex that is important for adaptation and growth of *Synechococcus* 7002 at extreme, high-light intensities.



Cyclic electron flow in *Synechococcus* wild type and NdhF grown at optimal and high light intensities. PSI P700 kinetics were monitored during 10 second illumination (green light, 530 nm), and during 'dark-decay' following illumination. The re-reduction phases are shown. A) Wild type (WT) and B) NdhF, ± DCMU (10µM). DCMU blocks electron flow from PSII and P700 re-reduction then relies on cyclic electron transfer (CET). CET in the WT was ~12% of total ET. C) Wild type and D) NdhF at high-light intensity (HLT), ± DCMU. The smaller extent of P700 oxidation indicates lower PSI contents. In NdhF in particular, CET increased dramatically to ~33% of total ET suggesting the induction of an alternative cyclic electron transfer pathway.

SPECTRAL RESOLUTION OF THE FIRST ELECTRON ACCEPTOR IN PHOTOSYSTEM I.

Adrien Chauvet[1], Naranbaatar Dashdorj[2], John H. Golbeck[3], T. Wade Johnson[4], and Sergei Savikhin[1]

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The reduced state of the first electron acceptor A_0- was spectroscopically resolved in its lowest energy Qy region for the first time without the addition of any harsh reducing chemical agent that may alter the properties of the protein, and without extensive manipulation of the data. In this study, we used the menB mutant of PS I, in which phylloquinone is substituted with plastoquinol in the position of the second electron acceptor A_1. This mutation can slow down electron transfer from A0 to A1 leading to long living A_0^- state and its spectral signature could be readily detected in time resolved optical pump-probe experiment. A maximum (A_0^- - A_0) bleaching was found at 684 nm with a corresponding extinction coefficient of 43 $mM^{-1}cm^{-1}$.Our data also show the evidence of electrochromic shift of the accessory pigment absorption suggesting that the latter Qy absorption band is centered at ~682 nm.

The authors gratefully acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-FG02-09ER16084 for funding studies on optical resolution of A_0 as well as the Susquehanna University for support. This work was also supported in part by a grant from the National Science Foundation (MCB-1021725 to J.H.G.).

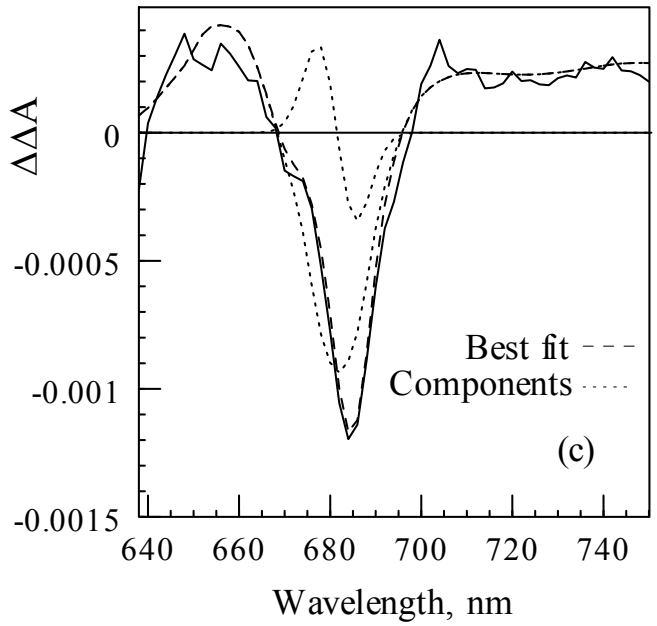

Figure: (200 ps – 4 s) spectrum of PSI menB (solid) and best fit (dashed) using the shifted in-vitro (Chl a^- - Chl a) spectrum from (Fujita 1978) (dotted) and a 1.7 nm elctrochromic blue-shift of a 682 nm Chl *a* pigment (dotted).

Poster Presentation Abstracts

(Listed alphabetically by first author)

(see also Author Index at the end of the book)

POSTER 1

RESPONSES TO COPPER EXCESS IN SYNECHOCYSTIS PCC 6803

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The pollution of the natural environment by heavy metals has become a serious problem, not only in industrialized countries, but also in developing countries in Southeast Asia. Many studies have been completed at different scales confirming the high probability of cyanobacterial species for removing nutrients that support the growth of cyanobacteria.

In this study, Synechocystis cells were cultured in BG-11 liquid medium supplemented with various concentrations of $CuCl_2$ (0.1, 0.2, 0.3, and 0.5 mg/l) for six days in normal light (50 µ mol photo m^{-2} s^{-2}) and high light (400 µ mole photon m^{-2} s^{-2}). The growth and pigment composition of the cells were analyzed. A light and time dependent increase in toxicity of Cu^{2+} to cyanobacterial cells was observed upon exposure to Cu^{2+} at concentrations higher than 0.3 mg/l. The effects of Cu^{2+} on cell growth differ in normal light and high light. In normal light Cu^{2+} appears to be a nutrient and stimulates the cell growth of cyanobacteria at concentrations lower than 0.3 mg/l, while Cu^{2+} toxicity was observed upon exposure of Synechocystis to Cu^{2+} at 0.5 mg/l. In high light Cu^{2+} appears to be toxic to Synechocystis at concentrations higher than 0.3 mg /l. The chlorophyll and carotenoid contents of the cells at normal light were reduced upon exposure to Cu^{2+} at concentrations higher than 0.3 mg/l. The chlorophyll and carotenoid contents were affected more severely in high light than in low light upon exposure of cyanobacterial cells to copper at concentrations higher than 0.3 mg/l. Clearly, high light aggravates Cu^{2+} toxicity and vice versa. The sensitivity of photosystem I and II to copper treatments appears to be different, and the PS II to PS I ratio appear to be reduced by Cu^{2+} (0.3 mg/l) treatment for 24 hours. Future the study will focus on dissecting Cu^{2+} toxicity to cells and using cyanobateria to remove heavy metals from sewage water and as sensor to detect the pollution by heavy metals.

THE K_M AND K_D VALUES OF CALCIUM AND CHLORIDE IN ACTIVATION OF OXYGEN EVOLUTION IN PHOTOSYSTEM II

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Photosystem II (PS II) is the membrane-bound protein complex at which oxygen is produced as a byproduct of electron transfer after light absorption. The site of oxygen evolution is a Mn_4Ca cluster, in which the Ca and four Mn ions form a di-μ-oxo-bridged cluster that cycles through a series of oxidation states during catalytic production of oxygen. Chloride is also known to play an important role as a cofactor in the formation of oxygen. Earlier experiments using X-ray diffraction of PSII crystals prepared with Br^- and I^- showed the presence of two Cl^- ions near the manganese cluster. In the study presented here, experiments were conducted to understand the dependence of oxygen evolution on chloride in the presence of varying concentrations of calcium. The preparations used were depleted of both the 23 and 17 kDa (PsbP and PsbQ) extrinsic subunits by high NaCl-washing so that Ca^{2+} and Cl^- would have access to the catalytic site. Calcium depletion was achieved by EDTA treatment during light exposure, which brought the activity down to about 20% in the absence of Ca^{2+} and Cl^- ions. Analysis of the data using an enzyme kinetics model for bisubstrate reactions, in which the activators Ca^{2+} and Cl^- were treated as substrates, showed that the data could fit the kinetics for either random sequential or ordered sequential reactions. Depending on the model used, values for the Michaelis constants (K_M) and dissociation constants (K_S) for each activating ion could be derived. Preliminary experiments to examine Cl^- binding directly have been carried out using Isothermal Titration Calorimetry (ITC), using intact PSII dialyzed to remove Cl^-. Given promising results, these experiments will be extended to examine other PSII preparations. This research will help to clarify the role of chloride binding and chloride activation in oxygen evolution by PSII. (Supported by the National Science Foundation).

POSTER 3

TEMPERATURE DEPENDENCE OF PHOTOASSEMBLY IN BBY PARTICLES

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Elucidating the mechanism of photoassembly of the oxygen-evolving complex (OEC) will lead to a better understanding of how the OEC catalyzes water oxidation. BBY particles for our measurements were prepared according to Berthold, Babcock, and Yocum [1], with modifications from Kolling *et al*.[2] The oxygen-evolution activity of intact BBY particles at a temperature range of 5–45°C was measured and found to have a maximum at 28°C, which is consistent with literature values[3]. We then generated OEC-depleted BBY particles by using an established aminosulfonic buffer (20 mM CHES/NaOH, pH 9.4) and a divalent cation (200 mM MgCl2) treatment[4]. These apo-BBY particles were combined with a mixture of Mn^{2+}, Ca^{2+}, HCO_3^-, and $K_3[Fe(CN)_6]$ and then subjected to light pulses within a temperature range of 5–45°C followed by oxygen-evolution measurements at 28°C. Approximately 90% of oxygen-evolution activity was recovered at each temperature with a maximum of photoassembly yield appearing at 28°C, almost identical to the temperature-maximum of oxygen evolution; in addition, the temperature-dependence curve does not deviate significantly from that of oxygen-evolution in untreated BBY particles. To our knowledge, this is the first report of the temperature-dependence of OEC photoassembly. Implications of the shared maxima between oxygen evolution and OEC photoassembly will be discussed.

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2. Kolling, D.R.J., Brown T.S., Ananyev G., Dismukes C.G. 2009. Photosynthetic Oxygen Evolution Is Not Reversed at High Oxygen Pressures: Mechanistic Consequences for the Water-Oxidizing Complex. Biochemistry. 48:1381-1389

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4.Baranov, S. V., Tyryshkin A. M., Katz D., Dismukes G. C., Ananyev G. M., and Klimov V. V. 2004. Bicarbonate Is a *Native* Cofactor for Assembly of the Manganese Cluster of the Photosynthetic Water Oxidizing Complex. Kinetics of Reconstitution of O_2 Evolution by Photoactivation. Biochemistry 43:2070-2079.

PERSISTENT α-HELICAL CONTENT AND LOCAL HELICAL STRUCTURAL FLUCTUATIONS FROM A HEME BINDING PEPTIDE'S MOLTEN GLOBULE TO ORDERED PEPTIDE TRANSITION

Mia Brown[†], *Andrew Mutter*[††], *Ronald Koder*[††], *Jason Cooley*[†], *Renee JiJi*[†]

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We have recently shown a unique spectroscopic potential for deep-UV resonance Raman (dUVRR) in simultaneously monitoring protein structure and solvation with respect to membranes [Halsey, C.M.; Xiong, J., Oshokoya, O.O.; Johnson, J.A.; Shinde, S.; Beaty, T.J.; Ghirlanda, G.; JiJi, R.D.; Cooley, J.W.; *ChemBiochem*. 2011, 12, 2125-2128]. A next logical step for this technique is to ascertain if dUVRR can monitor protein dynamics of membrane proteins. However, the ability of dUVRR to monitor subtle conformational changes of proteins not directly associated with gross secondary structural changes is still in its infancy. As a proof of concept we have measured a molten globule to ordered state transition of a soluble protein. A molten globule state is defined as having similar secondary structural content, but increased tertiary structural disorder. However, the proof the persistent secondary structure content is typically less than concrete due to a variety of technical challenges associated with available spectroscopic measurements. DUVRR spectroscopy has emerged as a means by which to monitor extremely subtle structural fluctuations in the secondary structure of a given protein, and here we will present an extension of this methodology to tertiary structural disorder related structural changes of a heme binding maquette, HP7. HP7 is a four-helix bundle that retains its helical secondary structure while altering its tertiary structure dependent upon the attachment of one or two heme groups. In studying the apo-, intermediate- and holo-proteins, we have determined that dUVRR spectroscopy can not only quantitatively confirm the persistence of total α-helical content and concurrent increase in disorder in the peptide ensemble structures going from molten to ordered states, but that it also indicates the presence of local fluctuations in the helical content itself in the steady state. Therefore, dUVRR is a promising tool for the analysis of subtle protein secondary and tertiary structure changes and may be a valuable tool for monitoring membrane protein structural dynamics.

SPECTRAL RESOLUTION OF THE FIRST ELECTRON ACCEPTOR IN TYPE I REACTION CENTERS OF *Heliobacterium modesticaldum* AND *Chlorobium tepidum*.

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[3]Department of Chemistry, Susquehanna University, Selinsgrove, Pennsylvania

Based on protein sequence and optical data it has been suggested that both accessory chlorophylls A_{-1} and primary electron acceptors A_0 of the Reaction Center (RC) from *Heliobacterium modesticaldum* and *Chlorobium tepidum* are Chl *a* molecules, while the special pairs (P) and rest of the antenna pigments are BChl *g* or BChl *a*, respectively for these species. It has been also suggested that the optical transient absorption signal at ~670 nm reflects the formation of relatively long living A_0^- possibly mixed with electrochromic shift of A_{-1}. However, the intensity of the A_0 signal in these studies has been too low compared to the oxidized special pair signal to unambiguously support this conclusion. Moreover, essentially no optical evidence exists to support the suggestion that Chl *a* pigment serves as an accessory pigment in these RCs.

In this work we clearly resolve the electrochromic shift signal of the Chl *a* pigment in optical (P^+- P) absorption difference spectra from which the position and orientation of the Chl *a* pigment is inferred. The character and intensity of this signal is consistent with the A_{-1} pigment (accessory) being Chl *a* molecule. We also refine the ($P^+A_0^-$ - PA_0) difference spectra and discuss the structural consequences for both species.

The authors gratefully acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-FG02-09ER16084 for funding studies on optical resolution of A_0 as well as the Susquehanna University for support. This work was also supported in part by a grant from the National Science Foundation (MCB-1021725 to J.H.G.).

