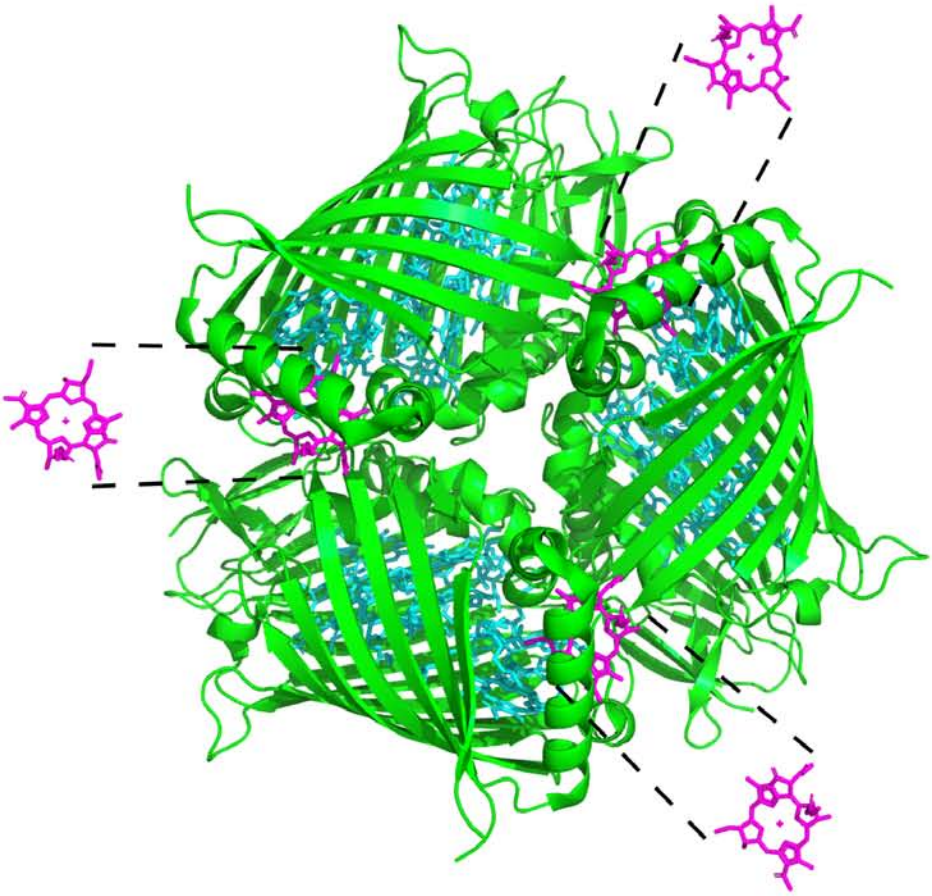
38th Annual Midwest/Southeast Photosynthesis Meeting

PROGRAM AND ABSTRACTS



Turkey Run State Park, Marshall, IN

November 9-11, 2012

PROGRAM AND ABSTRACTS

38th Annual Midwest/Southeast Photosynthesis Meeting

Turkey Run State Park
Marshall, Indiana

November 9-11, 2012

Organizers:

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Please note the dates of next year's MW/SE Photosynthesis Meeting: November 8-10, 2013

On the cover (insert): FMO protein showing position of newly discovered 8th BChl. Generously provided by Professor Robert Blankenship (Washington University at St. Luis)

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We gratefully acknowledge our sponsors:

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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 9

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 10

7:00 AM	Breakfast
9:00 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
8:00 PM	Mixer and Poster Viewing

Sunday, November 11

7:00 AM	Breakfast and preliminary checkout
9:00 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

Friday Evening, November 9

Session I. Keynote Lectures

Chair: Derrick Kolling

7:30 PM Opening Remarks and Welcome

7:40 PM Keynote Speaker: Charles Dismukes, Rutgers University
CONTROLLING PHOTOSYNTHETIC WATER SPLITTING: EFFICIENCY, MECHANISM AND BLUEPRINT FOR CATALYST DESIGN

8:30 PM Keynote Speaker: Asaph Cousins, Washington State University
ENHANCED RATES OF C_3 PHOTOSYNTHESIS IS LINKED TO PHOTORESPIRED CO_2 AND MITOCHONDRIAL RESPIRATION

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

Saturday Morning, November 10

Session II. Photosynthetic Acclimation and Regulation

Chair: Jason Cooley

9:00 AM CREATION OF FRET SENSORS TO PROBE INTRACELLULAR METABOLITES
Steven C. Holland, Alyson Colin, Robert L. Burnap

9:20 AM HIERARCHICAL REGULATION OF CYANOBACTERIOCHROMES IN *FREMYELLA DIPLOSIPHON*
Adam N. Bussell and David M. Kehoe

9:40 AM A PHYCOERTHRIN-SPECIFIC BILIN LYASE-ISOMERASE CONTROLS BLUE-GREEN CHROMATIC ACCLIMATION IN MARINE *SYNECHOCOCCUS*
Animesh Shuklaa, Avijit Biswas, Frédéric Partensky, Jonathan A. Karty, Loubna A. Hammad, Wendy M. Schluchter and David M. Kehoe

10:00 AM STATE-TRANSITION INDEPENDENT CYCLIC ELECTRON FLOW IN *CHLAMYDOMONAS REINHARDTII* IN RESPONSE TO CHANGING ENERGY REQUIREMENTS FOR CARBON ASSIMILATION
Ben F. Lucker and David M. Kramer

10:20 AM Coffee/tea Break

Session III. Structure, Modeling and Mechanism

Chair: William Cramer

10:50 AM MUTATIONS PERTURBING THE WATER CAVITY SURROUNDING THE MN CLUSTER HAVE A STRONG EFFECT ON THE WATER OXIDATION MACHANISM OF PHOTOSYSTEM II
Han Bao, Preston L. Dilbeck, Curtis L Neveu, and Robert L. Burnap

11:10 AM PROTON TRANSFER PATHWAYS THROUGH THE CYTOCHROME B_6F COMPLEX: ROLE OF WATER (*IN ABSENTIA AQUAE*)
S. Saif Hasan, Eiki Yamashita, Danas Baniulis, and William A. Cramer

11:30 AM COUPLING OF MOLECULAR MODELING WITH *IN VITRO* ENZYMATIC ANALYSIS TO ELUCIDATE HOW THE TOC GTPASE MECHANISM IS COUPLED TO THE PRE-PROTEIN IMPORT CYCLE
Kristen Holbrook, Jianzhuang Yao, L. Evan Reddick, Hong Guo, and Barry D. Bruce

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. Light Harvesting Systems

Chair: Himadri Pakrasi

7:00 PM BIOHYBRID ANTENNA COMPLEXES WITH NATIVE PEPTIDE ANALOGS AND TUNABLE SYNTHETIC CHROMOPHORES
Michelle A. Harris, Joseph W. Springer, Pamela S. Parkes-Loach, Kanumuri Ramesh Reddy, Michael Krayer, Jieying Jiao, Dariusz M. Niedzwiedzki, Phani Pavan, Elizabeth Martin, John D. Olsen, C. Neil Hunter, Christine Kirmaier, Dewey Holten, David F. Bocian, Jonathan S. Lindsey, and Paul A. Loach

7:20 PM SPECTROSCOPIC INSIGHTS INTO THE DECREASED EFFICIENCY OF CHLOROSOMES CONTAINING BACTERIOCHLOROPHYLL *F*
Gregory S. Orf, Marcus Tank, Kajetan Vogl, Dariusz M. Niedzweidzki, Donald A. Bryant, and Robert E. Blankenship

7:40 PM PHYCOBILISOMES FEED BOTH PHOTOSYSTEMS IN ONE MEGACOMPLEX IN *SYNECHOCYSTIS* PCC 6803
Haijun Liu, Hao Zhang, Dariusz M. Niedzwiedzki, Mindy Prado, Michael L. Gross, and Robert E. Blankenship

8:00 PM **Mixer and Poster Viewing**

Sunday Morning, November 11

Session V. Biofuels and Solar Fuels

Chair: Toivo Kallas

9:00 AM INACTIVATION OF GLYCOGEN SYNTHASE GENES FOR INCREASED ISOPRENE PRODUCTION IN *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA
Brandon Thomas, Andrea Felton, Olalekan Aremu, Sarah Black, Kevin Crawford, Eric L. Singsaas, Toivo Kallas, and Matthew E. Nelson

9:20 AM PHOTO-CATALYTIC CONVERSION OF CARBON DIOXIDE TO ORGANIC ACIDS BY A RECOMBINANT CYANOBACTERIUM INCAPABLE OF GLYCOGEN STORAGE
Damian Carrieri, Troy Paddock, Justin Ungerer, Charlie Broadbent, William Old, Pin-Ching Maness, Michael Seibert, and Jianping Yu

9:40 AM SUN-DRIVEN ELECTRICITY PRODUCTION VIA METAL-OXIDE COUPLED PSI
Richard F. Simmerman and Barry D. Bruce

10:00 AM Coffee/tea Break

Session VI. ROS and Photoinhibition

Chair: Robert Burnap

10:30 AM OXIDIZED AMINO ACID RESIDUES IN THE VICINITY OF Q_A AND $PHEO_{D1}$ OF THE PHOTOSYSTEM II REACTION CENTER: PUTATIVE GENERATION SITES OF REDUCING-SIDE REACTIVE OXYGEN SPECIES
Laurie K. Frankel, Larry Sallans, Patrick A. Limbach, and Terry M. Bricker

10:50 AM RPAA REGULATES THE ACCUMULATION OF MONOMERIC PHOTOSYSTEM I AND PSBA UNDER HIGH LIGHT CONDITIONS IN *SYNECHOCYSTIS* SP. PCC 6803
Waqar Majeed, Yan Zhang, Yong Xue, Saurabh Ranade, Ryan Nastashia Blue, Qiang Wang, and Qingfang He

11:10 AM Technical Break

11:25 AM Awards
Chair: Charles Yocum

11:30 AM Talk: Best undergraduate student poster

11:40 AM Talk: Best graduate student poster

11:50 AM Closing notes and announcements
Chair: Harvey Hou

12:00 PM Departure

Oral Presentation Abstracts

(In order of presentation in the program)

KEYNOTE TALK

PHOTOSYNTHETIC WATER SPLITTING: EFFICIENCY, MECHANISM AND BLUEPRINT FOR CATALYST DESIGN

Charles Dismukes

Rutgers University, Department of Chemistry & Chemical Biology,
The Waksman Institute of Microbiology, Piscataway, NJ 08854

The past several years have witnessed major advances in understanding both natural oxygenic photosynthesis and translation of the operating principles into the design of artificial photosynthetic devices and hybrid systems. Oxygenic photosynthesis is the most successful autotrophic metabolism in the biosphere. It is powered by the photosystem II (PSII) reaction center and water oxidizing complex (WOC). In this talk I intend to give an overview of PSII-WOC enzymes from the functional diversity of PSII-WOCs, how they contribute to redox and energy production (ATP, pmf), and the chemical basis for water oxidation capacity.

The intrinsic catalytic efficiency of PSII-WOCs differs both across different families of oxygenic phototrophs and within natural variants of the same organism, resulting in different fitness *in vivo*. Isolated PSII complexes from a handful of phylogenetically distinct phototrophs are available and exhibit remarkably similar light-saturated turnover rates *in vitro.* These rates are typically considerably slower than the fastest single turnover rate = sum of the Kok S-state lifetimes ~ 2ms. The maximum rate of PSII turnover *in vivo* differs by two orders of magnitude among distinct species. The fastest rate occur in cyanobacteria that feature rapid oxidation of the PQH_2 pool (coupling to AOX) and specifically those with the greatest (bi)carbonate buffering capacity of the lumen (rate limited by H^+ release?). *In viv*o rates at low light flux are limited by charge recombination reactions originating in many cases by decay of Q_A^- or Q_B^- with the S_2 and S_3 states. This can produce "period two" oscillations in O_2 yield at low frequency. Control of photosynthetic fitness occurs *in vivo* at the level of D1 subunit expression. At least two distinct isoforms of cyanobacterial D1 confer different photochemical efficiencies and charge recombination rates at high vs low light intensities. At low light intensity, a strain expressing exclusively LL-D1 grows faster and out competes a strain expressing only HL-D1, and vice versa at high light. When these cyanobacterial D1 isoforms are expressed in the chloroplast of a green alga (*C. reinhardtii*) lacking its natural D1, they assemble PSII complexes that work more efficiently than the native PSII. The molecular basis for this adaptability will be described.