DYNAMIC PHOTOSYNTHETIC PHENOMICS

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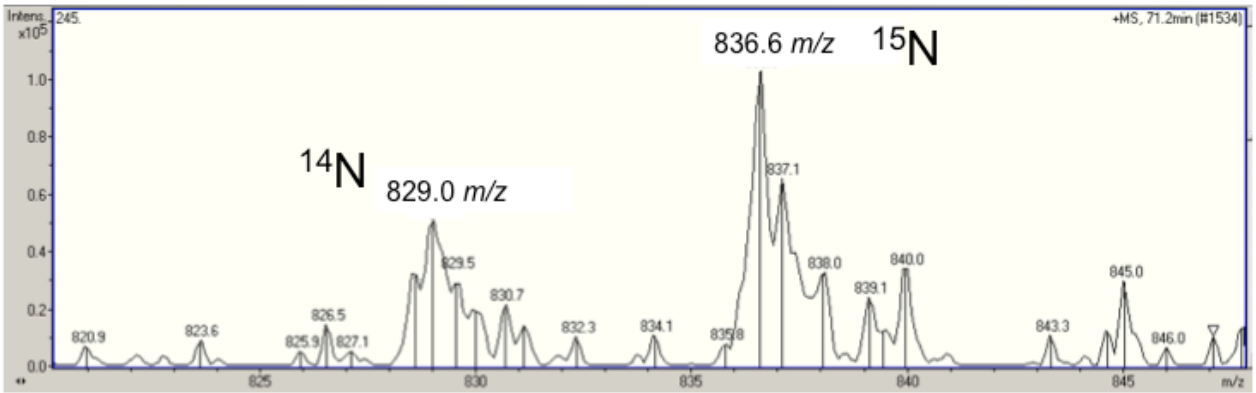
During photosynthesis, photon capture can exceed the rate at which the energy can be used, resulting in production of reactive oxygen species (ROS) and cell damage. Many mechanisms have evolved to cope with this challenge, some that are very fast responding (e.g., q_E response) and others that are much slower (e.g., induction of genes encoding proteins that can detoxify ROS). While these processes protect against damage, many of them also result in loss of efficiency. What is *not* known is how these different systems are integrated and the degree to which given mechanisms take precedence under specific environmental conditions. Furthermore, most of our knowledge of photosynthetic behavior has come from measurements on plants grown under highly controlled growth conditions (chambers or greenhouses) or by exposing plants to a change in a single condition and then following the effects over time on a specific photosynthetic parameter(s). While this reductionist experimental design is extremely powerful, it is becoming increasingly apparent that it is likely not to detect novel biochemical and regulatory mechanisms that have important roles in nature. For example, knocking out q_E quenching (*i.e., npq4*) has no visible developmental phenotype under static growth conditions, but severely limits growth under dynamically changing actinic light. In this poster we highlight recent advances in the development of dynamic photosynthetic "phenomics" technology, enabling us to measure a wide range of photosynthetic parameters in real time in response to an array of constant and fluctuating environmental conditions. We will use this technology to identify biophysical processes and genes that have key roles in photosynthetic acclimation and contribute to photosynthetic efficiency and productivity. These studies will include: (1) testing the importance of processes and genes that have been suggested to play roles in dynamic regulation of photosynthesis; and (2) using forward genetics to discover novel genes with roles in photosynthetic acclimation.

LC-MS/MS INVESTIGATION OF PROTEIN EXPRESSION IN *SYNECHOCOCCUS* SP. PCC 7002 DURING HIGH-LIGHT INTENSITY GROWTH

Jessica Dorschner & *Toivo Kallas*

Department of Biology-Microbiology, University of Wisconsin Oshkosh

The cyanobacterium *Synechococcus* sp. PCC 7002 grows rapidly, is amenable to genetic manipulation, and tolerates a wide range of salt, nutrient, and high-light stresses, making it an interesting model organism and candidate for biofuels applications. We are particularly interested in the ability of *Synechococcus* 7002 to grow with very little change in growth rate at extremely high light intensities of more than twice full sunlight (4500 μE m^{-2} s^{-1} light intensity) (Nomura *et al.*, 2006). To gain insight into adaptive mechanisms, we are using liquid chromatography tandem mass spectrometry (LC-MS/MS) to analyze the proteomes of ^{14}N- and ^{15}N-labeled cells grown under high light (2000 μE m^{-2} s^{-1}) and optimal light intensity (200 μE m^{-2} s^{-1}). By comparing the MS signal intensities of ^{14}N and ^{15}N versions of peptides, we can determine relative protein abundance under the two illumination conditions. One challenge is that peptides compete for detection by LC-MS/MS, resulting primarily in the detection of abundant peptides. We are interested in several techniques for improved proteome coverage, including preliminary protein fractionation by SDS-PAGE, repeated analyses over small *m/z* ranges, and the use of a more sensitive, LTQ Orbitrap mass spectrometer. Ultimately, we hope to perform absolute quantification of selected proteins of interest by spiking samples with known concentrations of isotopically-labeled peptides. Overall, we hope to characterize protein expression under optimal vs. high light intensity to gain insight into the mechanisms employed by *Synechococcus* 7002 to withstand extreme high-light stress.



^{14}N and ^{15}N phycocyanin α-subunit peptides detected by LC-MS/MS. Soluble proteins from ^{14}N-labeled high-light and ^{15}N-labeled optimal-light grown cells were mixed and digested with trypsin. Peptides were separated by LC and detected by MS/MS on a Bruker Esquire™ 3000*plus* ion trap mass spectrometer. MS/MS *m/z* signals were searched against a MASCOT database and identified as peptide TFDLSPSWYVEALK of the phycocyanin α-subunit (MOWSE probability score 1834, >32 indicates identity, $p<0.05$). ^{14}N and ^{15}N forms of peptides ionize with the same efficiency, thus MS signal intensity can be used to determine relative abundance under the two conditions.

Employing FUNCTIONAL GENOMICS TO study regulation of light independent and light dependent chlorophyll biosynthetic pathways IN THE MODEL UNICELLULAR GREEN ALGA *Chlamydomonas reinhardtii*

Phillip Grovenstein[1], *Kathryn Lankford*[1], *Justin Puckett*[1], *Kelsey Gaston*[1], *Darryel Wilson*[1] *Bernhard Grimm*[2] *and Dr. Mautusi Mitra*[1]

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Chlamydomonas reinhardtii is a green micro algae, which is haploid, easy to culture in the laboratory, can grow both photosynthetically and heterotrophically (can use acetate as the sole carbon source in the dark, like a heterotroph and also can use atmospheric CO_2 in the presence of light, like a photosynthetic autotroph), is amenable to both nuclear and chloroplast transformation and its genome has been completely sequenced. All of these traits make it an ideal, simple and elegant system to study photosynthesis. Additionally, *Chlamydomonas* has two different pathways to make chlorophyll, unlike angiosperms. It has a strictly light dependent chlorophyll biosynthetic pathway like all photosynthetic higher plants and a light independent chlorophyll biosynthetic pathway, which can operate both under dark and light, like in some cyanobacteria and gymnosperms. To isolate *Chlamydomonas* insertional mutants that are very light sensitive, selection of transformed cells in the dark is necessary. The 4A+ strain was selected for random DNA insertional mutagenesis because it has a better growth rate and has more chlorophyll per cell than other strains under dark. We have used the vector pBC1, which contains a paromomycin resistance gene for insertional mutagenesis of the 4A+ strain. The mutant library was screened by visual and spectrophotometric methods to isolate pigment deficient mutants.

To date, we have isolated two pigment deficient mutants. One of these mutants, namely F3(14), is yellowish green/brown under dim light (15μmole photons $m^{-2}s^{-1}$) but progressively turns bright yellow to white/whitish brown with increase in light intensity when grown on heterotrophic media. It cannot grow heterotrophically above 100μmole photons $m^{-2}s^{-1}$ and cannot grow autotrophically above 30μmole photons $m^{-2}s^{-1}$. Although low light adapted yellow cells of the mutant when grown under dark makes some amount of chlorophyll, the cells are still chlorophyll deficient compared to the dark adapted parental strain. This indicates the mutant has a defect, which affects both chlorophyll biosynthetic pathways. However, the light dependent pathway is more severely affected than the light independent pathway. The second mutant, 5A(7), is very light sensitive, and can only grow heterotrophically under dark to very dim light (15μmole photons $m^{-2}s^{-1}$) and appears "white/brownish" devoid of any carotenoids and chlorophyll. Photosynthetic pigment and pigment precursor analyses of the F3(14) mutant and wild type cells under different light intensities by high performance liquid chromatography (HPLC) are currently being performed to get information about the steady state pigment precursor levels. Genetic crossing experiments are also currently being performed to confirm the linkage of phenotype of the mutant with paromomycin resistance. We will be presenting our data on F3(14) and 5A(7) mutants.

*This project has been funded by the University of West Georgia, Carrollton, GA

The Photobioreactor Sensor Matrix: Bringing the Laboratory Closer the Field

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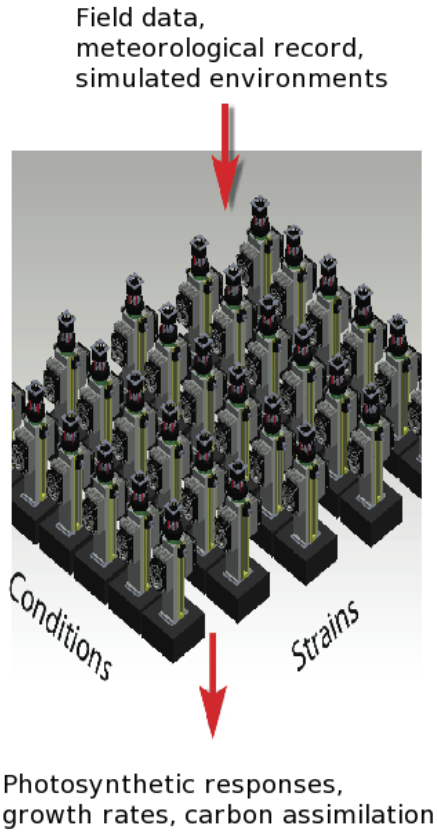
The disconnect between laboratory growth conditions and outdoor environments makes algal species selection for field conditions extremely difficult. The algal research field lacks a defined set of standard operating procedures and a uniform experimental platform. To address these issues, we have designed a novel, expandable photobioreactor sensor matrix designed to mimic outdoor conditions. Light, temperature, CO2 sparging and mixing are controlled by a computer, enabling programmed fluctuations of conditions based on environmental data and simulation. Controllers and sensors, including pH, turbidity, O2, CO2, PSII fluorescence, and absorbance changes, can be incorporated into the units. Here we present early results comparing the growth behavior of the micro alga *Chlorella sorokiniana* under constant and fluctuating light and temperature.



Left: Concept sketch of a photobioreactor sensor matrix set up for a 2-dimensional screen to identify photosynthetic phenotypes in mutant algal strains.

MAPPING TRIPLET STATE ENERGIES OF CHLOROPHYLLS.

Dan Hartzler and Sergei Savikhin

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Chlorophyll (Chl) molecules are known to produce highly toxic singlet oxygen under illumination as a result of energy transfer from their triplet excited states to oxygen. Our goal is to measure the triplet-state energy for all types of monomeric Chls as well as some aggregated forms. The most direct way of accomplishing this goal is measuring the phosphorescence spectrum emitted by Chls as they relax from triplet excited state back to the ground state.

The phosphorescence signal is extremely weak, with a quantum yield 10^{-5} to 10^{-6} times less than that of the fluorescence. Even though the fluorescence and phosphorescence are spectrally separate (670 nm vs. 970 nm for Chl *a*) the infrared tail of the fluorescence band is strong enough to overwhelm the phosphorescence. Fortunately, the fluorescence lifetime is ~10ns while the phosphorescence lifetime is ~1ms so the fluorescence can be additionally gated in the time domain.

In this work we report the progress in developing an optomechanical spectrometer capable of measuring weak phosphorescence signals. We will present first data on the phosphorescence characterization of several chlorophyll and chlorophyll-like molecules. The ultimate goal of this project is to catalog the triplet-state energies of all known Chls as well as investigate the role of triplet excitons in the photostability of the BChl aggregates forming the chlorosomal antenna of green sulfur bacteria.

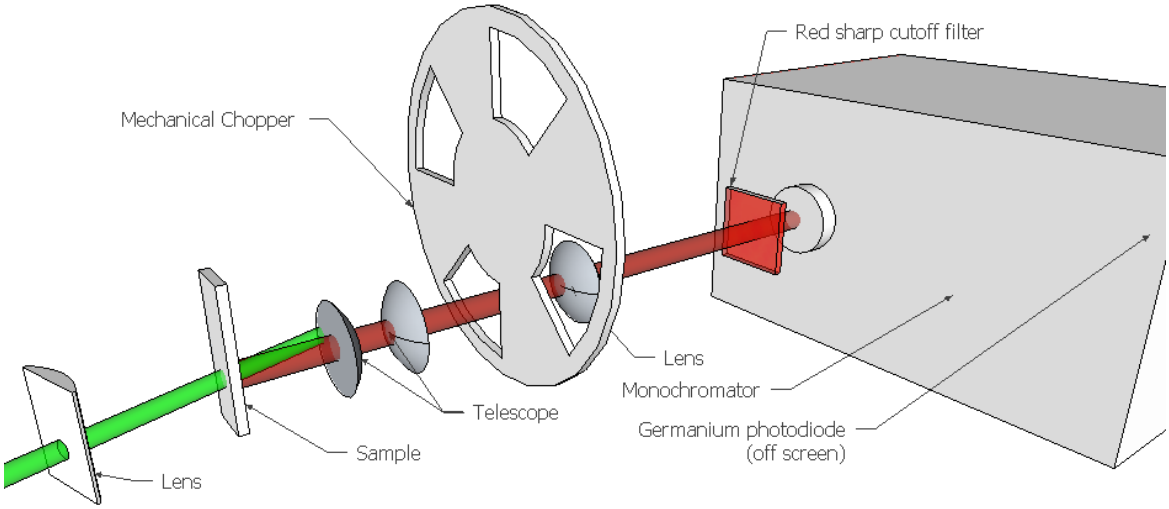

Figure: sample is excited by a nanosecond pulsed laser, while the mechanical chopper provides time domain gating, filters and monochromator provide spectral resolution. IR signal is measured by an ultra high-sensitivity Ge photodiode cooled down to liquid nitrogen temperature.