Since its introduction 43 years ago, multiple extensions of Kok's classic model for the damping of oscillations in flash oxygen yield in the catalytic cycle have been introduced. Additional S-states and additional fitting parameters have been included that increase the flexibility of the model to approximate experimental data through *numerical* solutions, albeit with added complexity. These iterative models do not permit constrained "fitting" of the parameters and thus unbiased goodness of fit cannot be determined. To date, *analytical* solutions have been found only for symmetric Kok cycles. However, it is well known that the water oxidation cycle is not symmetric. We introduce a new mathematical tool to solve the transition matrix for asymmetric Kok cycles. These asymmetric solutions allow realistic models that: 1)

limit backward transitions to exclusively the unstable S states (S_2 and S_3 recombination), 2) loss of PSII centers occurs at a single S state transition (inactivation linked to the O_2 releasing step), and 3) allow only thermodynamically reasonable transitions (no reversal of $S_0 \rightarrow S_3$). Applications will be shown. Supported by the DOE-BES Physical Biosciences Division.

KEYNOTE TALK

ENHANCED RATES OF C_3 PHOTOSYNTHESIS IS LINKED TO PHOTORESPIRED CO_2 AND MITOCHONDRIAL RESPIRATION

Asaph B. Cousins[1], Berkley J. Walker[1], Anthony Ghandin[1], Florian A. Busch[2,3], and Rowan F. Sage[2]

[1]School of Biological Sciences, Washington State University, Pullman, WA 99164 USA; [2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, M5S3B2 Canada; [3]Present address: Research School of Biology, Australian National University, Canberra, ACT, 0200, Australia; E-mail: acousins@wsu.edu

The majority of photosynthetic reactions occur within the chloroplast; however, photosynthesis does not operate in these organelles in isolation. In fact, chloroplasts exchange high-energy molecules and inorganic compounds (e.g., CO_2 and O_2) with other compartments within a cell. For example, photorespiratory recycling of carbon and nitrogen involves several enzyme-mediated reactions in the chloroplasts, mitochondria, peroxisomes, and possibly the cytoplasm. Additionally, the interaction of mitochondria and chloroplast are important for avoiding chloroplast over-reduction and balancing energy partitioning between photosynthesis, photorespiration, and respiration. Recently collected data will be presented demonstrating the importance of metabolic coordination between the chloroplast and mitochondria to optimize rates of photosynthesis in response to changing CO_2 availability, temperature, and light intensities.

The kinetic properties of Rubisco, the enzyme responsible for the initial fixation of CO_2 in C_3 plants, influence the rates of Rubisco uptake of CO_2 and O_2 under a given atmospheric condition. In C_3 plants, Rubisco oxygenation under current atmospheric CO_2 concentrations significantly inhibits the net uptake of CO_2 by photosynthesis. Traditionally, C_3 plants are not known to use specific mechanisms to reduce Rubisco oxygenation; however, data will be presented demonstrating that some C_3 plants trap and reassimilate 24 to 38% of photorespired and respired CO_2, enhancing photosynthesis by 8 to 11% under ambient atmospheric CO_2 concentrations.

In higher plants, the mitochondrial electron transport chain has non-phosphorylating pathways that include the alternative terminal oxidase (AOX). The AOX pathway has been suggested to act as a sink for dissipating excess reducing power, minimizing oxidative stress, and possibly optimizing photosynthesis in response to changing conditions. We demonstrate that AOX activity is also important for maintaining rates of photosynthetic CO_2 assimilation in response to increasing CO_2 concentrations by balancing the NAD(P)H/ATP ratio and rates of ribulose 1,5-bisphosphate regeneration within the chloroplast.

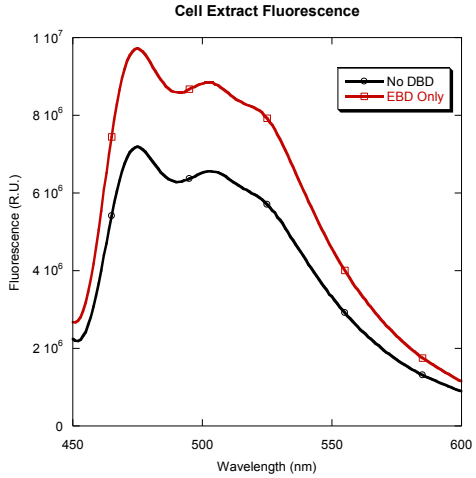
CREATION OF FRET SENSORS TO PROBE INTRACELLULAR METABOLITES

Steven C. Holland, Alyson Colin, and Robert L. Burnap

Oklahoma State University; 307 LSE, Stillwater, OK 74074

The cyanobacterial carbon concentrating mechanism (CCM) exists to saturate the active site of Rubisco with CO_2. When carbon becomes limiting, cyanobacteria increase their affinity for inorganic carbon (HCO_3^- and CO_2). The CCM accomplishes this through the induction and production of inorganic carbon transporters. By having both constitutive and inducible elements to the CCM, cyanobacteria conserve energy and nutrients.

Many CCM genes are regulated by two LysR-type transcriptional regulators (LTTRs): CcmR and CmpR. LTTRs regulate transcription through tetramerization of the LTTR proteins and the bending of DNA of substrate DNA. In addition, they are responsive to small metabolite cofactors. These cofactors are thought to induce a conformational change in the LTTR, relaxing DNA binding, and altering transcription. For CcmR, these cofactors are $NADP^+$ and α-ketoglutarate. (*1*)



Fluorescence of cell extracts of three protein constructs when excited with 435nm light. Peak maxima at ~485nm and ~510nm are indicative of CFP fluorescence. The shoulder at ~530nm is due to FRET to YFP.

The biophysical properties of CcmR and CmpR are not fully understood. In order to analyze metabolite binding and understand *in vivo* metabolite levels, biosensors created from CcmR and CmpR are being created. These sensors will rely on Förster resonance energy transfer (FRET) between two fluorescent proteins, cyan and yellow fluorescent proteins (CFP and YFP) sandwiching CcmR. In theory, conformational changes due to effector binding will move the fluorescent proteins, altering the fluorescent properties of the protein. This could allow analysis of effector binding properties, DNA binding properties, and sensing of internal metabolite concentrations within cells (*2*).

Currently, three protein constructs have been created, each containing varying degrees of LTTR protein. One construct contains full-length CcmR (not pictured). Another contains both the effector binding domain and connecting α-helix, but omits the DNA binding domain (No DBD trace in figure). The final construct contains only the effector binding domain (EBD Only trace in figure). These constructs are currently being assayed for conformational change due to metabolite binding *in vitro* and nutrient availability *in vivo*.

1. Daley, S. M., Kappell, A. D., Carrick, M. J., and Burnap, R. L. (2012) Regulation of the Cyanobacterial CO(2)-Concentrating Mechanism Involves Internal Sensing of NADP(+) and alpha-Ketogutarate Levels by Transcription Factor CcmR, *PLoS One 7*, e41286.
2. Bermejo, C., Haerizadeh, F., Takanaga, H., Chermak, D., and Frommer, W. B. (2011) Optical sensors for measuring dynamic changes of cytosolic metabolite levels in yeast, *Nature protocols 6*, 1806-1817.

HIERARCHICAL REGULATION OF CYANOBACTERIOCHROMES IN *FREMYELLA DIPLOSIPHON*

Adam N. Bussell and David M. Kehoe

Department of Biology, 1001 E. Third St.,
Indiana University, Bloomington, IN 47405

Cyanobacteriochromes, representing the most diverse and greatest number of phytochrome superfamily members, often utilize multiple light sensing GAF (cGMP-phosphodiesterases, adenylyl cyclases and FhlA) domains to detect wavelengths of light throughout the visible spectrum. IflA, a newly described cyanobacteriochrome, senses four distinct colors of light using two GAF domains and is strongly regulated by the two color sensing cyanobacteriochrome RcaE. Regulation of *iflA* expression occurs through RcaE activation of RcaC under red light, causing RcaC to repress expression of *iflA* by binding to a direct repeat sequence, called the L box, upstream of *iflA*. In green light, RcaC binding is absent and *iflA* expression increases 5-fold. IflA controls growth rate at low cell densities under natural sunlight conditions, possibly by sensing changes in the ratio of red:far-red light. Interactions among the photosensory domains of IflA after absorption of blue, green, red, and far-red light were examined and demonstrated how information from different light sensing domains within a single photoreceptor are integrated.

A PHYCOERTHRIN-SPECIFIC BILIN LYASE-ISOMERASE CONTROLS BLUE-GREEN CHROMATIC ACCLIMATION IN MARINE *SYNECHOCOCCUS*

Animesh Shukla[a], Avijit Biswas[b], Frédéric Partensky[c,d], Jonathan A. Karty[e], Loubna A. Hammad[e], Wendy M. Schluchter[b], and David M. Kehoe[a1]

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The marine cyanobacterium *Synechococcus* is the second most abundant phytoplanktonic organism in the world's oceans. The ubiquity of this genus is due to its use of a diverse set of photosynthetic light harvesting pigments called phycobiliproteins, which allow it to efficiently exploit a wide range of light colors. We have uncover a pivotal molecular mechanism underpinning a widespread response among marine *Synechococcus* cells known as "Type IV chromatic acclimation" (CA4). During this process, the pigmentation of the two main phycobiliproteins of this organism, phycoerythrins-I and II, is reversibly modified to match shifts in the ratio of ambient blue (BL) and green light (GL) so as to maximize photon capture for photosynthesis. CA4 involves the replacement of three molecules of the BL-absorbing chromophore phycourobilin that are present in BL with an equivalent number of the GL-absorbing chromophore phycoerythrobilin in GL. We have identified and characterized MpeZ, an enzyme critical for CA4 in marine *Synechococcus*. MpeZ attaches phycoerythrobilin to cysteine-83 of the alpha-subunit of phycoerythrins-II and isomerizes it to phycourobilin. *mpeZ* RNA is six times more abundant in BL, suggesting that its proper regulation is critical for normal CA4, and *mpeZ* mutants fail to normally acclimate to BL conditions. Moreover, we have identified a possible regulator of *mpeZ* that belongs to the AraC family of transcription regulators. This family of transcription factor directly/indirectly represses *mpeZ* in GL and the cells are locked in the BL state of pigmentation. These findings provide insights into the molecular mechanisms controlling an ecologically important photosynthetic process.

STATE-TRANSITION INDEPENDENT CYCLIC ELECTRON FLOW IN *CHLAMYDOMONAS REINHARDTII* IN RESPONSE TO CHANGING ENERGY REQUIREMENTS FOR CARBON ASSIMILATION

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.

The chloroplast must rapidly adjust its output of ATP and NADPH to meet the changing metabolic demands imposed by fluctuating environmental conditions or developmental stages. Cyclic electron flow (CEF) around photosystem I is thought to supplement ATP production to balance the chloroplast energy budget when the relative demand for ATP is high. Based on experiments performed on inhibited, dark-adapted or transiently illuminated *Chlamydomonas reinhardtii*, it was proposed that CEF is regulated by phosphorylation of light harvesting complexes associated with photosystem II (LHCII), followed by antenna 'state transitions' and formation of supercomplexes of photosystem I, cytochrome b_6f complexes and ancillary proteins. In this work, we test this regulatory model operates under continuous illumination in response to altered ATP/NADPH demands. Using noninvasive *in vivo* spectroscopic techniques, we found CEF was activated under conditions where ATP demand is expected to be altered, consistent with a role in balancing the ATP/NADPH budget of the cell. CEF activation was not correlated with antenna state transitions as measured by 77K fluorescence emission spectroscopy, in either wild type or the state transition mutant stt7. Instead, CEF responded to altering metabolic states within seconds, in contradiction with the longer times likely required for phosphorylation and macromolecular reorganizations for state transitions and supercomplex formation. These results favor models where CEF is activated by rapid redox changes or allosteric modulators of key steps, probably the plastoquinone reductase. Changes in CEF resulted in increased photosynthetic proton flux, and thus ATP synthesis, but did not dramatically alter the lumen pH-sensitive exciton quenching (q_E) response. We thus conclude that CEF in *Chlamydomonas* primaryily acts to balance ATP homeostasis rather than to initiate photoprotection.

MUTATIONS PERTURBING THE WATER CAVITY SURROUNDING THE MN CLUSTER HAVE A STRONG EFFECT ON THE WATER OXIDATION MACHANISM OF PHOTOSYSTEM II

Han Bao, Preston L. Dilbeck, Curtis L Neveu, and Robert L. Burnap

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater

The active site of water oxidation in Photosystem II (PSII) is a Mn_4CaO_5 cluster located in a cavity between two PSII subunits. The remainder of this cavity is filled with water molecules that participate in poorly understood hydrogen bond networks that may modulate the function of the Mn_4CaO_5. Mutations of the residue D1-Val185 were produced in *Synechocystis* sp PCC 6803, with the intention that the substitute residue would extend into the water cavity that includes H_2O molecules that interact with the Mn_4CaO_5, several amino acid ligands of the Mn_4CaO_5, Y_Z and the chloride cofactor of PSII (Fig.1). Four mutants were characterized by polarographic and fluorescent techniques to determine their ability to evolve oxygen and undergo charge separation. The asparagine substitution produced the most complex phenotype. While still able to evolve oxygen, it does so less efficiently than wild type. It increased the $t_{1/2}$ of O_2 release from 1.2 ms to 10.0 ms and increased the $t_{1/2}$ of lag phase prior to the onset of O_2 release from 200 μs to 2.8 ms. The combination of a long lag period and decreased rate of O_2 release has also been observed in the D1-D61N mutant and in iodide substituted PSII. Temperature dependence of the rate constant of O_2 production was also investigated with polarographic transients. The Arrhenius plot for the wild type is more likely to exhibit a straight line than that of the V185N, with similar temperature-independent activation energy to previous study[1]. The phenotype in the V185N mutant may be due to the perturbation of hydrogen bond networks surrounding the Mn_4CaO_5 cluster or via an interaction between the asparagine residue and the chloride cofactor.

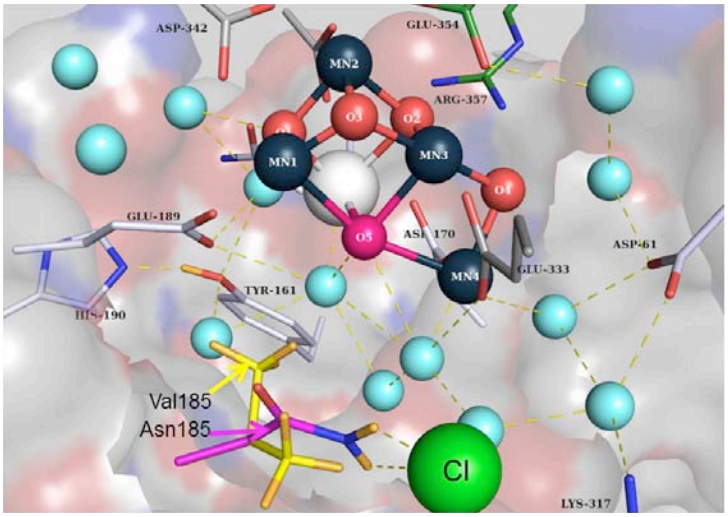

Figure 1. Structure of Mn_4CaO_5 cluster and surrounding hydrogen bond networks.

Reference

1. J. Clausen, R.J. Debus, W. Junge, *Biochim. Biophys. Acta*, 2004, 1655, 184-194.

PROTON TRANSFER PATHWAYS THROUGH THE CYTOCHROME B_6F COMPLEX: ROLE OF WATER (*IN ABSENTIA AQUAE*)

S. Saif Hasan[1], *Eiki Yamashita*[2], *Danas Baniulis*[3], *and William A. Cramer*[1,*]

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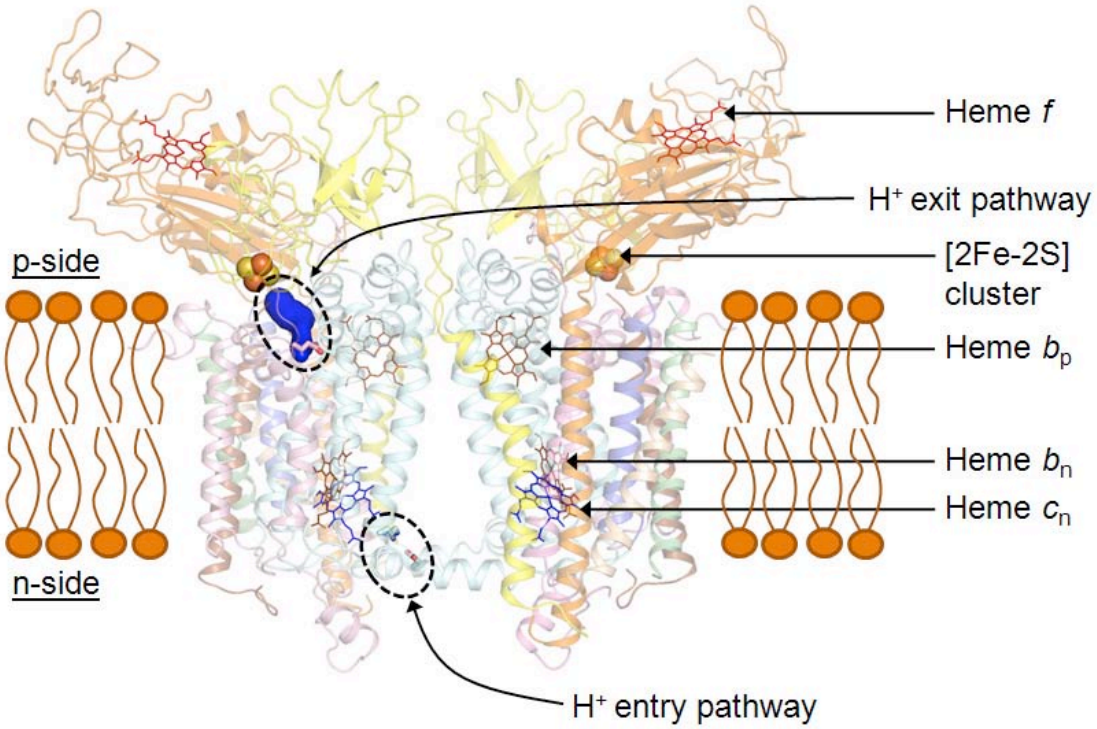
Cytochrome b_6f is a hetero-oligomeric membrane protein complex that is central to photosynthetic electron and proton transfer. The b_6f complex generates as much as two-thirds of the proton electrochemical gradient used for trans-membrane free energy storage and ATP synthesis in oxygenic photosynthesis. Based on a 2.70 Å crystal structure of the b_6f complex from the filamentous cyanobacterium *Nostoc* PCC 7120, the trans-membrane proton transfer pathways are defined within the b_6f complex. Proton uptake from the electrochemically negative (n) aqueous phase to the n-side quinone binding site of the complex occurs via Asp20 and Arg207 (cytochrome b_6 subunit). Crystal structures obtained with quinone analog inhibitors TDS (3.07 Å) and NQNO (3.25 Å) confirm the role of Arg207 as a ligand to the quinone bound at the n-side quinone binding site. In contrast to the anhydrous pathway on the n-side, the Glu78 residue of subunit IV on the electrochemically positive (p) side, which extracts a proton from the plastosemiquinone bound at the p-side quinol binding site, faces a 15.5 Å x 4-6 Å polar portal. A crystallographic water molecule (wat316) links the Glu78 side chain to the portal, thus providing a route for proton exit to the p-side aqueous phase. A role for the small subunit PetG in proton exit is also identified. These pathways provide the first structure based description of the mechanisms and pathways of proton transfer through the b_6f complex, which is outlined below. Financial support: NIH-GM038323 (WAC), Purdue University (SSH)



COUPLING OF MOLECULAR MODELING WITH IN VITRO ENZYMATIC ANALYSIS TO ELUCIDATE HOW THE TOC GTPASE MECHANISM IS COUPLED TO THE PRE-PROTEIN IMPORT CYCLE

Kristen Holbrook, Jianzhuang Yao, L. Evan Reddick, Hong Guo, and Barry D. Bruce

Department of Biochemistry, Molecular and Cellular Biology, University of Tennessee, Knoxville TN

Most plastid-localized proteins are nuclear-encoded and post-translationally imported from the cytosol. The mechanism for the selective translocation of precursor proteins appears to involve the recognition of the transit peptide by the Toc GTPases, which act as receptors or gatekeepers. It is widely hypothesized that the primary targeting specificity is mediated through specific interactions between the transit peptide and the Toc receptors, Toc34 and Toc159. Although it is known that both Toc34 and Toc159 are GTPases and bind precursor proteins, little is understood about how their intrinsic GTPase activity is involved in the import process.

To aid in the elucidation of this complex process, we have developed highly sensitive GTP-binding and hydrolysis assays to characterize the catalytic mechanism of the Toc34 GTPase component. We have extended our analysis of Toc34 to integrate molecular dynamics simulations and QM/MM calculations. Molecular modeling has allowed for the design of site-specific mutations near the active site of Toc34 so that we can investigate its catalytic mechanism in detail. We are currently testing mutant proteins using the soluble, truncated GTPase domain expressed and purified in *E.coli*. Next, we will enlist *in vitro* kinetic analyses to further test the characteristics of these mutants. Our work is expected to make a major step forward in the understanding of the Toc GTPase catalytic mechanism as well as its importance in the preprotein import cycle.

BIOHYBRID ANTENNA COMPLEXES WITH NATIVE PEPTIDE ANALOGS AND TUNABLE SYNTHETIC CHROMOPHORES

Michelle A. Harris,[1] Joseph W. Springer,[1] Pamela S. Parkes-Loach,[2] Kanumuri Ramesh Reddy,[3] Michael Krayer,[3] Jieying Jiao,[4] Dariusz M. Niedzwiedzki,[1] Phani Pavan,[3] Elizabeth Martin,[5] John D. Olsen,[5] C. Neil Hunter,[5] Christine Kirmaier,[1] Dewey Holten,[1] David F. Bocian,[4] Jonathan S. Lindsey,[3] and Paul A. Loach[2]

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Native light harvesting systems are highly efficient. When a photon is absorbed the energy is transferred to the reaction center with near unity quantum yield. However, the absorption spectrum leaves gaps in the visible and near IR regions. Using self assembly of LH1 peptide analogs, native bacteriochlorophyll and designer chromophores, this project affords antenna complexes that enhance solar spectral coverage and have high energy transfer efficiencies comparable to the native light harvesting systems.

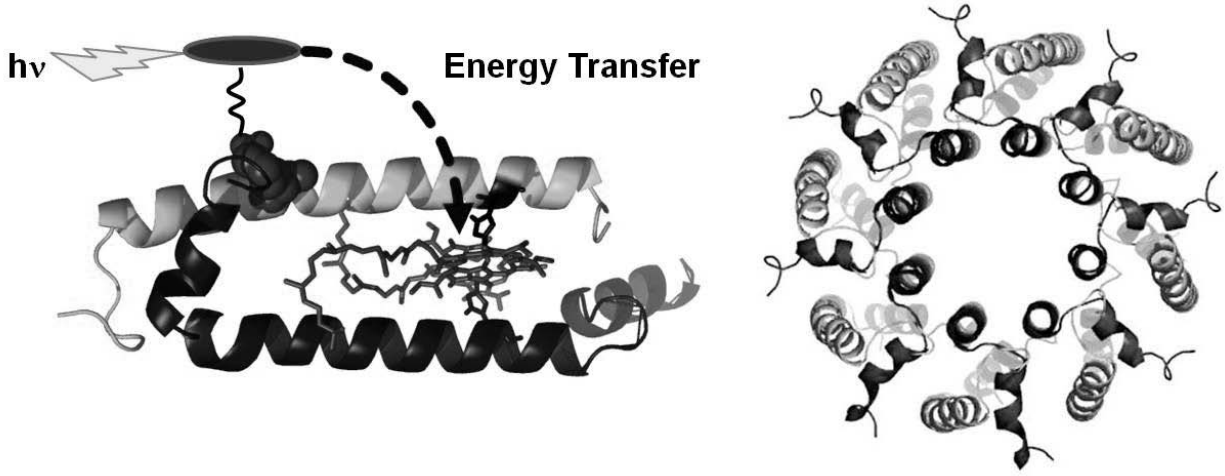

Figure: Left model depicts energy transfer from tunable synthetic chromophore to bacteriochlorophyll dimer. Right model depicts LH2 structure in *Phs. Molischianum* (Koepke, et al. *Structure* **1996**, *4*, 581–597.) . The self assembling peptide analogs likely form a similar cyclic structure.