This work was funded by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-FG02-09ER16084.

PREFERENTIAL REDUCTION OF THE MONOMER IN THE DIMERIC CYTOCHROME b_6f COMPLEX

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Cytochrome *bc* complexes that function in electron transport and coupled proton translocation are hetero-oligomeric structures of symmetric dimers. Outside of conferring structure stability, an obligatory function of the dimer in electron or proton transfer is not known. Studies of inter-monomer interactions that affect electron transfer have been carried out on bacterial and respiratory cytochrome bc_1 complexes (*1-4*). In the present study, electron transfer of purified monomeric and dimeric cytochrome b_6f complex, was compared through the rate and amplitude of chemical reduction and formation of a split circular dichroism (CD) spectrum in the Soret band associated with exciton interactions between the two hemes (*5*). In the dimeric complex, reduction of the *b*-hemes, although synchronous with formation of the split CD spectrum, was biphasic, with a relatively rapid phase occurring within the mixing time (seconds), but more than half the amplitude change occurring slowly in tens of seconds. A similar biphasic rate of heme reduction and generation of a split CD signal occurred with the dimeric complex using NADPH/ferredoxin as reductant. With monomeric b_6f complex, however, reduction by dithionite of the two trans-membrane *b*- hemes and synchronous formation of the split CD spectrum were monophasic and rapid, occurring within the mixing time. Along with the geometrical requirements for heme-heme exciton interaction, this implies that the split CD signal arises from intra-monomer heme-heme exciton interactions. Additional reduction of the *b*-hemes in the second monomer in the dimer occurs more slowly, as does inter-monomer cross-over of electrons. Reduction of the extra-membrane cytochrome *f* is rapid. It is proposed that the rate of reduction of the second monomer, and of inter-monomer electron transfer, is retarded because of repulsive inter-monomer electrostatic interactions that arise from reduction of the *b*-hemes in the 2 monomers (NIH GM-38323).

(1) Crofts, A. R. *et al*. (2008) *BBA*;
(2) Castellani, R., *et al.* (2010) *J. Biol. Chem.*;
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(5) Palmer, G., and Degli Esposti, M. (1994) *Biochem.*

POSTER 12

Monomeric turnover out competes inter-monomeric function in bc_1 complex

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The homodimeric bc_1 complexes are membrane proteins essential in respiration and photosynthesis. The ~11 Å distance between the two b_L-hemes of the dimer opens the possibility of electron transfer between them, but contradictory reports make such inter-monomer electron transfer controversial. We have constructed in *Rhodobacter sphaeroides* a heterodimeric expression system in which the bc_1 complex can be mutated differentially in the two copies of cyt *b*, but found that genetic recombination by cross-over then occurs to produce wild-type homodimer. Selection pressure under photosynthetic growth always favored the homodimer over heterodimeric variants enforcing inter-monomer electron transfer, showing that the latter are not competitive. These results, together with kinetic analysis of myxothiazol titrations, demonstrate that inter-monomer electron transfer does not occur at rates competitive with monomeric turnover.

CHARACTERIZATION OF PERIDININ-CHLOROPHYLL A-PROTEIN COMPLEX IN THE DINOFLAGELLATE *SYMBIODINIUM*

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Peridinin-chlorophyll a-protein (PCP), a water-soluble light-harvesting complex found in photosynthetic dinoflagellates, has the carotenoid peridinin absorbing at 470nm to 550nm as its primary pigment. This work identified and characterized the PCP of *Symbiodinium* sp. CS-156-b1. SDS-PAGE revealed a single band at a mass of 33kDa, which was confirmed by LC/MS. Spectroscopic experiments suggested the ultrafast energy transfer from the peridinin S_2 state to Chl-*a* Q_y (~0.13 ps), and the high energy transfer efficiency of 94.9%.

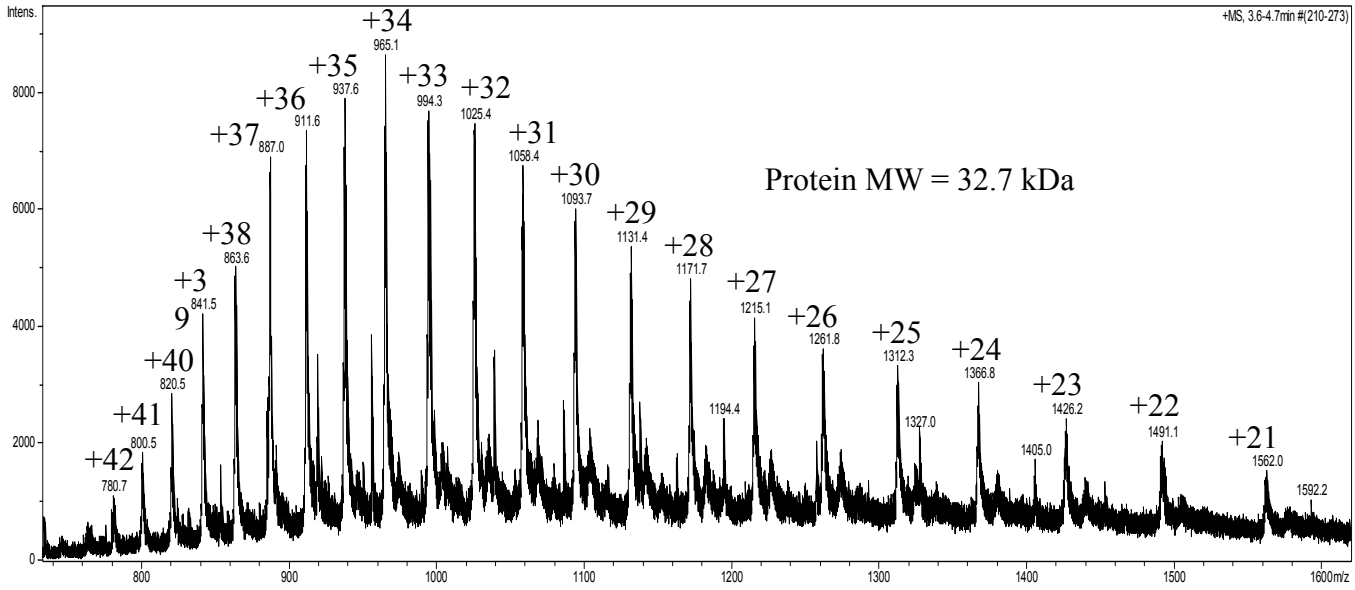

Fig 1. LC/MS of PCP

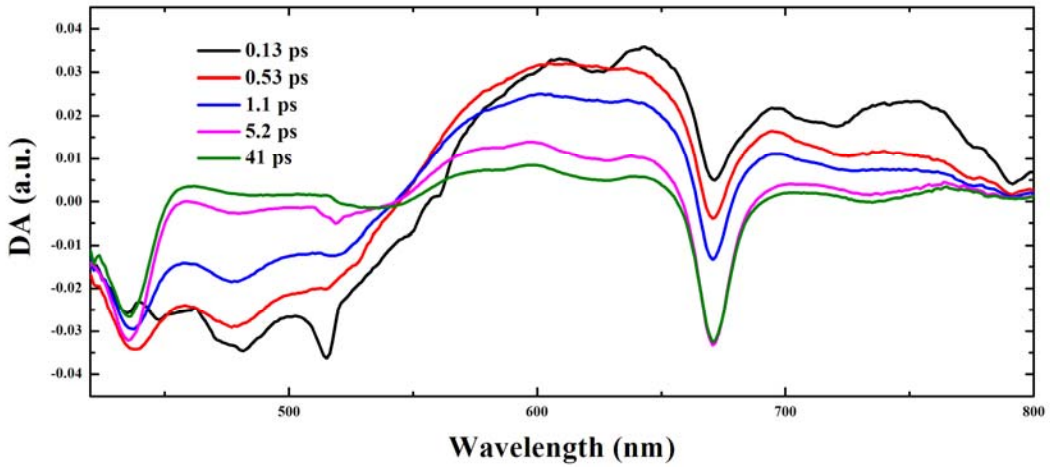

Fig 2. The transient absorption spectrum of PCP. The sample was excited at 520 nm.

THE CHLOROPLAST ATP SYNTHASE AS THE GOVERNOR OF PHOTOSYNTHESIS

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The light and dark reactions of photosynthesis must be finely co-regulated to optimize efficiency while avoiding deleterious side reactions, resulting in up to 80% of absorbed solar energy being 'purposely' dissipated as heat to protect plants from photodamage. In wild-type plants, lowering CO_2 initiates a regulatory cascade in which the ATP synthase is down-regulated, slowing proton efflux from the thylakoid lumen, resulting in buildup of thylakoid proton motive force (*pmf*), acidification of the lumen, leading to slowing of electron transfer at the cytochrome b_6f complex and activation of photoprotective excitation quenching in the light-harvesting complexes (qE). In contrast, the *cfq* mutant of Arabidopsis, which has a single amino acid change in the γ-subunit of the chloroplast ATP synthase, has lost the ability to down-regulate the ATP synthase, so at low CO_2, *pmf* decreases rather than increasing, allowing uncontrolled electron transfer through b_6f and preventing full activation of qE. The buildup of reduced electron transfer species results in rapid inactivation of photosystem I, which does not recover over the many hours time scale. This photodamage is distinct from that caused by inactivation of photoprotection of photosystem II. We conclude that the chloroplast ATP synthase acts as an essential regulatory governor, regulating lumen pH and thus maintaining the redox balance of the electron transfer chain. Our work also confirms that photosystem I is a distinct site of photodamage.

This research is supported by funding from the U.S. Department of Energy (Office of Science, Basic Energy Sciences Program).

DEVELOPMENT OF AN FMO MUTAGENESIS SYSYTEM IN *CHLOROBIUM TEPIDUM*

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The Fenna-Matthews-Olson (FMO) proteins in green sulfur bacteria transfer the excitation energy from the light-harvesting chlorosomes to the reaction center. Here, we report the preliminary progress on the development of an FMO mutagenesis system in *C. tepidum* to better understand how the protein environment determines and influences the electronic properties of the complex and also facilitate the expression, purification and characterization of foreign FMO proteins whose native host are challenging to grow. We have successfully introduced a second copy of the *fmoA* gene into *C. tepidum* by inserting it into a neutral site on the chromosome. The His-tag at the C-terminal end of the introduced FMO has enabled the separation of the mixed trimers of FMO. Further refining of the mutagenesis system is underway.

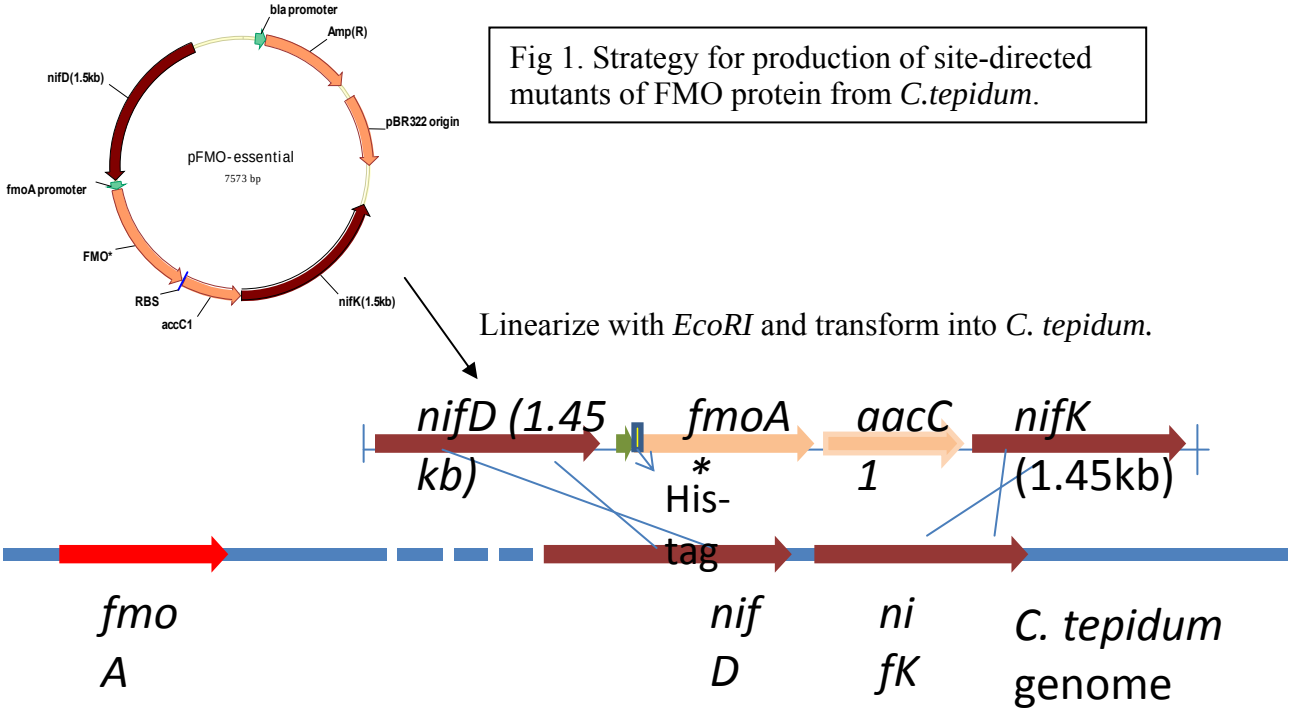

Fig 1. Strategy for production of site-directed mutants of FMO protein from *C.tepidum*.