SPECTROSCOPIC INSIGHTS INTO THE DECREASED EFFICIENCY OF CHLOROSOMES CONTAINING BACTERIOCHLOROPHYLL *F*

Gregory S. Orf[a], *Marcus Tank*[b], *Kajetan Vogl*[b], *Dariusz M. Niedzweidzki*[c], *Donald A. Bryant*[b,d], *and Robert E. Blankenship*[a]

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Chlorosomes are light-harvesting antenna complexes that occur in green bacteria which have only been shown naturally to contain bacteriochlorophyll (BChl) *c*, *d*, or *e* as the principal photosynthetic pigments. BChl *f* has long been thought to be a fourth member of these so-called *Chlorobium* chlorophylls because it possesses a C-7 formyl group like BChl *e* and lacks a methyl group at C-20 like BChl *d*. In organisms that synthesize BChl *c* or *e*, the *bchU* gene product encodes for the enzyme that methylates the C-20 position of these molecules. A *bchU* null mutant of the green sulfur bacterium *Chlorobaculum limnaeum,* which normally synthesizes BChl *e,* has recently been reported[1], which produces chlorosomes containing BChl *f*. In this study, chlorosomes containing BChl *f* and monomeric BChl *f* in pyridine were characterized using a variety of spectroscopic techniques, including fluorescence emission and excitation spectra, fluorescence lifetime and quantum yield determinations, and circular dichroism. These spectroscopic measurements, as well as Gaussian simulation of the data, show that chlorosomes containing BChl *f* are less efficient in energy transfer than those with BChl *e*. This can primarily be attributed to the decreased spectral overlap between the oligomeric BChl *f* (energy donor) fluorescence emission and the BChl *a* (energy acceptor) absorption in the chlorosome baseplate. This study yielded clues that help to evaluate the evolutionary consequences of BChl *f* utilization by green sulfur bacteria.

PHYCOBILISOMES FEED BOTH PHOTOSYSTEMS IN ONE MEGACOMPLEX IN *SYNECHOCYSTIS* PCC 6803

Haijun Liu[a,b], *Hao Zhang*[b,c], *Dariusz M. Niedzwiedzki*[c], *Mindy Prado*[a,b], *Michael L. Gross*[c], *and Robert E. Blankenship*[a,b,c]

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Phycobilisomes capture light energy and feed into reaction centers in cyanobacteria and red algae. Their ability to capture light across a broad spectral range, distinct from those of the chlorophylls and carotenoids, expands photosynthetic solar energy transformation globally. The energy transfer from phycobilisomes to reaction centers has been established for decades, however, remarkably little is known about the structural interface between the phycobilisome and the thylakoid membrane and reaction centers. We have isolated a megacomplex composed of a phycobilisome with its energy acceptors, i.e., Photosystem I (PSI) and Photosystem II (PSII). Functional analysis showed highly active oxygen-evolving PSII activity and O_2 consumption activity from PSI in the megacomplex. Steady-state fluorescence spectroscopy analysis indicated efficient excitation energy transfer from phycobilisomes to both photosystems. LC-MS analysis preceded by chemical cross-linking identified the close association of ApcE and CP43, and of ApcD and PsaA. Mass spectrometry also identified the structural proximity of ApcD and ApcE. Femtosecond time-resolved fluorescence spectroscopy was employed to study the kinetic energy transfer from phycobilisomes to both reaction centers in the megacomplex. Results from global and target analysis are presented and discussed. A model is proposed that the phycobilisome covers both photosystems structurally and feeds them energetically as well in one megacomplex. Regulation of phycobilisome-reaction center interactions and the role of CP43 in the megacomplex assembly in the context of PSII biogenesis are also discussed.

Acknowledgements: This research is funded by the Photosynthetic Antenna Research Center (PARC) (DOE).

INACTIVATION OF GLYCOGEN SYNTHASE GENES FOR INCREASED ISOPRENE PRODUCTION IN *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA

Brandon Thomas[1], Andrea Felton[1], Olalekan Aremu[1], Sarah Black[2], Kevin Crawford[3], Eric L. Singsaas[4], Toivo Kallas[1], and Matthew E. Nelson[1]

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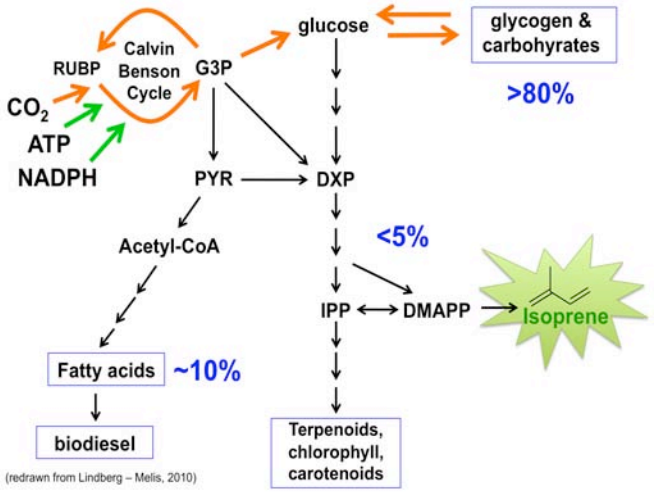
Because of escalating costs, climate change, and political uncertainty associated with fossil fuels, the development of carbon-neutral biofuels is a national-security imperative. Microalgae can help meet this need because they efficiently capture enormous amounts of solar energy and convert atmospheric 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. 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* sp. PCC 7002, a fast-growing, high-light and halotolerant cyanobacterium, and obtained isoprene production at rates promising for commercial development. To further improve isoprene production, we have inactivated glycogen synthase, *glgA1* and *glgA2*, genes for a major, competing carbon pathway. Results of these studies will be presented. U.S. patent 20110039323 (A.E. Wiberley, E.L. Singsaas, and T.D. Sharkey) has been issued for isoprene production work and a provisional patent application (T120019US) has been filed for inventions specific to cyanobacteria.



Carbon partitioning in cyanobacteria. Carbon is stored primarily as glycogen in reactions catalyzed by glycogen synthases GlgA1 and GlgA2. Only a small fraction of carbon typically flows into the MEP pathway. Adapted from Lindberg et al., 2010 *Metabolic Engineering 12, 70.*

PHOTO-CATALYTIC CONVERSION OF CARBON DIOXIDE TO ORGANIC ACIDS BY A RECOMBINANT CYANOBACTERIUM INCAPABLE OF GLYCOGEN STORAGE

Damian Carrieri, Troy Paddock, Justin Ungerer, Charlie Broadbent, William Old, Pin-Ching Maness, Michael Seibert, and Jianping Yu

National Renewable Energy Laboratory
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Oxygenic photosynthetic microbes (algae and cyanobacteria) have great potential to produce fuels and other valuable organic chemicals from sunlight, water, and carbon dioxide. However, efficient conversion of carbon dioxide to products of interest is in direct competition with cellular biomass accumulation. A better understanding of how cells regulate partitioning of photosynthetically fixed carbon between target products, native storage compounds, and cellular growth is needed. Deletion of the gene encoding glucose-1-phosphate adenylyltransferase (Δ*glgC*) in the non-nitrogen-fixing cyanobacterium, *Synechocystis* sp. PCC 6803, (A) disables glycogen synthesis, and under nitrogen deficiency: (B) arrests cellular biomass accumulation, (C) redirects photosynthetically fixed carbon to organic acids (α-ketoglutarate and pyruvate) that appear in the extracellular medium, and (D) does not appear to degrade it phycobillin proteins as is typical for wild-type under N-starved conditions. While we have recently published our initial observations of these responses,[1] continuing work is focused on deciphering their mechanistic origins.

Reference:

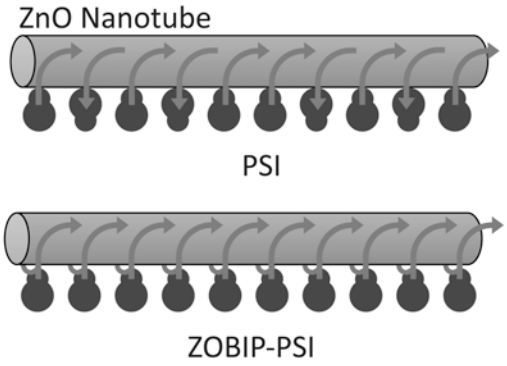
1. Carrieri, D., T. Paddock, et al. (2012). *Energy and Environmental Science* doi: 10.1039/c2ee23181f.

SUN-DRIVEN ELECTRICITY PRODUCTION VIA METAL-OXIDE COUPLED PSI

Richard F. Simmerman[1,2] *and Barry D. Bruce*[1,2]

1) Department of Biochemistry and Cellular and Molecular Biology, The University of Tennessee, Knoxville
2) Tennessee Office of Experimental Program to Stimulate Competitive Research (TN-SCORE)

Due to the growing demand for power and detrimental effects of harnessing fossil fuels (e.g. pollution, climate change, political instability), photosynthetic organisms are being explored for alternative energy production. Strategies to utilize the high efficiency photochemical charge separation of Photosystem I (PSI) while eliminating lower efficiency processes involved in the conversion of NADPH to biomass are being developed. This project focuses on engineering and incorporation of PSI into a bio-inorganic photovoltaic device (BIPV) that will produce electricity. Antibodies have been raised against PsaD and PsaE of *Thermosynechococcus elongatus* (*T.e*.) and Synechocystis PCC #6803 (*Syn* 6803). A ZnO-binding peptide (ZOBIP) has been introduced onto the N-termini of PsaD and PsaE, and these ZOBIP-PSI subunits have been produced *in vitro*. WT and ZOBIP PsaD and PsaE subunits have been characterized with Western blotting, circular dichroism, and MALDI-TOF. Wild-type (WT) PsaD and PsaE subunits have been replaced *in vitro* with ZOBIP-PsaD and ZOBIP-PsaE with around 50% efficiency. While WT PsaD and PsaE bind quite well to ZnO nanoparticles, WT PSI does not. ZOBIP-PSI will bind ZnO, and the results of binding will be compared to WT PSI ZnO binding. Plasmids are under construction that will allow for the transformation of *T.e*. and *Syn* 6803, and *in vivo* production of ZOBIP-PsaD and ZOBIP-PsaE. The ZOBIP will allow for self-orientation of PSI onto ZnO, while ZnO nanowires will increase the volume of the biological heterojunction, and will increase the amount of electricity produced and harnessed from the BIPV.

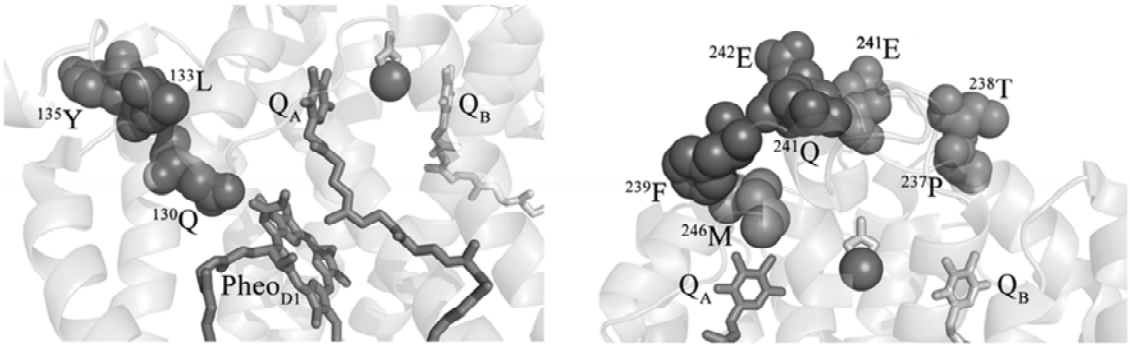


OXIDIZED AMINO ACID RESIDUES IN THE VICINITY OF Q_A AND $PHEO_{D1}$ OF THE PHOTOSYSTEM II REACTION CENTER: PUTATIVE GENERATION SITES OF REDUCING-SIDE REACTIVE OXYGEN SPECIES

[a]Laurie K. Frankel, [b]Larry Sallans, [b]Patrick A. Limbach, and [a]Terry M. Bricker

[a]Department of Biological Sciences, Division of Biochemistry and Molecular Biology, Louisiana State University, Baton Rouge, LA 70803; [b]The Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221.

Under a variety of stress conditions, Photosystem II produces reactive oxygen species on both the reducing and oxidizing sides of the photosystem. Recently, we have identified oxidized amino acid residues located in the vicinity of the Mn_4CaO_5 cluster (Frankel, L.K. et al. 2012 *Biochemistry* 51, 6371-6377) which may be associated with an oxygen/reactive oxygen species exit pathway. In this communication, also using Fourier-transform ion cyclotron resonance mass spectrometry, we have identified several residues on the D1 and D2 proteins from spinach which are oxidatively modified and are in close proximity to Q_A (D1 residues ^{239}F, ^{241}Q, ^{242}E and the D2 residues ^{238}P, ^{239}T, ^{242}E and ^{247}M) and $Pheo_{D1}$ (D1 residues ^{130}E, ^{133}L and ^{135}F). These residues may be associated with reactive oxygen species exit pathways located on the reducing side of the photosystem, and their modification may indicate that both Q_A and $Pheo_{D1}$ are sources of reactive oxygen species on the reducing side of Photosystem II.



Modified Residues Mapped onto the *Thermosynechococcus vulcanus* Structure. Please note that *T. vulcanus* contains a one amino acid residue deletion in the D2 protein. Oxidized residues are shown as spheres (D1:dark, D2:light) and are labeled. Relevant cofactors are shown as sticks and are labeled.