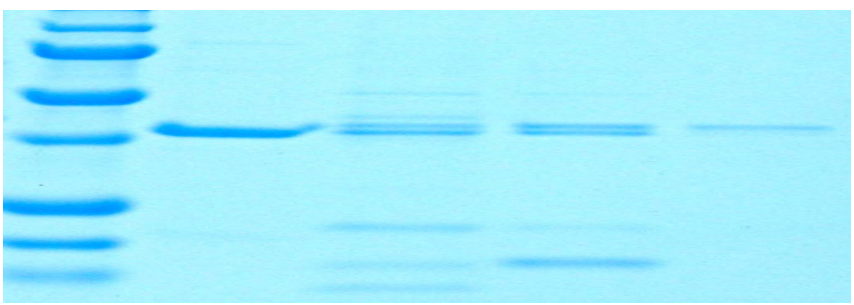

Fig.2 Purification of His-tagged mutant FMO protein from *C. tepidum*.

THE EFFECTS OF CARBON LIMITATION ON NADPH IN *SYNECHOCYSTIS* SP. PCC 6803

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Inorganic carbon (Ci) is an essential and often limiting resource for the activity of aquatic organisms performing oxygenic photosynthesis, including cyanobacteria. The carbon concentrating mechanism (CCM) emerged for the progenitors of contemporary cyanobacteria adapting to cope with an increase in photorespiration and low efficiency carbon fixation. The cyanobacterium, *Synechocystis* sp. PCC 6803, exhibits a basal, low affinity form of Ci transport when grown under Ci sufficient conditions. Under limiting Ci conditions there is an induced increase in transporter affinity achieved through transcriptional up-regulation of transport activities and carboxysome components, as well as the kinetic modification of existing transporters[1]. It has been shown that these induced transporters are controlled through two LysR-type transcriptional regulators, the activator CmpR and the repressor CcmR, where the activity of CmpR increases with increased levels of 2PG and RuBP[2], indicators of RuBisCO efficiency; and the activity of CcmR increases with increased levels of α-KG and NADPH[3], possible indicators of carbon metabolism. We looked at the physiological changes of *Synechocystis* and a strain bearing the deletion of *ccmR* during carbon limitation by simultaneous measurement of Chl *a* and NADPH fluorescence within a PAM fluorometer. The Δ*ccmR* mutant showed a higher level of NADPH fluorescence prior to Ci deprivation and a lower level of NADPH fluorescence after, compared to wild-type. Since CcmR represses the expression of transporters which are coupled to specialized NAD(P)H-dependent hydrogenase complexes, the results suggest an increased demand for NADPH to drive the transporters within the mutant which caused an increase in the steady state level of NADPH compared to wild-type, and caused a smaller increase as Ci became limiting. Wild-type also showed an increase in relative NADPH fluorescence prior to decrease in photochemical quenching suggesting that NADPH levels increased before the oxygenase activity of RuBisCO became detrimental due to lack of Ci. These results and further photosynthetic parameters are discussed.

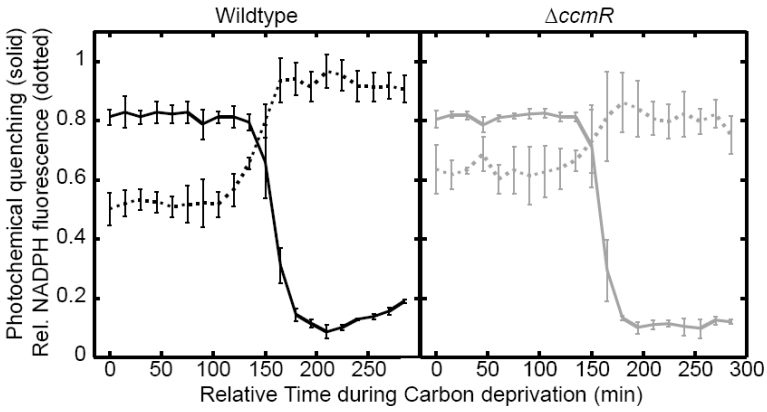

Figure: Photochemical quenching and relative NADPH fluorescence during carbon deprivation. From Chl *a* and NADPH fluorescence traces taken every 15 minutes during carbon depletion of a high carbon adapted sample of wild type or Δ*ccmR* (n=4).

Ref1) Reviewed in: Price GD, et al. (2008) J Exp Bot; 59(7): 1441-61
Ref2) Nishimura T, et al. (2008) Mol Micro; 68(1):98-109
Ref3) Daley S, et al., unpublished

The role of oxygen in long-distance triplet energy transfer from *Chlorophyll a* to β-*Carotene* in the *Cytochrome b_6f complex* of Oxygenic photosynthesis

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[1]Department of Physics and [2]Department of Biological Sciences, Purdue University, West Lafayette, Indiana

The cytochrome b_6f (Cyt b_6f) complex of oxygenic photosynthesis mediates electron transfer between photosystem II (PSII) and photosystem I (PSI) and facilitates coupled proton translocation across the membrane. High resolution X-ray crystallographic structures of the Cyt b_6f complex show that a chlorophyll molecule (Chl) is an intrinsic component of the complex. The functional role of this Chl *a* remains unknown. However, a Chl molecule is known to produce highly toxic singlet oxygen ($^1O_2$) under light illumination as the result of energy transfer from its triplet excited state to oxygen. To prevent the formation of $^1O_2$, carotenoids (Car) are typically positioned close (~4 Å) to Chl to ensure rapid triplet-triplet energy transfer from Chl to Car, which can then safely dissipate this energy. However, the β-Car in Cyt b_6f is positioned too far (~14 Å) from the Chl *a* for direct triplet energy transfer. From earlier work by our group, it is suggested that oxygen molecules in the intraprotein pathway of Cyt b_6f mediate the energy transfer from ^{3}Chl to Car. To test this hypothesis, we present the study of the oxygen dependence of the energy transfer from Chl to Car using transient pump-probe technique under aerobic and anaerobic conditions and at low temperature where the mobility of O_2 is impeded.

This work was funded by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-FG02-09ER16084.

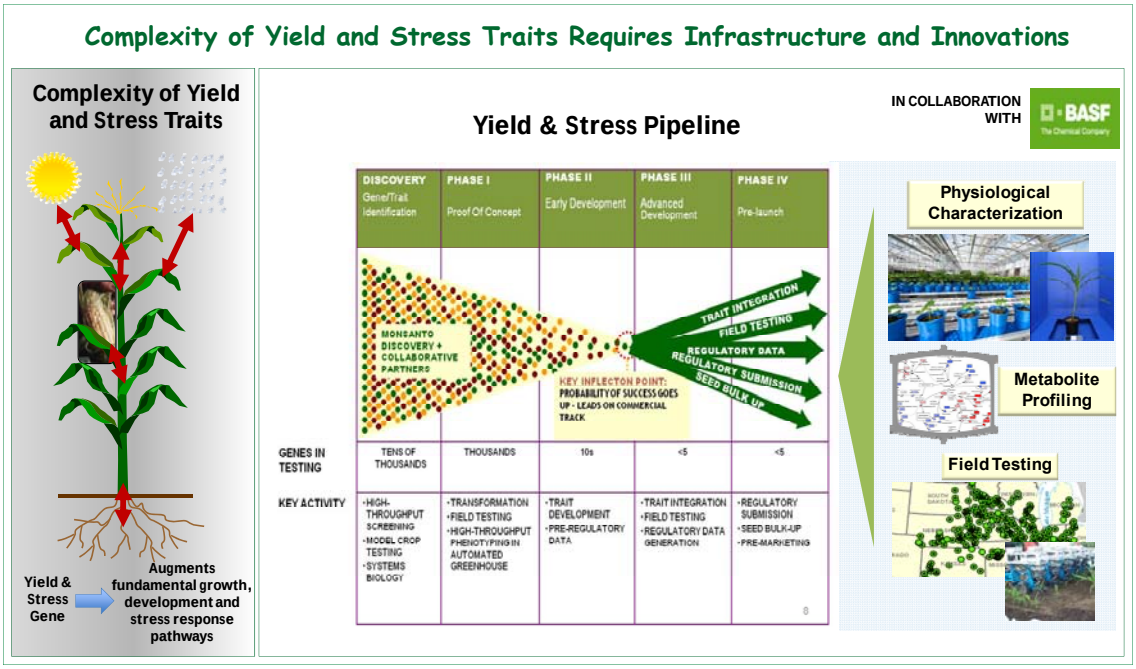
Monsanto Biotech Efforts Toward Doubling Yield of Corn in the US by 2030

JAE KIM AND EDWARDS ALLEN

MONSANTO COMPANY, 700 CHESTERFIELD PARKWAY WEST, CHESTERFIELD, MO 63017

The demands on world agriculture are greater than ever and continue to grow. At Monsanto, we are working to meet the world's growing food needs while protecting the environment and conserving natural resources. In June 2008, we issued this three-fold commitment that we call our Commitment to Sustainable Yield. We use breeding, biotechnology, and improved agronomic practices to work toward our pledge of doubling yield, reducing inputs, and improving farmer's lives. Specifically, we will help farmers with improved seeds and agronomics to double the yields of corn, soy, cotton, and canola crops while reducing the aggregate use of key resources (land, irrigation water, and energy) by 1/3 per unit of output over the 30 year interval from 2000-2030.

Achieving all three of these commitments is challenging. Monsanto's Research and Development (R&D) has developed a very strong biotechnology pipeline focusing on increasing crop stress tolerance and yield potential. For example, our Yield and Stress efforts on doubling yield of corn focus on improving nitrogen use efficiency, water use efficiency, light use efficiency, and general stress tolerance of corn through modification of gene expression and activity. Our goal is for our transgenic corn products to give high yield with less irrigation, less nitrogen application, and under high density planting conditions. Monsanto has implemented a high-throughput transgenic field screening program to evaluate Yield and Stress traits under broad acre conditions to measure yield. The quantitative nature of yield traits is highly complex; our field screening of transgenes will enable discovery of novel traits that bring value to our customers that can be observed in farmer's fields. Our gene discovery program combines collaborative efforts from Monsanto scientists, academic and industry collaborator innovations to ensure delivery of new products.



STRUCTURE-FUNCTION ANALYSIS OF BLUE COPPER PROTEINS FROM *CHLOROFLEXUS AURANTIACUS*

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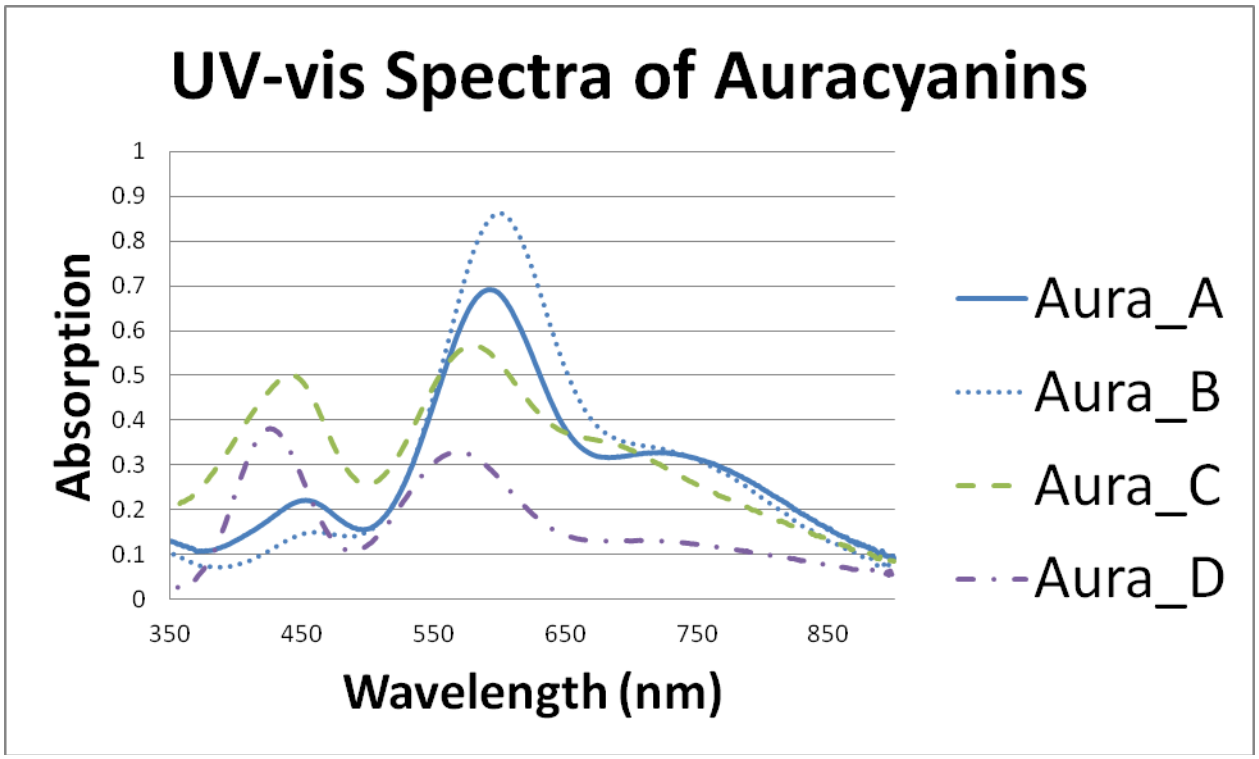
[1]Graduate Program in Plant Biology [2]Departments of Biology and Chemistry Washington University in St. Louis, St. Louis, MO 63130

Blue copper proteins are critical to the function of many electron transport chains. They typically perform electron transfers at high potentials. This makes blue copper proteins well-suited for donating electrons to terminal oxidases or reaction centers. In the Filamentous Anoxygenic Phototroph *Chloroflexus aurantiacus*, a novel group of blue copper proteins, called auracyanins, are thought to perform such a role. *C. aurantiacus* encodes four auracyanins, labeled A-D. Auracyanin A is thought to function in respiration and auracyanin B in photosynthesis. Both are thought to accept electrons from Alternative Complex III (ACIII). Auracyanins C and D have unknown roles.