Talk: Sunday 10:50 AM

RPAA REGULATES THE ACCUMULATION OF MONOMERIC PHOTOSYSTEM I AND PSBA UNDER HIGH LIGHT CONDITIONS IN *SYNECHOCYSTIS* SP. PCC 6803

Waqar Majeed[1], Yan Zhang[2], Yong Xue[1], Saurabh Ranade[1], Ryan Nastashia Blue[1], Qiang Wang[3], and Qingfang He[1, 2]

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[2]High Tech Research Center, Shandong Academy of Agricultural Sciences, Jinan, Shandong Province, 250100 China
[3]Institute of Hydrobiology, Chinese Academy of Sciences, 7 South Donghu Road,Wuhan, Hubei Province, 430072 China

The response regulator RpaA was examined by targeted mutagenesis under high light conditions in *Synechocystis* sp. PCC 6803. A significant reduction in chlorophyll fluorescence from photosystem I at 77K was observed in the RpaA mutant cells under high light conditions. Interestingly, the chlorophyll fluorescence emission from the photosystem I trimers at 77K are similar to that of the wild type, while the chlorophyll fluorescence from the photosystem I monomers was at a much lower level in the mutant than in the wild type under high light conditions. The RpaA inactivation resulted in a dramatic reduction in the monomeric photosystem I and the D1 protein but not the CP47 content. However, there is no significant difference in the transcript levels of *psaA* or *psbA* or other genes examined, most of which are involved in photosynthesis, pigment biosynthesis, or stress responses. Under high light conditions, the growth of the mutant was affected, and both the chlorophyll content and the whole-chain oxygen evolution capability of the mutant were found to be significantly lower than those of the wild type, respectively. We propose that RpaA regulates the accumulation of the monomeric photosystem I and the D1 protein under high light conditions. This is the first report demonstrating that inactivation of a stress response regulator has specifically reduced the monomeric photosystem I. It suggests that PS I monomers and PS I trimers can be regulated independently for acclimation of cells to high light stress.

Poster Presentation Abstracts

(Listed alphabetically by first author)

TOWARD BIOLOGICAL TREATMENT OF SEWAGE WATER -- RESPONSES OF CYANOBACTERIA TO HEAVY METAL EXCESS

Mohammed Al-Said Jalel and Qingfang He

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

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. Especially in developing countries, the spread of disease such as diarrhea, cholera, hepatitis type A, leptospirosis, schistosomiasis, as well as various parasites, are caused mainly by water pollution resulted from the release of raw sewage into rivers.

In recent years, it has become necessary to find alternatives to traditional methods of chemical or physical removal of toxic metals from wastewater. Microorganisms, including bacteria, fungi and as well as algae, are considered as cheap materials and bio-absorbents, which can be applied, cost effectively, for removal of metals from contaminated water.

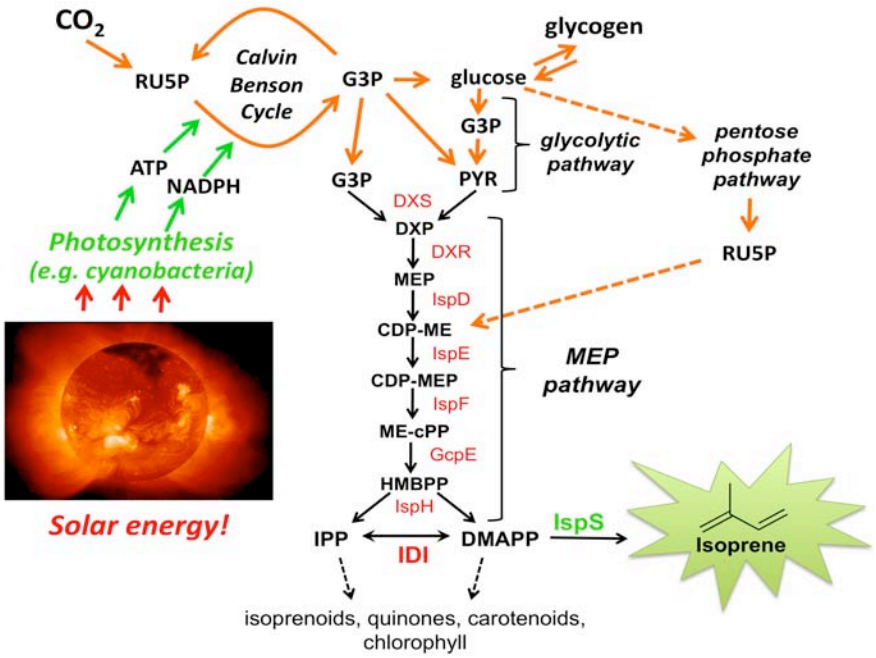
In this study, we explore the possibility of using two strains of cyanobacteria, *Synechocystis* PCC 6803 and *Synechococcus* PCC 7002, as biosensor and/or biosorbent of heavy metals. We began by studying the responses of cyanobacteria to excess copper. *Synechocystis* 6803 and *Synechococcus* 7002 were grown in the presence of copper at various concentrations and under normal light or high light conditions for 5 days. The growth and pigment composition of the cells were analyzed. A light and time dependent increase in toxicity of Cu^{2+} to cyanobacteria cells was observed. The effects of Cu^{2+} on cell growth differ in normal light and high light on both species. 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 and 30 mg/l for *Synechocystis* and *Synechococcus*, respectively. Clearly, high light aggravates Cu^{2+} toxicity and vice versa. The sensitivity of photosystem I and photosystem II to copper treatments, as well as the adjustment of PS II to PS I ratio, appears to be different between the two species. Further study will help us understand the toxicity of heavy metal to cyanobacteria, laying the foundation for application of the two cyanobacteria for removing heavy metals from sewage water or as sensors to detect the pollution by heavy metals.

METABOLIC ENGINEERING OF ISOPRENE PRODUCTION IN FAST-GROWING, HIGH-LIGHT TOLERANT *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA

Olalekan Aremu[1], *Brandon Thomas*[1], *Andrea Felton*[1], *Sarah Black*[2], *Kevin Crawford*[3], *Eric L. Singsaas*[4], *Toivo Kallas*[1], *and Matthew E. Nelson*[1]

[1]Dept. of Biology-Microbiology, UW Oshkosh, [2]Pomona College, [3]Dept. of Chemistry, UW Oshkosh, and [4]Wisconsin Institute for Sustainable Technology, UW Stevens Point

Development of carbon-neutral biofuels is an imperative for economics, global ecology, and national security. 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. We have expressed 'codon-optimized' isoprene synthase and 2-C-methyl-D-erythritol 4-phosphate, MEP, pathway genes in *Synechococcus* PCC 7002 and obtained production of isoprene (a precursor for synthetic rubber and liquid biofuel) at rates promising for development. Further improved isoprenoid production will require strategies such as further engineering of carbon flux through the MEP and other pathways, down-regulation of competing carbon pathways, and down-regulation of light-harvesting antenna for increased cell density and volumetric yields. Toward that end, we have inactivated *glgA1* and *glgA2* genes for synthesis of glycogen, the major storage carbohydrate of cyanobacteria, as well as genes for phycobilisome light-harvesting complexes. The halotolerant *Synechococcus* 7002 grow well under 100% CO_2 indicating that waste agricultural and industrial water and gas effluents may be used as nutrient and CO_2 sources. The work on isoprene production is covered by U.S. patent 20110039323 (A.E. Wiberley, E.L. Singsaas, and T.D. Sharkey) and a provisional patent application (T120019US) has been filed for inventions specific to cyanobacteria.



Methyl-D-erythritol-4-phosphate (MEP) pathway of cyanobacteria and isoprene synthesis.
Or -- Inactivation and replacement of glycogen synthase genes in *Synechococcus* PCC 7002.

UNDERSTANDING SHIFTS BETWEEN LINEAR AND CYCLIC ELECTRON TRANSPORT DURING CYANOBACTERIAL ADAPTATION OF LOW INORGANIC CARBON AVAILABLITY

Juliana Artier, Steven C. Holland, and Robert L. Burnap

Department of Microbiology and Molecular Genetics, Oklahoma State University

In natural habitats, cyanobacteria, an aquatic photoautotrophic organism, have to adapt and survive to several adverse situations. Changes in the metabolism and gene expression are necessary to increase the selective fitness. Under low carbon (LC) conditions, cyanobacteria rely on the CO_2 concentration mechanism (CCM) to overcome the low rates of inorganic carbon (Ci) provided to Rubisco. CcmR, a lysR-type transcriptional regulator is known to control its own expression and down-regulate the expression of Ci transporters under normal conditions (*1*). However, in LC conditions, the cell internally senses changes in [α-ketoglutarate] and [$NADP^+$] that act as co-repressors of CcmR (*2*). These allow an increased expression of the inorganic carbon transporters. This way, the increased concentration of Ci enables fixation by Rubisco in the Calvin cycle (CBB). We are studying the integration of these processes with photosynthetic electron transport producing ATP and NADPH. The electron transport can be linear (LET), involving an ending reaction with ferredoxin:NADP oxidoreductase (FNR) producing NADPH, that will be used in CBB to assimilate carbon. Also, the electron transport can be cyclic (CET), where the electron flow involves the NDH-1 complex and leads mainly to production of ATP. Under LC, cyanobacteria are known to favor cyclic electron flow. We are interested in understand what triggers this switch between LET and CET, and its regulation. Different subunits of FNR are supposed to be involved with LET and CET (*3, 4*)). Both large (FNR_L) and small (FNR_S) subunits are present in *Synechocystis* sp. strain PCC6803 wild type (WT). However, FNR_S is predominant under stress conditions such as N- starvation, high light (*3*) and LC (*4*). Two mutants for FNR have specific characteristics. FSI only present FNR_S and is supposed to have mainly the cyclic photosynthetic reactions, while MI6 have FNR_L, which would have the linear photosynthetic reactions. We studied the changes in chlorophyll fluorescence and P700 oxidation using Dual-PAM (pulse amplitude modulated fluorometry and absorbance spectroscopy) for WT and the mutants FS1, MI6, ΔccmR and ΔtcpA (a mutant of ndhP). The results showed interesting patterns and will be here presented. Supported by US Department of Energy, Office of Basic Energy Sciences DE-FG02-08ER15968

1. Wang, H. L., Postier, B. L., and Burnap, R. L. (2004) Alterations in global patterns of gene expression in Synechocystis sp. PCC 6803 in response to inorganic carbon limitation and the inactivation of *ndhR*, a LysR family regulator, *J Biol Chem 279*, 5739-5751.
2. Daley, S. M., Kappell, A. D., Carrick, M. J., and Burnap, R. L. (2012) Regulation of the Cyanobacterial CO(2)-Concentrating Mechanism Involves Internal Sensing of NADP(+) and alpha-Ketogutarate Levels by Transcription Factor CcmR, *PloS one 7*, e41286.
3. Thomas, J. C., Ughy, B., Lagoutte, B., and Ajlani, G. (2006) A second isoform of the ferredoxin : NADP oxidoreductase generated by an in-frame initiation of translation, *Proc Natl Acad Sci U S A 103*, 18368-18373.
4. Korn, A. (2010) Respective roles of the ferredoxin:NADP-oxidoreductase isoforms in the cyanobacterium *Synechocystis* sp. PCC 6803, In *Service de Bioénergétique, Biologie Structurale et Mécanismes*, Institut de Biologie et de Technologies de Saclay, Saclay, France.

SUPEROXIDE PRODUCTION IN THE CYTOCHROME B_6F COMPLEX: ROLE OF CHLOROPHYLL-*A* IN RATE LIMITATIONS OF THE CANONICAL Q-CYCLE

Danas Baniulis[1], Jason T. Stofleth[2,4], Eiki Yamashita[1], S. Saif Hasan[4], and William A. Cramer[4,]*

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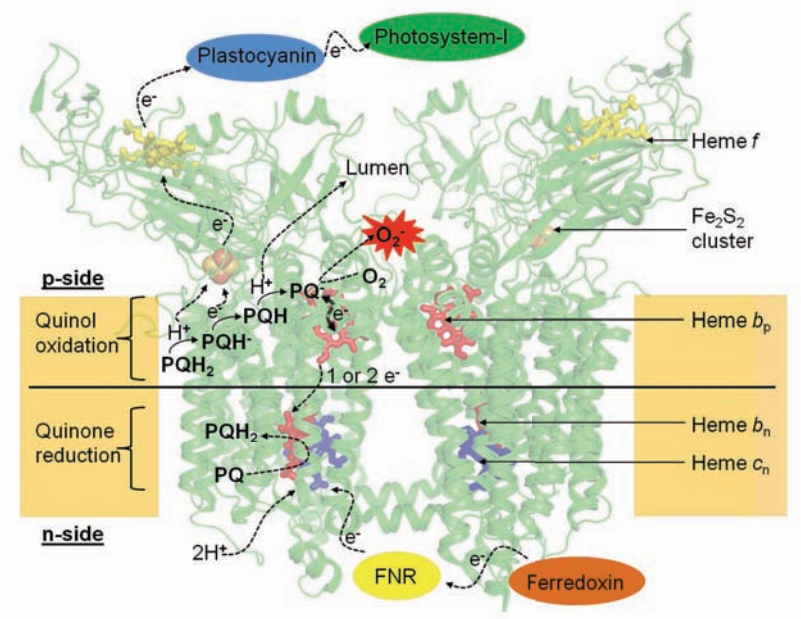
The mechanism of superoxide production in cytochrome *bc* complexes of the respiratory and photosynthetic electron transport chains is relevant to biologically deleterious effects of reactive oxygen species as well as partition of electron transfer in the branched pathway of quinol oxidation in these complexes. The specific rate of superoxide production, normalized to the rate of electron transfer through the respiratory or photosynthetic electron transport chain, was determined in the yeast mitochondrial cytochrome bc_1 complex and in the photosynthetic cytochrome b_6f complex from spinach thylakoid membranes and the cyanobacteria *M. laminosus* and *Nostoc* sp. PCC 7120. In the absence of inhibitors, electron transfer rates were comparable in bc_1 and b_6f. However, the specific rate of superoxide production, normalized to the electron transport rate, was 10-20 fold greater in the b_6f complex. Whereas antimycin A, a specific n-side quinone analogue inhibitor of the bc_1 complex, caused a >50-fold increase in the superoxide production rate in the bc_1 complex, no comparable effect was found for b_6f in the presence of NQNO, also an n-side quinone analogue inhibitor and an axial ligand of heme c_n. Based on crystal structures of cyt b_6f from the cyanobacterium *Mastigocladus laminosus* with the plastoquinone analog inhibitor, TDS, and the ubiquinone analog inhibitor, stigmatellin, it is proposed that the chlorophyll-*a* molecule in the b_6f complex is responsible for steric restriction of plastosemiquinone within the Q_p-site and this retards conformational changes required for electron transfer from semiplastoquinone to heme b_p, thereby decreasing the efficiency of electron transfer to heme b_p and increasing efficiency of a bypass reaction, such as superoxide production. Financial support: NIH-GM038323 (WAC), Purdue University Fellowship (SSH)



MAPPING THE TEMPERATURE DEPENDENCE OF PSII WATER OXIDATION AND OF OEC PHOTOASSEMBLY

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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. While the temperature dependence of oxygen evolution has been described in the literature [for example, see 1], we have performed a comprehensive study that covers PSII activity in the presence and absence of DCBQ from 5–45°C. In addition, we performed similar measurements for OEC photoassembly in the absence of DCBQ. The BBY particles used for our measurements were prepared according to Berthold, Babcock, and Yocum [2], with modifications from Kolling *et al*.[3] The oxygen-evolution 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[4]. We then generated OEC-depleted BBY (apo-BBY) particles by using an established aminosulfonic buffer (20 mM CHES/NaOH, pH 9.4) and a divalent cation (200 mM $MgCl_2$) treatment with minor modifications. [1] 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. The OEC-depleted BBYs were then subjected to different $K_3[Fe(CN)_6]$ concentrations and a range of pulses with varying flash number and frequency in order probe the rate limiting step of photoassembly. We found that PSII oxygen evolution and OEC photoassembly have similar temperature-dependence profiles and share a maximum; inclusion of DCBQ during PSII water oxidation flattens the temperature-dependence profile in the range of 10–32°C; and protease activity within the BBY preps also showed a temperature dependence and was responsible for some loss of activity over 28°C. To the best of our knowledge, this is the most comprehensive study of the temperature dependence of PSII activity and of OEC photoassembly. Mechanistic implications of these findings will be discussed.

1. Baranov, S. V., A. M. Tyryshkin, D. Katz, G. C. Dismukes, G. M. Ananyev, and V. V. Klimov. 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.
2. Berthold, D. A., G. T. Babcock, and C. F. Yocum. 1981. A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes : EPR and electron-transport properties. *FEBS Lett*. 134:231-234.
3. 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
4. Schiller, H.; Dau, H. 2000. Preparation protocols for high activity Photosystem II membrane particles of green algae and in higher plants, pH dependence of oxygen evolution and comparison of the S_2-state multiline signal by X-band EPR spectroscopy. *Journal of Photochemistry and Photobiology*. 55:138-144.

EXPLORING THE PHOTOSYNTHETIC ROLE OF A CHLOROPLAST NUCLEOTIDE TRANSPORTER (NTT)

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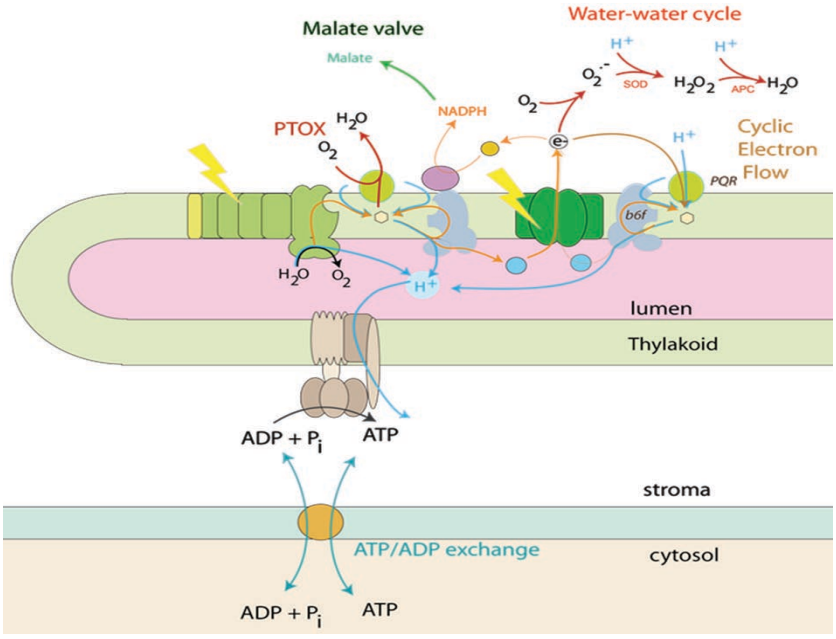
The ratio of ATP: NADPH produced by the photosynthetic light reactions has to be tightly regulated. Perturbation of this ratio can lead to ATP depletion and the failure of downstream processes (such as the Calvin-Benson cycle). Several mechanisms have been proposed to modulate the ATP: NADPH production ratio, namely the water-water cycle (Mehler reaction), malate valve, plastid terminal oxidase (PTOX), and cyclic electron flux (CEF) around Photosystem I. Using the firefly luciferase assay to monitor ATP production during CEF, we discovered unexpectedly high rates of ATP export in intact chloroplasts (100 µmol ATP mg^{-1} Chl hr^{-1}), 25-fold higher than previously reported rates. Chloroplast intactness was assessed by monitoring rates of membrane-impermeant ferricyanide reduction and phase contrast microscopy. In *Arabidopsis,* ATP transport across the plastid inner membrane envelope has been proposed to be due to the ATP/ADP antiporters NTT1 and NTT2. NTT has previously been thought to function primarily as a nocturnal importer for cytosolic ATP into the chloroplast. Our observation of high rates of ATP export in the light has novel biological implications, which are likely to impact the plastid and cellular energy budget. Further work is being conducted on the photosynthetic apparatus involved in the light-dependent reactions that produce ATP in the *Arabidopsis* NTT1, NTT2, and double knock-out mutant lines.



HIGH LIGHT INDUCIBLE CAROTENOID BINDING PROTEIN COMPLEX FROM *SYNECHOCYSTIS* SP. PCC 6803

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Department of Applied Science, University of Arkansas at Little Rock, AR-72204

Photosynthetic organisms provide food and oxygen that are essential for life on earth by harvesting solar energy and converting it into chemical energy. However, exposure of these organisms to high light affects the photosynthetic performance, growth and viability of the organism. To survive, photosynthetic organisms have evolved several protective processes. Cyanobacteria, like other photosynthetic organism, protect themselves from light-induced stress by dissipating excess absorbed energy as heat. It is well known that in cyanobacteria carotenoid plays an essential role in photoprotective mechanisms. In this study we investigated the effect of different light intensity on *Synechocystis* 6803. Cells of wild type, *IsiA* and *PsaL* less strain of *Synechocystis* 6803, where culture under normal light (40µmol m^{-2} s^{-1}), and high light (400µmol m^{-2} s^{-1}) for various length of time. The thylakoid protein complexes were then isolated, solubilized in mild detergents and fractionated by sucrose gradient ultracentrifugation. The results indicate the presence of an orange carotenoid protein binding complex after 24hours high light exposure of *PsaL* less strain of *Synechocystis* 6803.

X-RAY EMISSION AND ABSORPTION ANALYSIS OF THE PHOTOSYSTEM II PROTEIN COMPLEX AT RT & LT

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Large-scale implementation of man-made systems based on artificial photosynthesis to harvest solar energy could lead to an abundant chemical storage of solar power in the form of hydrogen. The process of splitting water ($2H_2O \rightarrow O_2+4e^- +4H^+$) in Photosystem II, the trans-membrane metalloprotein complex responsible for splitting water in the photosynthetic process, requires a catalyst, the Oxygen-Evolving Complex (OEC) Mn_4Ca cluster. Characterization of sub-millisecond reactive intermediates in this system is central to understanding the catalysis involved in water splitting. We present results from two different X-ray spectroscopy techniques.

1) *X-ray emission spectroscopy (XES)* is an informative technique for the analysis of the oxidation and spin states of the Mn center, as well as its ligand environment.[1] Following the progression of x-ray induced damage by Mn Kβ x-ray emission spectroscopy, we demonstrate the feasibility of collecting room temperature (RT) data on the electronic structure of the dark stable S_1 state of Photosystem II. Two different beam structures were tested during these measurements: continuous monochromatic beam (at 20-ID), and pulsed pink beam (22μs pulse-width – at 14-ID). The dosage/damage relation for continuous beam measurements matches preliminary RT models well;[2] the XES spectral shape obtained at RT and low T are similar indicating that no significant charge redistribution is happening within the OEC upon freezing PS II.[3] The determined damage threshold (~100 ms for continuous monochromatic beam; ~100 μs in pulsed pink beam mode) is sufficient for the analysis of electron dynamics and the catalytic mechanism. The reported timeframes are expected to be representative of other metalloproteins. In addition, preliminary time-resolved Kβ emission laser pump (to advance the catalytic cycle to the subsequent S_2 state), x-ray probe experiments conducted in pulsed pink beam mode show promise when compared to previously published cryogenic data.[4]
2) *X-ray absorption spectroscopy (XAS)* is also sensitive to the oxidation state of Mn (X-ray absorption near edge structure - XANES), but in addition, can obtain structural information such as bond lengths and ligand identities from directly around the Mn atoms (X-ray absorption fine structure - EXAFS). We present results indicating the RT structure of PS II in comparison to those at LT. In addition, EXAFS were performed on S_2, S_3, and S_4/S_0 samples frozen on ms-timescales in the hopes of catching short-lived intermediate states.

1 Glatzel, P. & Bergmann, U. High resolution 1s core hole X-ray spectroscopy in 3d transition metal complexes - electronic and structural information. *Coord. Chem. Rev.* **249**, 65-95 (2005).

2 Grabolle, M., Haumann, M., Muller, C., Liebisch, P. & Dau, H. Rapid loss of structural motifs in the manganese complex of oxygenic photosynthesis by x-ray irradiation at 10-300 K. *J. Biol. Chem.* **281**, 4580-4588, doi:10.1074/jbc.M509724200 (2006).

3 Yano, J. *et al.* X-ray damage to the Mn_4Ca complex in photosystem II crystals: A case study for metallo-protein X-ray crystallography. *P. Natl. Acad. Sci. USA* **102**, 12047-12052 (2005).

4 Messinger, J. *et al.* Absence of Mn-centered oxidation in the S_2 to S_3 Transition:implications for the mechanism of photosynthetic water oxidation. *J. Am. Chem. Soc.* **123**, 7804-7820 (2001).