The auracyanins are unique blue copper proteins. They share high sequence similarity, but are spectrally different (See Figure). This makes them great candidates for understanding the blue copper site. Typically, copper is coordinated by two histidines, one methionine, and one cysteine. A charge-transfer from the cysteine results in a strong blue color. Auracyanins A and B both show typical blue copper spectra, but auracyanins C and D appear gray. Here, we report the initial characterization of auracyanins C and D.



LIGHT-DARK AND METABOLISM-RELATED REGULAYION OF THE CHLOROPLAST ATP SYNTHASE HAVE DISTINCT

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The chloroplast ATP synthase is regulated at several levels. In the dark, that ATP synthase is down-regulated, presumably to prevent wasteful hydrolysis of ATP. It has also been shown that ATP synthase activity is regulated during steady-state photosynthesis. This "metabolism-related" regulation modulates the thylakoid proton motive force, thus affecting the regulation of light capture and electron transfer. The γ-subunit of the chloroplast ATP synthase possesses two cysteine residues which are involved in thioredoxin-modulated light-dark regulation. The γ subunit, coded by *ATPC1*, has three highly conserved acidic amino acids (D^{211}, E^{212}, and E^{226} in *Arabidopsis thaliana*) near the regulatory cysteine residues. We generated a mutant, *mothra*, in which these were modified to neutral residues V^{211}, L^{212}, and L^{226}, respectively. Flash induced relaxation kinetics (FIRK) of the electrochromic shift, a measure of the activity of the ATP synthase *in vivo*, demonstrated that the modified γ-ATP synthase in *mothra* is defective in light-dark down-regulation. *In situ* equilibrium redox titrations using oxidized and reduced DTT, showed that *mothra* ATP synthase is insensitive to physiologically relevant changes in thiol redox poise. These results suggest that the highly conserved acidic residues in γ subunit are important for adjusting thiol redox properties. Importantly, though *mothra* ATP synthase was defective in light-dark regulation it retained its regulation in response to changing CO_2 levels during steady-state photosynthesis. This result clearly demonstrates that metabolism-related regulation of ATP synthase does not involve thiol modulation of the γ-subunit.

POSTER 21

ARE PHOTOSYNTHETIC REACTION CENTERS OPTIMIZED FOR HIGH EFFICIENCY?

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Photosynthesis evolved after life had already established much of its core biochemical species and reactions. Thus, photosynthesis can be viewed as an “add-on module’ to life, with pre-existing, or ‘legacy’ biochemistry, forming the biochemical/biophysical foundations allowing photosynthesis to rapidly evolve. However, these same foundations may limit currently the overall efficiency of photosynthesis, e.g. the slow rates and O_2 sensitivity of CO_2 assimilation. In this work, we explore the apparent mismatch between energy available from efficiency visible photons and that able to be stored in the biological “energy batteries,” NADPH and ATP. To address this issue, we are simulating the competition between energy conversion efficiency, losses in energy due to recombination, and redox properties of the electron acceptors in photosystem I. We will present preliminary results, suggesting that the establishment of NADPH as the de facto biological battery of life significantly may limit the energy conversion efficiency of modern photosynthesis.
Supported by Department of Energy

CHARACTERIZATION OF 4(1H)-QUINOLONE (4-1HQ), A NOVEL INHIBITOR OF THE CYTOCHROME *bf* COMPLEX IN *SYNECHOCOCCUS* SP. PCC 7002

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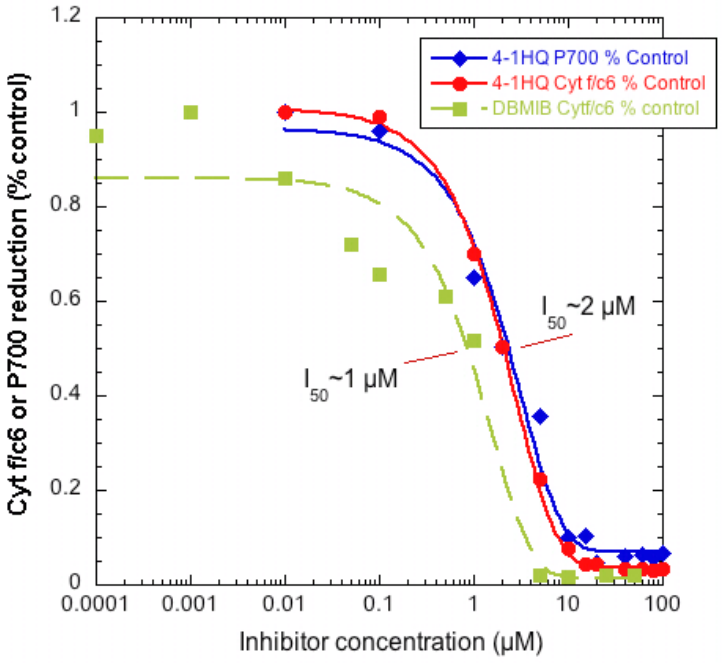
Department of Biology-Microbiology, University of Wisconsin Oshkosh

Photosynthesis converts solar radiation into chemical energy as ATP and reducing power as NADPH. In this process, the cytochrome *bf* complex generates a proton gradient for ATP synthesis and functions in redox sensing and signaling. There are relatively few useful inhibitors of cytochrome *bf* complexes. 1-hydroxy-2-nonyl-4-quinolone NQNO binds the quinone-reductase (Q_n) site and slows electron flow through the low potential chain. Tridecylstigmatellin (TDS), binds the quinol-oxidation (Q_p) site, but is largely ineffective in some cyanobacterial *bf* complexes because of a constrained portal for access to this site (Yamashita et al., 2007 *JMB* 370, 39). 2,5-dibromo-3-methyl-6-isopropylbenzoquinone (DBMIB) is an effective inhibitor of quinol-oxidation but its activity depends on its oxidation state. We have previously synthesized a precursor of NQNO, 4(1H)-Quinolone (4-1HQ) and found that in contrast to NQNO, 4-1HQ is an inhibitor of quinol-oxidation by the cytochrome *bf* complex (Zulegar, Ott, Rivera, Coplien, Johnson, Kedrowski, Xie, & Kallas, unpublished). We have now further characterized 4-1HQ as an electron transfer inhibitor in *Synechococcus* PCC 7002 cyanobacteria. Titrations of cytochrome *f*/c_6 and photosystem I (PS I) P700 reduction kinetics (with a BioLogic JTS-10 spectrophotometer) showed that 4-1HQ has an I_{50} (50% inhibition) value of ~2 µM for these reactions relative to ~1 µM for DBMIB. These data demonstrate that 4-1HQ is nearly as effective as DBMIB and will be useful for studies of the cytochrome *bf* high- and low-potential chains, cyclic electron flow around PS I, and regulation of gene expression by the *bf* complex.



4-1HQ and DBMIB inhibitor titrations in *Synechococcus* sp. PCC 7002. Cytochrome *bf* and photosystem I activities were measured as cytochrome *f*/c_6 and P700 re-reduction rates, respectively, after 9 ms, 530 nm actinic illumination at 300 µmol photons m^{-2} s^{-1}.

POSTER 23

FLUORIDE AS AN INHIBITOR IN OXYGEN EVOUTION BY PHOTOSYSTEM II

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Plants, algae, and cyanobacteria all contain an enzyme complex called Photosystem II (PSII), which carries out the fundamental process of splitting water into molecular oxygen using energy gained by light absorption. Chloride and calcium are known to be important cofactors required for oxygen evolution, which takes place at a catalytic Mn_4Ca complex. In previous studies fluoride was found to be a competitive inhibitor of the chloride cofactor in oxygen evolution. It appears that F^- interferes with Ca^{2+} function although the question of whether F^- further damages the oxygen formation site still remains unclear. Because F^- is known to form an insoluble complex with Ca^{2+} in aqueous solution, one might suspect that it could remove calcium from the Mn_4Ca cluster. In our experiment, we have tested for the inhibition caused by F^- in NaCl-washed PSII using oxygen evolution assays and a spectrophotometric assay to determine the electron transfer rate through the observation of a blue-colored electron acceptor, 2,6-dichlorophenolindophenol. NaCl-washed PSII lacks the extrinsic PsbP and PsbQ (23 kDa and 17 kDa) subunits, which results in a greater access of the Ca^{2+} and Cl^- ions to the active site. Using NaCl-washed PSII with a partial requirement for Ca^{2+}, experiments were designed to distinguish between inhibition caused by Ca^{2+} loss and inhibition associated with permanent damage. The PSII samples were pretreated with fluoride or chloride in the presence of an electron acceptor and exposed to light to promote catalytic turnover. Results indicated that under these conditions, some permanent damage occurred in both the presence of F^- or Cl^- and that the loss of activity appeared to be associated with the portion of the sample that required Ca^{2+}. No evidence was found that Ca^{2+} was removed by exposure to F^-. These results suggest that the mode of inhibition by F^- in NaCl-washed PSII may be limited to competition with Cl^- activation, as in intact PSII. (Supported by the UNCG Office of Research and a grant from the National Science Foundation).

Psb27, a transiently associated protein, binds to chlorophyll binding protein CP43 in photosystem II assembly intermediates

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Photosystem II (PSII), a large multisubunit pigment-protein complex localized in the thylakoid membrane of cyanobacteria and chloroplasts, mediates light-driven evolution of oxygen from water. Recently, a high-resolution x-ray structure of the mature PSII complex has become available. Two PSII polypeptides, D1 and CP43, provide many of the ligands to an inorganic Mn_4-Ca center, essential for water oxidation. Because of its unusual redox chemistry, PSII often undergoes degradation followed by stepwise assembly. Psb27, a small lumenal polypeptide, functions as an important accessory factor in this elaborate assembly pathway. However, the structural location of Psb27 within PSII assembly intermediates has remained elusive. Here we report that Psb27 binds to CP43 in such assembly intermediates. We treated purified genetically tagged PSII assembly intermediate complexes from the cyanobacterium *Synechocystis* 6803 with chemical cross linkers to examine intermolecular interactions between Psb27 homologue protein and various PSII proteins. First, the water-soluble 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was used to cross-link proteins with complementary charged groups in close association to one another. In the His27Δ*ctpA*PSII preparation, a 58 kDa cross-linked species containing Psb27 and CP43 was identified. This species was not formed in the HT3Δ*ctpA*Δ*psb27*PSII complex in which Psb27 was absent. Second, the homobifunctional thiol-cleavable cross-linker 3,3'Dithiobis(sulfosuccinimidylpropionate) (DTSSP) was used to reversibly cross-link Psb27 to CP43 in His27Δ*ctpA*PSII preparations. This allowed the use of LC-MS/MS to map the cross-linking sites as $Psb27K^{63}\leftrightarrow CP43D^{321}$ (trypsin) and $CP43K^{215}\leftrightarrow Psb27D^{58}AGGLK^{63}\leftrightarrow CP43D^{321}$ (chymotrypsin), respectively. Our data suggest that Psb27 acts as a checkpoint protein during PSII assembly through close and specific interactions with the lumenal domain of CP43.

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Elucidating the energy-transfer mechanisms at the interface of the chlorosome and FMO protein in green sulfur bacteria

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The light harvesting complexes in green bacteria have been heavily studied and are of particular interest to the areas of biohybrid nanomaterials and photovoltaics. In green sulfur bacteria, the cytoplasmic Fenna-Matthews-Olson (FMO) protein acts as a wire to funnel excitations generated by solar photons in the chlorosomes to the membrane-bound reaction center (RC). The chlorosome, FMO protein, and RC have been individually studied in depth but the specifics of the interface between the chlorosome and FMO protein are still under debate. It is proposed that, *in vivo,* the CsmA "baseplate" protein-pigment complex of the chlorosome interacts with the FMO protein and serves as the energy-transfer bridge. This work seeks to uncover the exact energy-transfer mechanisms and structure of this interface using an *in vitro* reconstitution of the CsmA-FMO interaction from *C. tepidum* using both solution and surface methods. The reconstituted complex will be probed with spectroscopic, fluorescence, and crystallographic techniques. Insights gleaned from these findings will be beneficial to constructing more efficient bio-inspired photovoltaics that incorporate photosynthetic machinery or synthetic analogs.