USING STREAMLINED MUTAGENESIS AND SCREENING TO INCREASE ELECTRON TRANSFER TO THE B-BRANCH PATHWAY IN BACTERIAL PHOTOSYNTHETIC REACTION CENTERS

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Photosynthetic reaction centers (RCs) convert light energy into chemical energy in a series of extremely efficient electron transfer reactions, accomplishing transmembrane charge separation. The structures of RCs reveal two symmetry-related branches of cofactors that are functionally asymmetric; bacterial RCs use the A pathway exclusively. Using current knowledge of the architectural and energetic factors that underlie the directionality and yields of electron transfer, our goal is to generate a mutant RC that uses the B pathway preferentially and efficiently. Site-specific mutagenesis has produced transmembrane charge separation solely via B-branch activity, but the best overall yield of state $P^+Q_{B^-}$ is still low, ~15% in the F(L181)Y-Y(M208)F-L(M212)H-W(M250)V (YFHV) mutant. This rational design approach has provided neither the means nor understanding necessary to engineer an efficient B-branch electron-transfer pathway. Therefore, the goal of this project is to produce such an RC via directed molecular evolution, implementing streamlined mutagenesis and high-throughput spectroscopic screening steps to sample a large number of RC variants. The primary experimental vehicle is the RC from the purple photosynthetic bacterium *Rhodobacter capsulatus*. Using cassette-based mutagenesis and a time-resolved millisecond-seconds spectroscopic assay, we have produced and screened 321 mutants, of which 57 exhibit $P^+Q_{B^-}$ yields greater than YFHV. The primary photochemistry (using femtosecond transient absorption spectroscopy) and P redox potentials have been examined in a subset of these RCs.

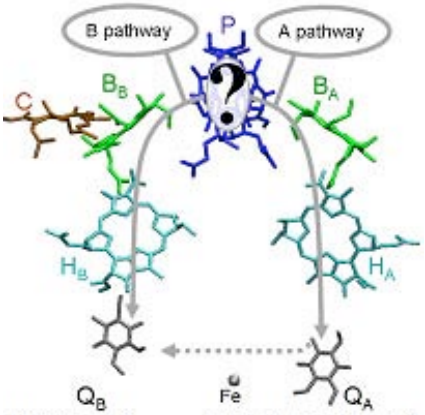


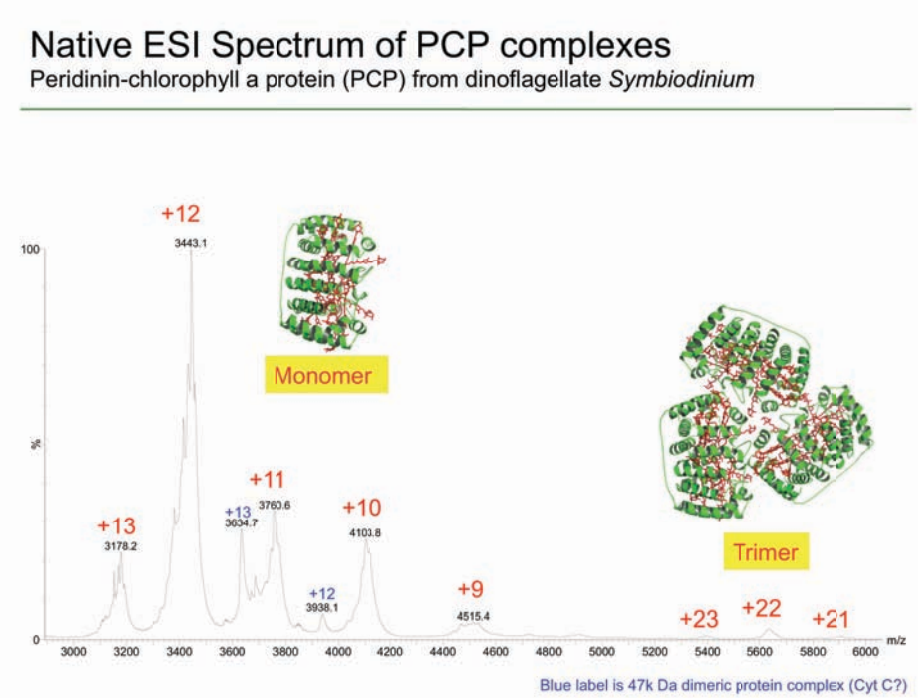
Figure 1. *Arrangement of cofactors in the bacterial RC.*

NATIVE MASS SPECTROMETRY AS A TOOL TO ELUCIDATE COFACTOR ASSOCIATION OF PHOTOSYNTHETIC COMPLEXES

Lucas B. Harrington[1], *Hao Zhang*[1], *Jing Jiang*[2], *Michael L. Gross*[1], *and Robert E. Blankenship*[1,3]

[1]Department of Chemistry, [2]Department of Energy, Environmental & Chemical Engineering, [3]Department of Biology, Washington University, St. Louis, MO 63130

Native electrospray ionization mass spectrometry (NESI-MS) continues to develop as a technique to probe multi-subunit complexes in their near-native form. This tool holds particular promise towards understand systems in which non-protein cofactors play a critical role in the complex's function. However, continued work is needed to refine this technique so that it may be used on previously uncharacterized protein-cofactor systems. To this aim, we have used NESI-MS to analyze the cofactor association of two photosynthetic complexes from the endosymbiotic dinoflagellate symbiodinium, which have varying degrees of characterization and membrane association. The first of the two, peridinin-chlorophyll-protein (PCP), is a well characterized soluble protein. In contrast, the second is an integral membrane protein called chlorophyll *a* /*c* protein (ACP) for which multiple cofactor stoichiometries have been suggested. Our analysis of PCP suggests that careful optimization of instrument parameters is necessary to limit gas-phase cofactor dissociation. Making use of this information, we obtained a spectrum of the native or near-native ACP complex allowing us to suggest a protein-cofactor stoichiometry. These data add to the growing number of photosynthetic systems that have been characterized using NESI-MS and demonstrate this technique's potential to be used in concert with existing tools to elucidate non-protein cofactor associations.



MAPPING TRIPLET STATE ENERGIES OF CHLOROPHYLLS

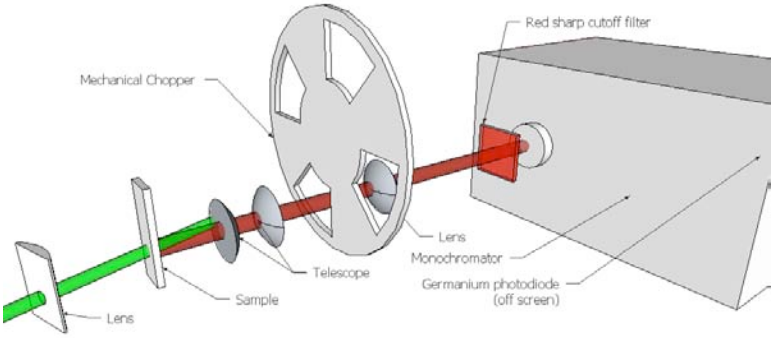
Dan Hartzler[1], *Dariusz Niedzwiedzki*[2], *Robert Blankenship*[2], *Sergei Savikhin*[1]

1. Department of Physics, Purdue University, West Lafayette, Indiana,
2. Department of Chemistry, University of Washington, St. Louis, Missouri

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 the 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 (e.g. 670nm vs. 970nm for Chl a) the infrared tail of the fluorescence band is strong enough to mask 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.



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. Currently chlorophylls a, b, d and bacteriochlorophylls a, b, c, and e have been measured with Chl c2 and BChl d and g to be completed soon.

(Figure: sample excited by nanosecond pulsed laser, mechanical chopper provides time domain gating, filters and monochromator provide spectral resolution.)

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 DEFG02-09ER16084

CONFORMATIONAL CHANGES INDUCED BY LIPID CHARGE WITHIN THE CYTOCHROME B_6F COMPLEX

S. Saif Hasan[1], *Eiki Yamashita*[2], *Jason T. Stofleth*[1,3], *and William A. Cramer*[1,*]

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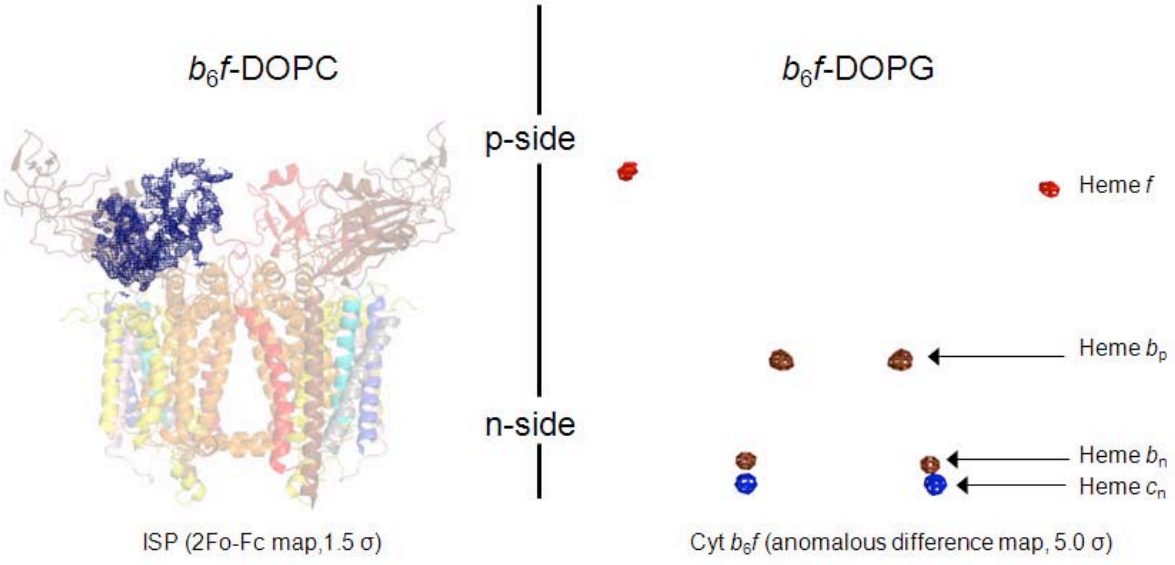
Internal lipids are essential for proper structural and functional stability of membrane proteins, especially well documented for those that are oligomeric. To understand the exact role of such lipids, their substitution and reconstitution in the purified protein is required. However, most membrane proteins undergo destabilization and loss of biological activity upon the removal of natural lipids, precluding study of these lipid-protein interactions. Cytochrome b_6f of oxygenic photosynthesis is a hetero-oligomeric membrane protein complex in thylakoid membranes that catalyzes proton coupled redox reactions involving plastoquinone(ol). Crystallographic studies of the b_6f complex from prokaryotic cyanobacteria, *Mastigocladus laminosus* and *Nostoc* PCC 7120, and the eukaryotic green alga *Chlamydomonas reinhardtii*, show well defined lipid and detergent binding sites. The cyanobacterial crystal structures have been obtained in the presence of the synthetic neutral lipid DOPC by a unique method of lipid reconstitution that allows the substitution of natural lipids. This methodology allows systematic testing of the effect of variations in lipid charge, tail length and the degree of unsaturation on the structure and function of the b_6f complex. We report a 2.80 Å crystal structure of the b_6f complex obtained in the presence of the natural acidic lipid DOPG from *Mastigoclaudus laminosus*. In the presence of the acidic lipid, the soluble domain of the Rieske Iron-Sulfur Protein (ISP), which has a well defined electron density in a neutral lipid environment, could not be traced beyond the trans-membrane helix. The anomalous difference map does not locate the Fe-atoms of the ISP [2Fe-2S] cluster, thereby indicating a long-range lipid-mediated conformational change, resulting from steric and/or electrostatic effects, which is transduced from lipid binding sites within the hydrophobic trans-membrane domain of the b_6f complex to the peripheral domain of the Rieske iron-sulfur protein subunit of the complex. Financial support: NIH-GM038323 (WAC); Purdue University (SSH).



ENTROPY DRIVEN REACTION IN THE *MENG* NULL MUTANT OF *SYNECHOCYSTIS* SP. PCC 6803

Harvey J.M. Hou[1], *John H. Golbeck*[2], *and David Mauzerall*[3]

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We have previously reported the thermodynamic parameters of electron transfer in the *menA* and *menB* null mutants of *Synechocystis* sp. PCC 6803 [1]. However, the deconvolution analysis on *menA/B* PS I failed to resolve any convincing parameter for the plastoquinone anion-to-$F_{A/B}$ step. The main reason is due to its lifetime of 15-300 μs, which is beyond the 10-μs time window. Inactivating the *menG* gene causes 2-phytyl-1,4-naphthoquinone (Q) to be located in the quinone binding pocket in PS I. The electron transfer from Q^- to F_X is slowed to 600 ns in *menG* PS I [2]. In this work, we conducted photoacoustic measurements on *menG* PS I from *Synechocystis* sp. PCC 6803 and carried out simulations by deconvolution analysis on the photoacoustic signals. The fit by deconvolution of *menG* PS I photoacoustic waves revealed that the enthalpy changes were –0.7±0.2 eV for the P_{700}* → Q step and +0.5±0.2 eV for the Q^- → $F_{A/B}$ step, respectively. Taking the free energy of –0.7 eV and –0.1 eV for these steps, the entropy changes are about zero and +0.5 eV, respectively. The data indicate that the Q^- to $F_{A/B}$ electron transfer step in *menG* PS I is entropy driven, which is similar to that in wild type PS I and in the *menA/B* PS I (Fig. 1).