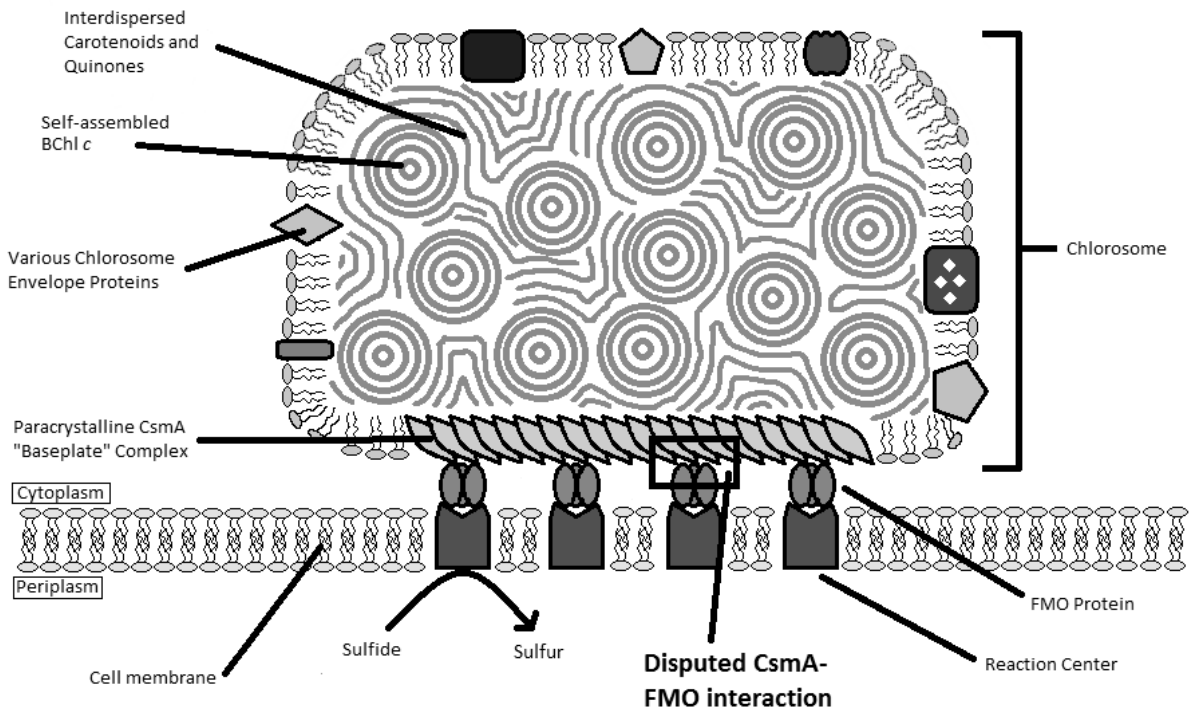

Figure 1: the typical photosynthetic system of green sulfur bacteria. Photons entering the chlorosome excite the self-assembled BChl *c* molecules. The excitations are funneled down to the baseplate, through the FMO protein, and into the reaction center, where charge separation occurs. The site of the disputed baseplate-FMO interaction is indicated.

CHEMICAL PROBES OF HYDROGEN BONDING NEAR THE PHOTOSYNTHETIC OXYGEN-EVOLVING COMPLEX

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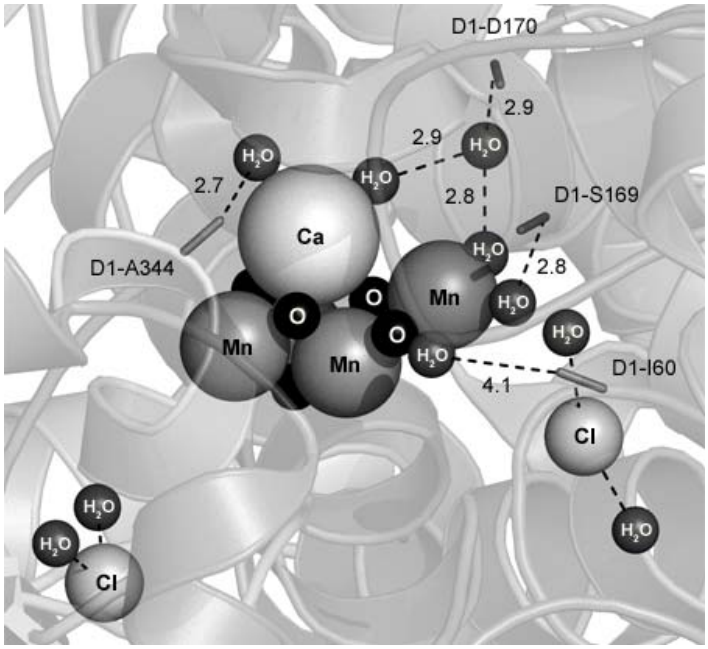
Photosystem II (PSII) is the enzyme complex responsible for the oxidation of water to molecular oxygen in oxygenic photosynthesis. Water oxidation is accomplished by procession through light-induced, charge-separated states at the PSII oxygen-evolving complex (OEC). The OEC is composed of a Mn_4CaO_5 cluster and nearby chloride ion(s). Each successive turnover of the PSII reaction center generates an oxidizing equivalent at the OEC. Four oxidations of the Mn cluster are required to release O_2. Each intermediate oxidation state is assigned an S_n state, where n = 0-4. S_1 is the dark stable state of the OEC, and oxygen release occurs on the transition from S_3 to [S_4] to S_0. Extensive hydrogen bond networks surrounding the OEC and extending to the lumen have been illustrated in a recent crystal structure of bacterial PSII[1]. Contributing to the hydrogen bond network are several carbonyls donated by amino acid residues surrounding the OEC within hydrogen bonding distance of putative water molecules. Ammonia, an analog of substrate water, has been used previously to probe the OEC[2-4]. We have employed reaction-induced Fourier transform infrared (FTIR) spectroscopy to monitor changes in the OEC hydrogen bond network during the S_1 to S_2 transition. Long-lived dynamics associated with relaxation of the OEC are probed by this technique. Ammonia and trehalose were used as perturbants of the hydrogen-bonding network. The results of these investigations will be discussed.



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PROBING THE TOPOGRAPHY OF THE PHOTOSYSTEM II OXYGEN EVOLVING COMPLEX: PSBO IS REQUIRED FOR EFFICIENT CALCIUM PROTECTION OF THE MANGANESE CLUSTER AGAINST DARK-INHIBITION BY AN ARTIFICIAL REDUCTANT.

Hana Popelkova, Nicholas Boswell, and Charles Yocum,

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The photosystem II (PSII) manganese-stabilizing protein (PsbO) is known to be the essential PSII extrinsic subunit for stabilization and retention of the Mn and Cl^- cofactors in the oxygen evolving complex (OEC) of PSII, but its function relative to Ca^{2+} is less clear. To obtain a better insight into the relationship, if any, between PsbO and Ca^{2+} binding in the OEC, samples with altered PsbO-PSII binding properties were probed for their potential to promote the ability of Ca^{2+} to protect the Mn cluster against dark-inhibition by an exogenous artificial reductant, *N*,*N*-dimethylhydroxylamine. In the absence of the PsbP and PsbQ extrinsic subunits, Ca^{2+} and its surrogates (Sr^{2+}, Cd^{2+}) shield Mn atoms from inhibitory reduction [Kuntzleman et al. (2004), Phys. Chem. Chem. Phys 6: 4897]. The results presented here show that PsbO exhibits a positive effect on Ca^{2+} binding in the OEC by facilitating the ability of the metal to prevent inhibition of activity by the reductant [Popelkova et al., Photosynth. Res. (In Press)]. The data presented here suggest that PsbO may have a role in the formation of the OEC-associated Ca^{2+} binding site by promoting the equilibrium between bound and free Ca^{2+} that favors the bound metal.

EMPLOYING FUNCTIONAL GENOMICS TO IDENTIFY NOVEL GENES THAT PROVIDE PHOTO-PROTECTION UNDER HIGH LIGHT STRESS IN THE GREEN MICROALGA *CHLAMYDOMONAS REINHARDTII*

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The purpose of this research project is to obtain a better understanding of how plants cells are able to sense high light stress, translate such information to the biosynthetic machinery in the nucleus and the chloroplast and provide photo-protection to the cells. The model green micro-alga C*hlamydomonas reinhardtii* can be easily cultured in the laboratory, can grow both photosynthetically and heterotrophically, is amenable to both nuclear and chloroplast transformation and its genome has been sequenced. All of these traits make it an ideal and elegant eukaryotic system to study oxygenic photosynthesis.

Although *Chlamydomonas* mutants incapable of photoautotrophic growth can be isolated and maintained as acetate-requiring mutants in the light, this approach does not allow the recovery of photosynthetic mutants that are light sensitive. Hence, to isolate DNA insertional mutants in all aspects of *C. reinhardtii*, it is necessary to select mutant cells in the dark. After comparison of the growth of four different wild-type *Chlamydomonas* strains in the dark, the cell-walled strain $4A^+$ was selected as the parental strain for the population of insertional mutants due to its ability to grow well and remain green in the dark. The linearized bacterial plasmid pBC1, conferring paromomycin resistance, was used to transform the strain $4A^+$ to generate a random insertional mutant library. The generated mutants were selected in the dark on paromomycin plus acetate-containing media to recover light-sensitive and non-photosynthetic mutants. The insertional mutant library is currently being subjected to a battery of primary and secondary phenotypic screens to identify photosynthetic mutants that are pigment-deficient and hypersensitive to high light stress (specifically high light induced-reactive oxygen species stress). We will be presenting a research summary of these high light sensitive photosynthetic mutants generated by our laboratory. (This project has been funded by University of West Georgia)

CONSTRUCTION OF RECOMBINANT STRAINS OF SYNECHOCYSTIS SP. PCC 6803 ABLE TO GROW ON WOOD SUGARS

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Wood sugars D-xylose and L-arabinose together consist of a large fraction of agricultural waste which can be used as an inexpensive carbon source. Model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) lacks the ability to utilize wood sugars due to absence of genes/ operons coding for specific transporters as well as the enzymes required for funneling of the wood sugars into pentose phosphate pathway. This fact was verified by conducting growth experiments under heterotrophic as well as mixotrophic conditions. For this study, we chose four transporter genes/operons for xylose namely *xylE*, *xylFGH*, *galP* and *glf* and three transporter genes/ operons for arabinose namely *araE*, *araFGH* and *araJ*. Out of these seven transporters, *xylE*, *galP*, *glf, araE* and *araJ* were independently cloned into a plasmid to create five separate recombinants of *Synechocystis* by integration of these genes/ gene sets into the host genome. Reverse transcriptase-polymerase chain reactions have revealed expression of the genes in the host. Work is underway to also generate the transformants for *xylFGH* and *araFGH* gene sets. After detection of sugar transport, the gene sets for utilization of xylose and arabinose namely *xylAB* and *araABD* would be independently cloned into another plasmid to be integrated into the genomes of previously created strains containing xylose or arabinose transporter gene/gene sets respectively. Except gene *glf* which comes from *Zymomonas mobilis* ZM4; all other transporter as well as utilization genes/gene sets source from *Escherichia coli* K-12. Further characterization and comparison of the transporters expressed in the new host is possible once homozygous transformants are obtained. An attempt is being made to further improve the potential of cyanobacteria by arming them with the ability to utilize wood sugars for future biotechnological applications.

POSTER 30

ENGINEERING OF THE BIOSYNTHETIC PATHWAY FOR THE PRODUCTION OF RESVERATROL, A PLANT SECONDARY METABOLITE, INTO TWO MICROBIAL PLATFORMS AND ITS POSSIBLE ROLE IN PHOTOPROTECTION

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Department of Applied Science, University of Arkansas Little Rock

This project focuses on the metabolic engineering of the biosynthetic pathway of resveratrol (a phytoalexin produced naturally by several plants while under attack). Phytoalexins are anti-bacterial and anti-fungal chemicals produced by plants as a defense against infection by pathogens. Resveratrol has been reported to have a number of beneficial health effects in humans, such as anti-cancer, anti-viral, neuroprotective, anti-aging and anti-inflammatory effects. The catalysis of p-coumaric acid to resveratrol requires two enzymes: 4-coumarate: coenzyme A (CoA) ligase (4CL) and stilbene synthase (STS). The biosynthesis of resveratrol from p-coumaric acid begins with the coupling of CoA to the p-coumaric acid molecule by the action of the enzyme 4CL. Subsequently, the intermediate coumaroyl-CoA molecule is converted into resveratrol by the sequential addition of three malonyl-CoA units with the release of carbon dioxide through the enzymatic action of STS.

cDNA encoding 4CL from *Nicotiana tabacum* and cDNA encoding STS from *Vitis vinifera* were assembled together into *E. coli* expression plasmid pAC-4CL-ST (a kind gift from Dr. Beekwilder), which was transformed to *E. coli* BL21 DE3 strain. Resveratrol synthesis from p-coumaric acid was verified and monitored in this *E. coli* strain via HPLC and LC-MS analyses. Because of the beneficial effects of resveratrol and the ease of working with *synechocytis*, one of our objectives is to explore the possibility of using *Synechocystis* PCC 6803 as a cell factory for the production of resveratrol from p-coumaric acid. We constructed three plasmids expressing genes encoding 4CL and STS under the control of three different *Synechocystis* promoters (*psa*A, *psb*AII, *hli*A). Two positive mutants (*psa*AKres and *hli*AKres) were identified and verified by PCR. Transcription of 4CL and STS genes were confirmed in these two mutants by RT-PCR. These two mutants also seemed to be able to convert p-coumaric acid to resveratrol derivatives. The appearance of an unknown peak showing up only in *psa*AKres mutant type and not in the control is under progress for identification.

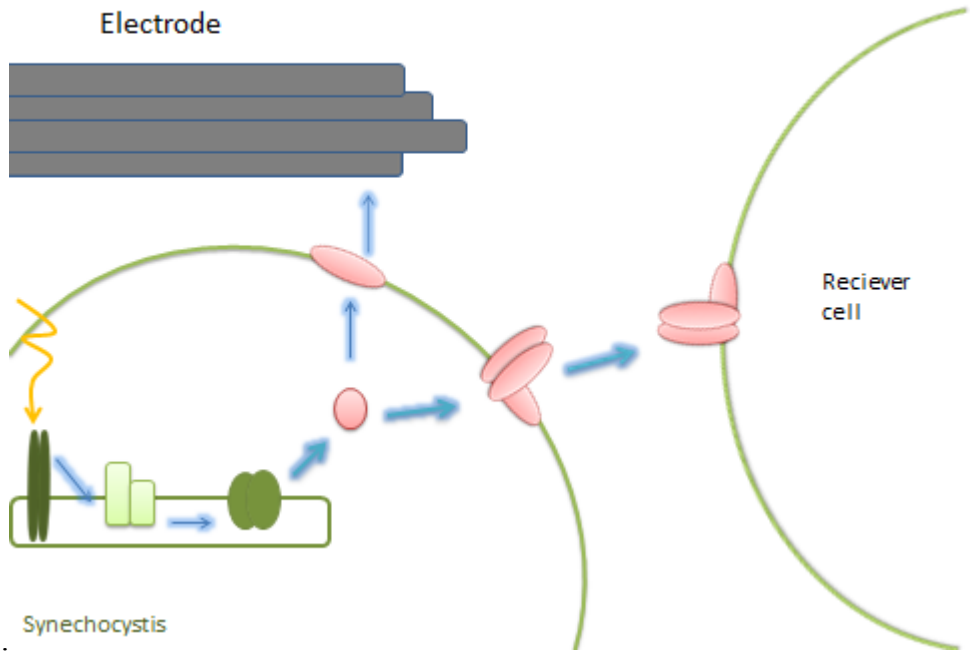
Several photoprotection studies were performed using *Synechocystis* PCC 6803 wild type cells grown in the present of resveratrol while grown under high light conditions. Our results showed that resveratrol provided a partial photoprotective effect to these cells when grown under high light conditions

Engineering biophotovoltaic organisms

Eliezer Schwarz and David Kramer.

Michigan State University, Plant Research Laboratory.
106 Plant Biology Building, East Lansing, MI 48824

Full sunlight provides about ten times more energy than photosynthetic organisms can successfully use for carbon fixation under ambient CO2 concentrations. The great majority of the solar energy absorbed throughout the day therefore never enters the photosynthetic electron transport chain, but is instead dissipated as heat to avoid oxidative damage to the photosystems. In considering strategies to increase photosynthetic yields, the enormously disproportionate abundance of solar energy with respect to the availability of substrates for its utilization is widely recognized as the greatest source of inefficiency, and the most promising area for improvement. While most approaches focus in one way or another on increasing the effectiveness of carbon fixation, this project instead attempts to harvest unused solar energy by exporting excess reducing potential from the cell as electric current. To do this, an alternative electron transport pathway from Photosystem I to the exterior of the cell will be engineered into the cyanobacterium Synechocystis PCC 6803 using components from the electrogenic bacterium Shewanella oneidensis MR-1



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Multiheme cytochromes from Shewanella Oneidensis form an electron transport pathway from Photosystem I to the outside of the cell, where electrons are harvested as electricity or fed to other cells to power biosynthesis.

HYDROGEN PEROXIDE ACTIVATES CYCLIC ELECTRON FLOW AROUND PHOTOSYSTEM I *IN VIVO*

Deserah D. Strand[1,2], *Aaron K. Livingston*[2], *and David M. Kramer*[1,2]

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Cyclic electron flow around photosystem I (CEF1) is important for maintaining the ATP/NADPH budget of photosynthesis. Despite its important role, there are many open questions about the mechanism and regulation of this process. We show that our previously described Arabidopsis high CEF1 mutant (*hcef1*) [1], which showed very high rates of CEF1 through the chloroplast NDH complex, also produces high levels of H_2O_2. We tested whether H_2O_2 was a cause or an effect of high CEF1. Introduction of H_2O_2 into leaves, either by infiltration or expression of glycolate oxidase in the chloroplast, resulted in strong activation of CEF1. The effect was suppressed by co-infiltration of lincomycin, suggesting that *de novo* synthesis of plastid-encoded genes is required. We propose a model in which H_2O_2 is poduced when PSI electron acceptors are strongly reduced, under conditions of ATP/NADPH imbalance. H_2O_2, in turn, may induce synthesis of NDH genes and activate the NDH complex, perhaps involving a phosphorylation cascade as suggested by previous work [2].

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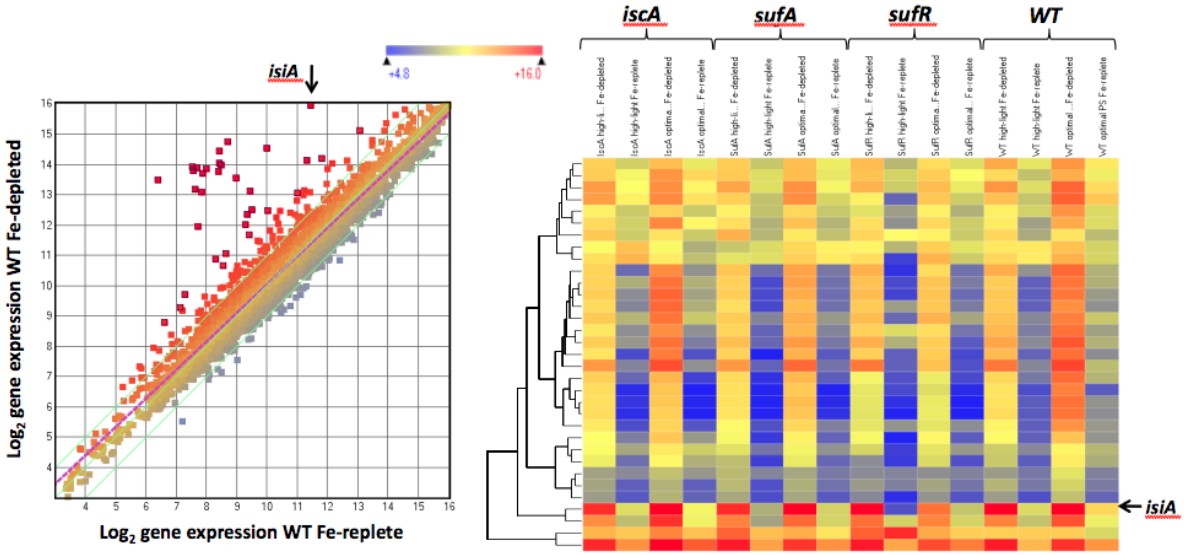
520 NM ELECTROCHROMIC SIGNAL AND GLOBAL GENE EXPRESSION IN WILD TYPE, *SUFR, SUFA, ISCA* MUTANTS OF *SYNECHOCOCCUS* SP. PCC 7002

Junlei Sun[1], *George L. Weir IV*[2], *Linda K. Vuorijoki*[3], *Matthew Nelson*[2], *David Furcy*[4], *Patrik R. Jones*[3], *John H. Golbeck*[1] & *Toivo Kallas*[2],

[1]Department of Biochemistry & Molecular Biology, Pennsylvania State University; [2]Department Biology-Microbiology, University of Wisconsin Oshkosh; [3]Department Biochemistry & Food Chemistry, University of Turku, Finland; [4]Department of Computer Science, University of Wisconsin Oshkosh

There are three known systems for iron-sulfur clusters assembly, *nif* (*n*itrogen *f*ixation), *isc* (*i*ron *s*ulfur *c*luster) and *suf* (*s*ulfur *u*tilization *f*actor). The *suf* system predominates in cyanobacteria and SufA and IscA are implicated in regulation. Fe-limited and/or high-light (2000 µE m^{-2} s^{-1}) conditions were established for *Synechococcus* sp. PCC 7002 wild type, *iscA, sufA* and *sufR* mutants to investigate their impacts on global gene expression. Time-resolved optical spectroscopy of Fe-starved cells revealed a light-induced 520 nm spectral signal that is associated with the IsiA, Fe-stress induced, antenna protein and arises from electron accumulation on the photosystem I A_1 phylloquinone. This signal serves as a useful indicator of Fe-starvation. The microarrays for gene expression analysis have 72,000 probes including >43,000 probes for high-density, up-stream un-translated (UTR) regions of all predicted genes to map transcription start sites and define regulatory regions. Genes up-regulated in response to Fe-depletion, included *isiA* (chlorophyll-binding protein), *isiB* (flavodoxin) and three clusters (SYNPCC7002_G18-25, SYNPCC7002_G79-84, SYNPCC7002_G98-104) on plasmid pAQ7. Under high-light, most of the genes on plasmid pAQ4 were down-regulated in the wild type and SufA, SufR mutants, but not in the IscA mutant, suggesting that IscA may be involved in sensing or responses to high-light stress. Further analysis of the microarray data sets is in progress.



Gene expression in wild type Fe-depleted vs. Fe-replete cells during optimal photosynthesis. Panel A: Scatter plot, 33 genes differentially expressed at ≥ 4-fold change ($p \leq 0.05$). Cultures were grown in 3% CO_2, 200 µmol m^{-2} s^{-1} light intensity). **Panel B:** Hierarchical cluster comparison of 33 differentially expressed genes.

EXPRESSION AND CHARACTERIZATION OF CYTOCHROME c6 FROM CHLAMYDOMONAS REINHARDTII USING A DESIGNER GENE

Nicole L. Vanderbush, Brian St. Clair, Marilyn Davis, and Dan J. Davis

Dept of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR,

Cytochrome c6 is a lumenal redox carrier in oxygenic photosynthesis. The cytochrome is a class I cytochrome with histidine and methionine serving as axial ligands. Its redox potential ranges from +340mV to +390mV and is found to be pH dependent in all species examined. We have constructed a synthetic gene, expressed, purified, and conducted an initial characterization for cytochrome c6 from Chlamydomonas reinhardtii.

The synthetic gene, constructed by the removal introns and the substitution to E. coli biased codons, was incorporated into a pUCF2 plasmid downstream of the lac operon and a pelA leader sequence. The protein is expressed by cotransformation in E. coli of the pUCF2 plasmid and the PEC86 plasmid, which contains genes for the covalent attachment of the heme to the protein. The spectral characteristics were determined by UV-Vis spectrophotometry and include a reduced α peak at 553nm, β peak at 523nm, Soret band at 417nm, the oxidized peak at 423nm and one at 693nm indicative of the His-Met ligation of the heme. In contrast to other c-type cytochrome, C. reinhardtii c6 also exhibits an atypical circular dichrosim spectrum, lacking the commonly found negative peak near 420 nm. C.Mutants K29I and K57I were constructed using site-directed mutagenesis and show a shift in the α peak to 552nm. The midpoint potentials at pH 7 as determined by redox titrations are 365 ± 5mV for the wild type and +322 ± 5mV and +335 ± 5mV respectively for the K29I and K57I mutants. The redox potentials of the wild type and mutants do not show a dependence on pH. Differential scanning calorimetry experiments reveal the folding of the wild-type protein and mutants to be irreversible. The Tm for the wild type is 78°C and 70°C and 71°C for the K29I and K57I mutants respectively.

Cytochrome c6 from C. reinhardtii shows a typical spectrumand redox potential, but the midpoint potential is not pH dependent as far as pH 10 differing from previously described c6. The observed Tm value for C. reinhirdtii is also higher than for mitochondrial cytochrome c indicating a greater thermostability. Also, the conserved K29 and K57 residues contribute significantly to the midpoint potential and stability of the protein.

POSTER 35

REVERSE GENETICS AND GENE DISCOVERY: UNCOVERING A POTENTIAL CARBON-SENSING REGULATOR, *YrdC*, IN THE MARINE CYANOBACTERIUM *SYNECHOCOCCUS* SP. PCC 7002

George L. Weir IV[1], *Justin Zangl*[1], *Stephanie Krueger*[1], *David Furcy*[2] & *Toivo Kallas*[1]

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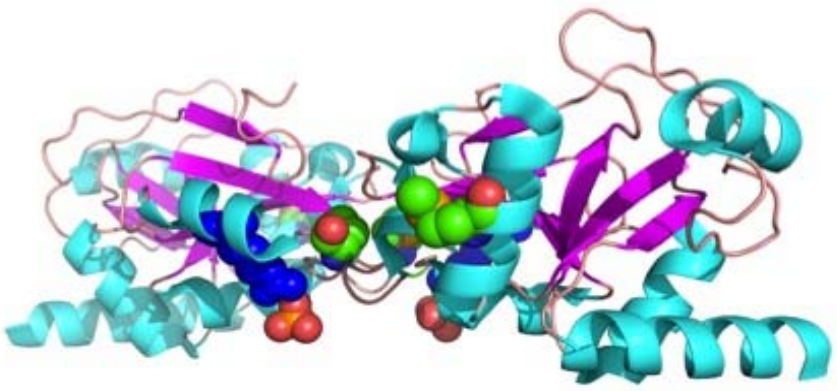
Cyanobacteria fix carbon dioxide (CO_2) into carbohydrates via the Calvin-Benson cycle in which ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the key CO_2 fixation reaction. CO_2 availability can vary greatly and several adaptive mechanisms have evolved for carbon acquisition and concentration. Mechanisms for carbon accumulation in the cyanobacterium *Synechocystis* PCC 6803 include two CO_2 uptake systems (NDH-1M, NDH-1S) and three HCO_3^- (SbtA, BicA, CmpABCD) transporters. Similar mechanisms are predicted in the model cyanobacterium *Synechococcus* sp. PCC 7002, but not completely understood. Our high-density microarray investigation of global gene expression responses in native and electron transport mutants of *Synechococcus* 7002 revealed a hypothetical *yrdC*-like gene (SYNPCC7002_A1732) up-regulated in low-CO_2 grown cells. Upstream, untranslated region (UTR) probes further indicated an alternative transcription start site for the *rbcR/ccmR* (RubisCO Regulator/Carbon Concentration Mechanism) gene, a known regulator of the low-CO_2 induced *ndhFIII-ndhDIII-cupA* operon. These data and bioinformatic tools (CyanoBIKE, MEME, and TOMTOM) indicated that *rbcR* is part of this operon and has an upstream binding site for the YrdC-like (_A1732) gene product. We hypothesize that YrdC_A1732 is a gene-regulatory factor that binds upstream to initiate transcription of the *rbcR-ndhFII-ndhDIII-cupA* and other operons in carbon limiting conditions. *E. coli* YrdC has a helix-turn-helix motif, and phosphorylation sites, indicating a potential role in regulated DNA binding. To further investigate the role of YrdC_A1732 in *Synechococcus* 7002, we have constructed a knockout mutant (ΔYrdC). This mutant grows normally in 3% CO_2 but extremely poorly in low (ambient) CO_2, consistent with an important role for YrdC_A1732 in mediating responses to carbon limitation.



***E. coli* YrdC protein structure and suggested role in DNA-binding**. The protein is a dimer of two asymmetric subunits totaling ~370 amino acids. Shown bound to two Arg are phosphate clusters typical of some DNA-binding proteins (Teplova et al., 2000 *Protein Science* 9, 2557).

COMPARISON OF LIPID ACCUMULATION IN PHOTOMIXOTROPHICALLY AND HETEROTROPHICALLY GROWN *CHLORELLA VULGARIS* CULTURES

Benjamin D. Woodworth, Courtney Nichols, Rebecca Mead, Leslie M. Frost and Derrick R. J. Kolling

Marshall University, Department of Chemistry,
490 Science Hall, Huntington, WV 25755, USA

Microalgae are an attractive biofuel feedstock because of their lipid products that are suitable for biodiesel production. *Chlorella vulgaris* is a green microalga capable of growing photoautotrophically, photomixotrophically, and heterotrophically. We studied photomixotrophic and heterotrophic growth in order to determine the influence of energy source and potentially, carbon source, on lipid metabolism. In both cultures, growth occurred in a medium supplemented with dextrose, which is an environmental source of carbon and chemical energy that can be used following cellular uptake. Photomixotrophic cultures, however, may utilize light energy in addition to chemical energy. These cultures may also photosynthetically fix inorganic carbon in addition to incorporating dextrose. Cell densities, dry weights, lipid extractions, and chlorophyll *a* levels were taken daily to track growth and lipid composition. We observed a lag in the onset of exponential growth for heterotrophic cultures, but found equivalent cell densities within the medium/late stationary phases. Chlorophyll *a* levels in the photomixotrophic cultures spiked in correspondence with the exponential growth, but then decreased as the cells progressed through stationary phase. In the heterotrophically grown cells, chlorophyll *a* was present in higher concentrations than were expected for the light deprived conditions (reaching almost 65% of levels obtained in photomixotrophic cells). Most importantly, cells grown photomixotrophically accumulated almost two times as many lipids (relative to biomass) as those grown heterotrophically. This difference in accumulation may be a result of the addition of light energy and the resultant increase in NADPH:NADP+ favoring the most reduced of the common biomolecules, lipids; alternatively, photosynthetic fixation of inorganic carbon and its subsequent integration into metabolic pathways may also allow cells to produce more lipids as they enter stationary phase sooner than heterotrophically grown cells.

STRUCTURE AND FUNCTION OF THE ALTERNATIVE COMPLEX III FROM THE PHOTOSYNTEHTIC BACTERIA *Chloroflexus aurantiacus and Roseiflexus castenholzii*

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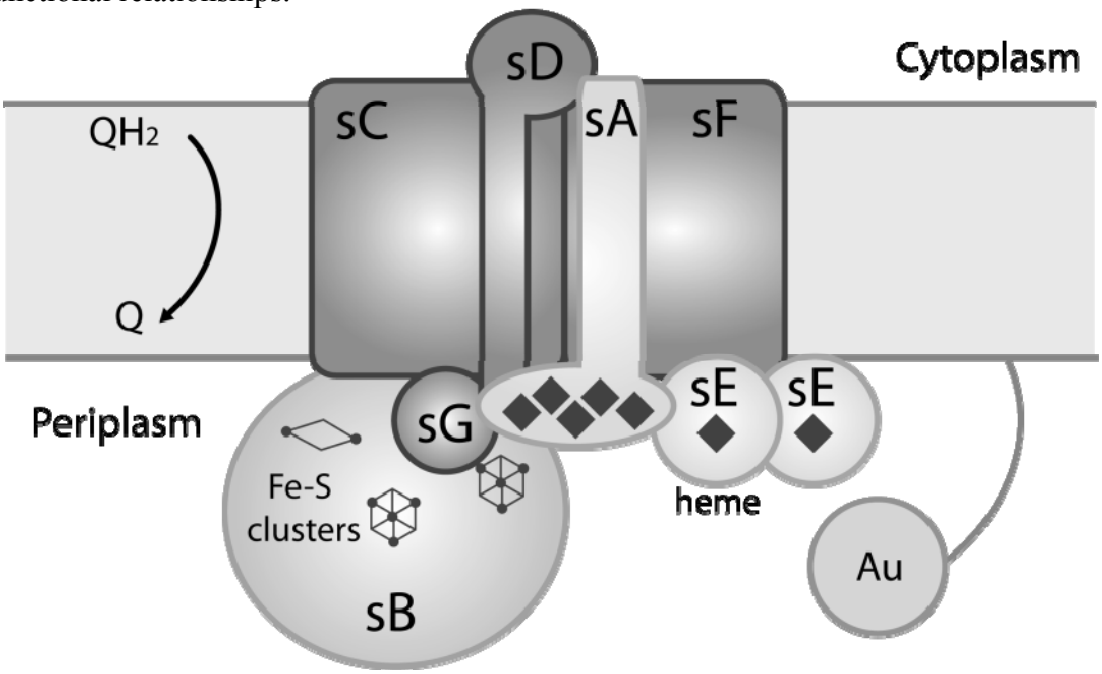
The structure and function of the energy-conserving alternative complex three (ACIII) from the electron transport chains of two related photosynthetic bacteria, *Roseiflexus castenholzii* and *Chloroflexus* aurantiacus is under investigation. *Roseiflexus* is a Filamentous Anoxygenic Phototroph, FAP, with ACIII like *Chloroflexus*, but lacks a chlorosome for light harvesting. ACIII is theorized to be a functional replacement for complex III, cytochrome bc_1 or cytochrome b_6f complex, in mitochondria and chloroplasts respectively, which is absent in these bacteria. ACIII in *Chloroflexus* has previously been purified, identified and characterized. However, it has not been shown if the protein pumps protons across the membrane. Experiments described here probe the proton pumping function of this protein by reconstituting ACIII in liposomes with entrapped pH sensitive fluorescent dye pyranine. Additionally, the complex in *Roseiflexus* is being purified and characterized. Comparisons between the two complexes reveal evolutionary and functional relationships.



The current model of the Alternative Complex III based on work by X. Gao. The protein is postulated to pump protons across the membrane to the periplasm as a functional replacement for Complex III in organisms that lack a cytochrome bc-type complex. sA-sG are the seven subunits in the order of the operon. The diamonds are c-type hemes. The Au in the periplasm is the electron acceptor blue copper protein, auracyanin. QH_2 is reduced menaquinone and Q is oxidized menaquinone.

MASS SPECTROMETRY BASED CHARACTERIZATION OF FMO ANTENNA PROTEIN COMPLEX: NATIVE ELECTROSPRAY, TOP-DOWN MS AND ION MOBILITY

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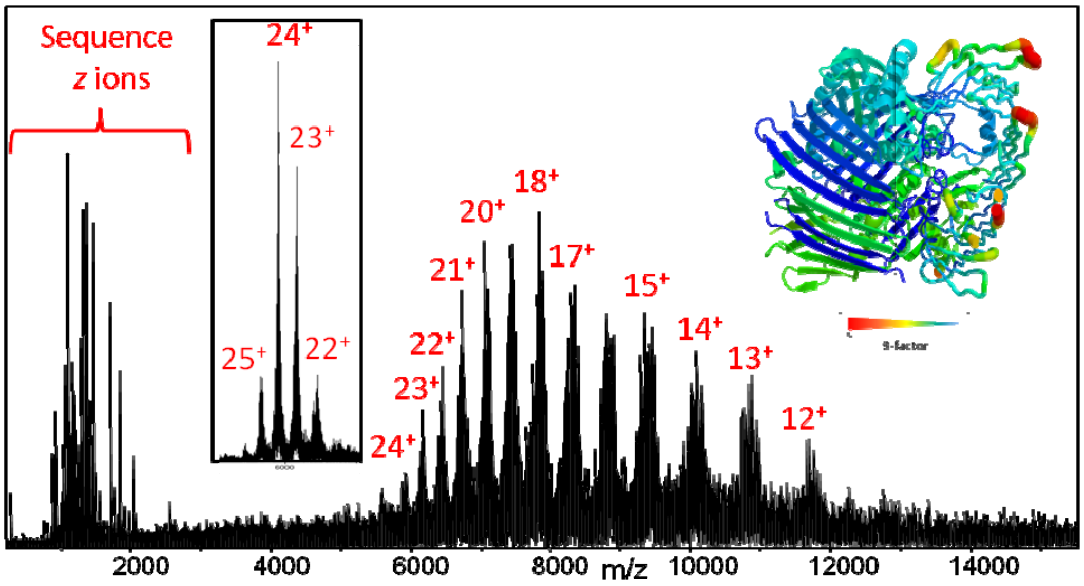
Native ESI mass spectrometry and ion mobility are playing an increasingly important role in structural studies of protein complexes. We report here the ion mobility measurement and a top-down approach that integrates ECD and native ESI of photosynthetic Fenna-Matthews-Olson antenna complex (FMO, 140 kDa). The MW and subunit identities can be obtained in one experiment. Top-down fragmentation patterns and ion mobility of the protein complexes activated at different collision energies provide structural information. We conducted ECD and CID top-down experiments show that the relatively free and flexible regions of the subunits can be sequenced by activated ion ECD in which the protein ions are collisionally activated prior to ECD. The fragments correlate well with the B-factor from X-ray crystallography, which is a measure of the extent an atom can move from its coordinated position as a function of temperature or crystal imperfections[1].



1.Zhang H, Cui W, Wen J, Blankenship RE, Gross ML. Native electrospray and electron-capture dissociation FTICR mass spectrometry for top-down studies of protein assemblies. *Anal Chem.* 2011 Jul 15;83(14):5598-606

PROTON COUPLED ELECTRON TRANSFER: TYROSINE Z IN THE PHOTOSYNTHETIC OXYGEN-EVOLVING COMPLEX

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Redox-active tyrosine Z (YZ) within photosystem II (PSII) plays an essential role in the catalysis of water into protons and O_2 by oxidizing the oxygen evolving center (OEC). Four flashes are required to produce oxygen from water (Joliot, p.; et al.; *Bioenergetics of Photosynthesis* 1975). The OEC cycles among four S_n states, where n refers to the number of oxidizing equivalents stored. The S-state cycle has a unique temperature dependence at each transition with the half inhibition temperature of the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow S_0$ transitions being 220-225, 135-140, 230, 235 K respectively (Styring, S.; et al.; *Biochim. Biophys. Acta* 1988). YZ, Y161 of the D1 polypeptide, is essential for oxygen evolution. YZ is surrounded by an extensive hydrogen bonding network that is believed to play a major role in the translocation of substrate and products from the active site (Umena, Y.; et al.; *Nature* 2011). In this report we investigate the proton coupled electron transfer reactions (PCET) of YZ in oxygen evolving PSII set in the S_0 state. The recombination reaction of $YZ^{\bullet}$ and Q_A^- shows a similar kinetic profile throughout the pL range (5.5-7.5) compared to the S_2 state (Keough J.; et al.; *J. Am. Chem. Soc.* 2011). At pH 5.0 the recombination reaction slows nearly threefold. We attribute this change to a pH dependent conformational change (Figure 1).

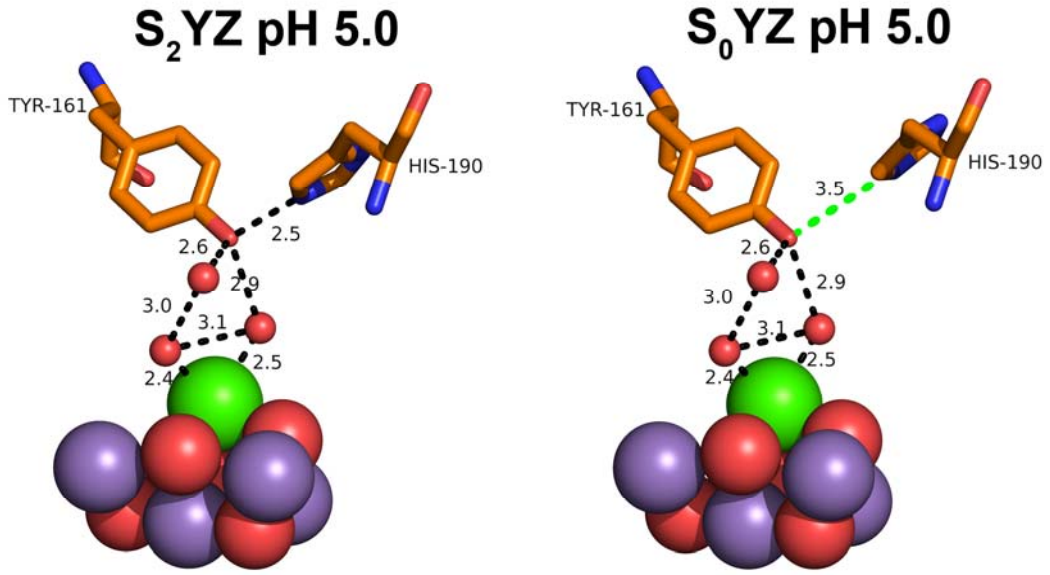

Figure 1. A speculative model showing changes in the hydrogen-bonding network around YZ in the S_2 (Left) and S_0 (Right) states at pH 5.0. Black dashed lines show the predicted hydrogen bonds with distances shown in angstroms (Umena, Y.; et al.; *Nature* 2011). Green dashed line shows that the distance between YZ and His190 can easily exceed hydrogen bond distance if the His is rotated about its C-alpha bond.

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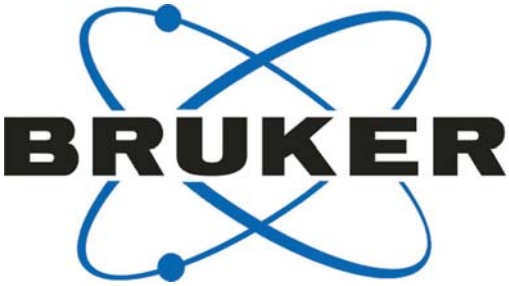

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