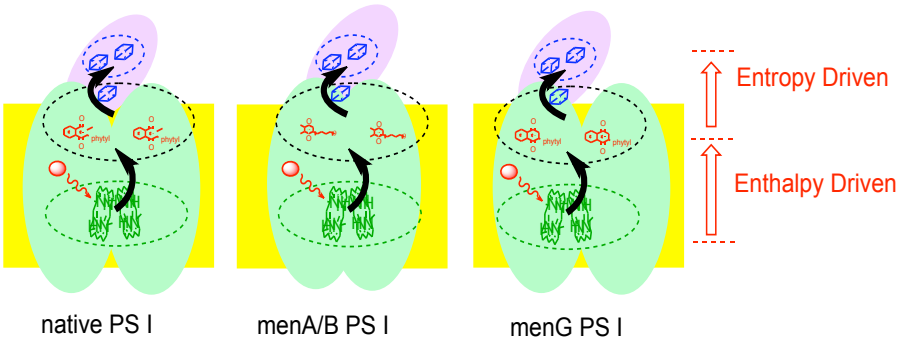

Fig. 1 Entropy driven reaction in photosystem I of *Synechocystis* 6803

1. Harvey J.M. Hou, Gaozhong Shen, Vladimir A. Boichenko, John H. Golbeck and David Mauzerall (2009) *Biochemistry*, 48, 1829-1837
2. Sakuragi, Y., B. Zybailov, G. Shen, A.D. Jones, P.R. Chitnis, A. van der Est, R. Bittl, S. Zech, D. Stehlik, J.H. Golbeck, and D.A. Bryant. (2002) *Biochemistry*, 41:394-405

REGULATION OF MRNA ENCODING PHYCOBILIPROTEINS IN RESPONSE TO LOW SULFATE CONDITIONS IN *FREMYELLA DIPLOSIPHON*

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Indiana University, Department of Biology. 1001 E. 3rd St. Bloomington, IN 47405

At sulfate levels below 150 uM, *Fremyella diplosiphon* carries out a "sulfur sparing" response by replacing the sulfur-rich phycobiliprotein isoforms PC1 and PC2 with PC3, which possesses less sulfur containing amino acids. The goal of my project is to understand the regulation of this response. The PC3-encoding *cpc3* operon is transcriptionally up-regulated during this response, and the posttranscriptional down-regulation of *cpc1* and *cpc2* via RNA degradation depends on a specific intergenic region of *cpc3* mRNA (referred to as the A3H3 region). Several regions of *cpc1* and *cpc2* mRNA potentially base pair with *cpc3* mRNA, and I am currently testing the *cpc1* regions for their ability to confer sulfate-dependent degradation of *atpD* mRNA, which is normally non-sulfate regulated. I am also performing experiments with in-vitro transcribed RNA to study the interaction between the A3H3 region and other RNAs that are either sulfur-regulated or nonsulfur-regulated.

It has not been previously investigated whether the sulfur sparing response gives *F. diplosiphon* a growth advantage in sulfate-limited conditions. I performed a competition experiment between wild type and Δ*cpc3* cells. I have used DNA blot analysis to determine the ratio of wild type to Δ*cpc3* cells when they are grown in sulfur-replete and sulfur-limited conditions. After twenty two days, wild type cells make up a large portion of the mixed population grown in sulfur-limited conditions.

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

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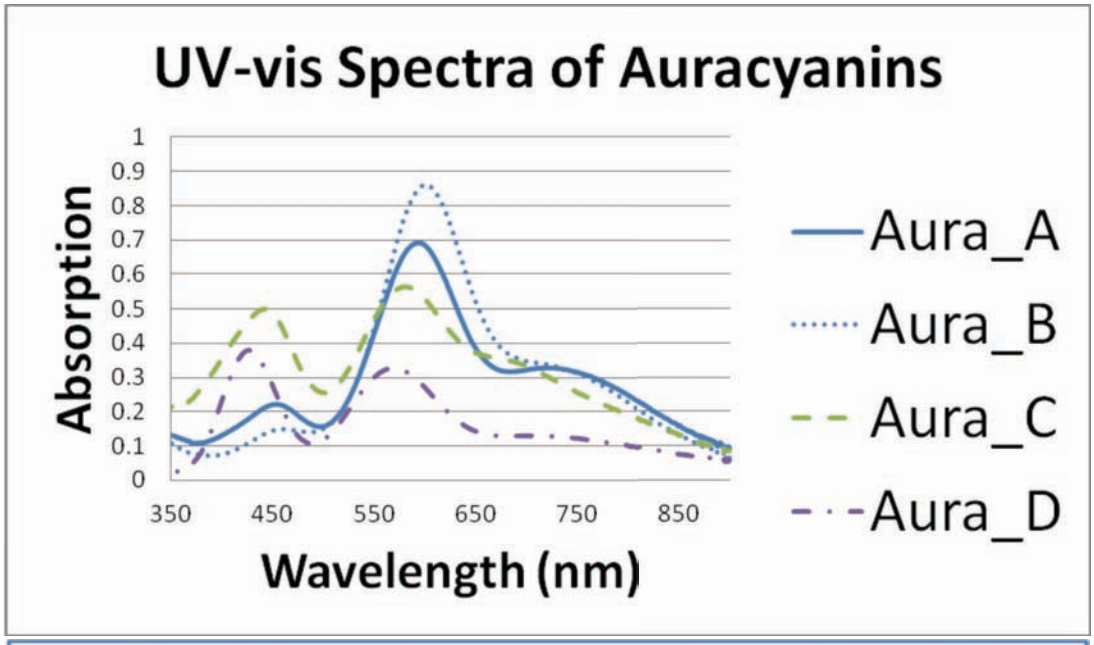
[4]Department of Chemistry, University of Missouri-Columbia, Columbia MO 65211

Blue copper proteins participate in electron transfer reactions in all domains of life. Classical blue copper proteins have an intense blue color. However, the color of blue copper proteins can vary from blue to green to red. Typically, green copper protein sites occur as part of larger metal center, such as in nitrite reductase. The green color appears due to a greater overlap of σ orbital from the cysteine ligand than normal (An even greater overlap leads to a red color). The function and cause of tuning a copper protein to the green and red colors are unknown.

The auracyanins are a family of blue copper proteins from *Chloroflexus aurantiacus*. *C. aurantiacus* encodes four duplications of the auracyanin gene, labeled A-D. Expression in *E. coli* of auracyanins A-D revealed tuning from blue to green despite high sequence homology (Figure). This high sequence homology combined with the single copper nature of auracyanins will allow us to probe the green copper site's function and origin. Here, we present UV-vis spectra, circular dichroism spectra, electron paramagnetic resonance, spectroelectrochemical redox titrations, and temperature dependency of green copper sites. We also hypothesize on the origin of the green copper site in auracyanins.



Most Blue — Most Green

Auracyanin B → Auracyanin A → Auracyanin C → Auracyanin D

APPLICATIONS OF THE ENVIRONMENTAL PHOTOBIOREACTOR (EPBR) MATRIX FOR ALGAL STRAIN CHRARACTERIZATION AND SELECTION UNDER DYNAMIC ENVIRONMENTAL CONDITIONS

Ben Lucker[1,2], *Christopher Hall*[1,2], *and David M. Kramer*[1,2]

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

Algal growth, cell cycle, and metabolism rapidly adapt to the ever-changing environment, whereas they are typically are cultured and studies under unnaturally static conditions, leading to laboratory results, which are not applicable to outdoor growth conditions. Compounding the complexities, dynamic environmental conditions (light, temperature, carbon dioxide etc.) interact in complex ways that differ in different species and strains. To address these challenges, we have developed the environmental Photobioreactor (ePBR), designed to operate as a matrix of programmable photobioreactors, each capable of simulating multiple dynamic environmental conditions. In this work, we demonstrated the utility of the ePBR for rapid screening and strain selection by analyzing the growth of a range of different species matrix under a range of distinct environmental conditions. This work was funded by the US Department of Energy under contract DE-EE0003046 awarded to the National Alliance for Advance Biofuels and Bioproducts.

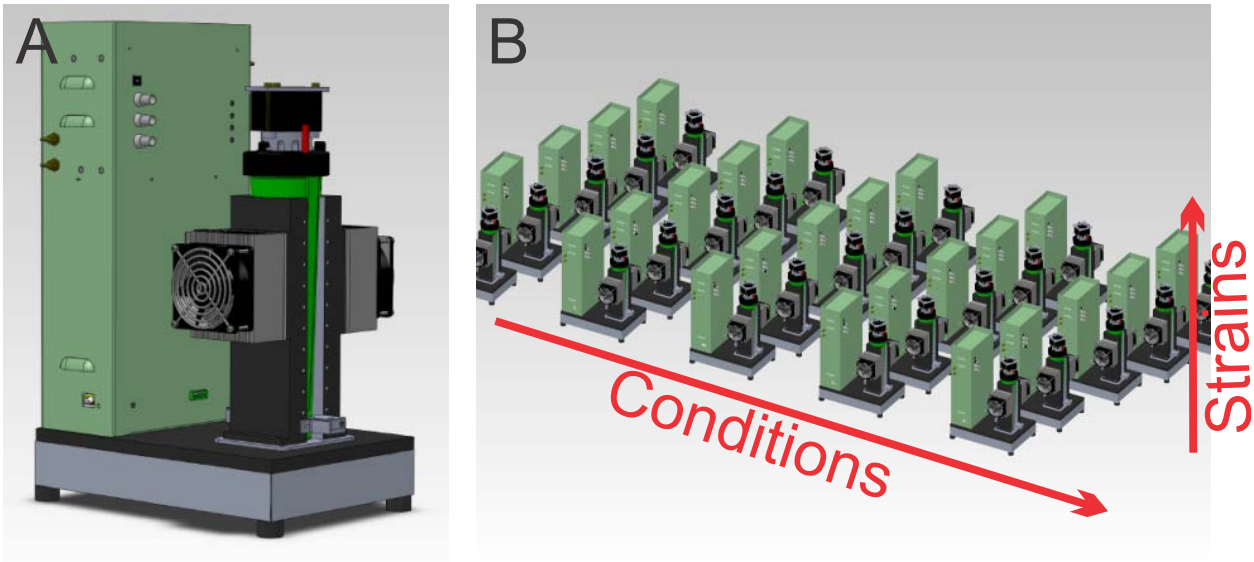

Figure 1. 3-dimentional rendering of the environmental photobioreactor. B An example of how a matrix of ePBRs can be used to select and monitor algal strains under predefined environmental conditions.

NEW INSIGHTS INTO ENZYMATIC ACTIVITY AND MECHANISM IN THE ALTERNATIVE COMPLEX III FROM THE PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

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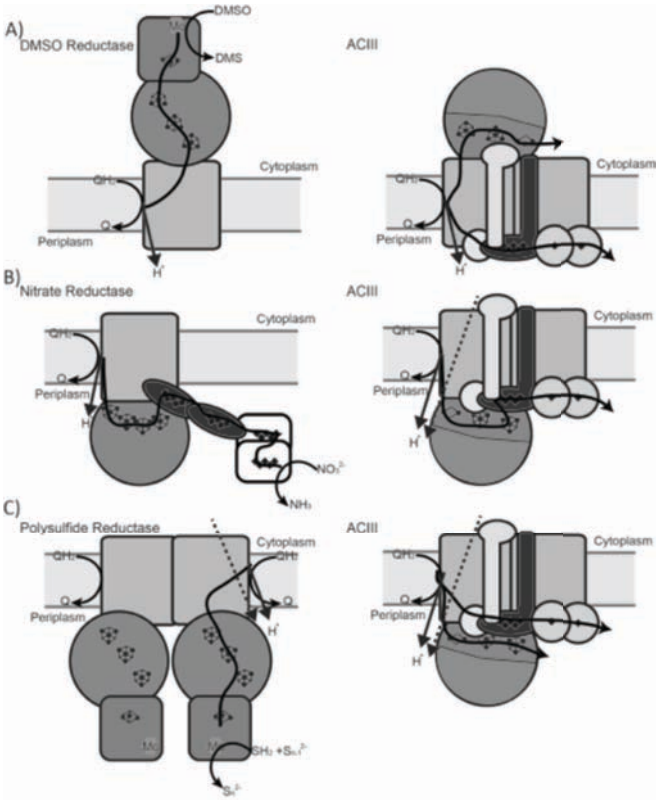
The Alternative Complex III (ACIII) is an energy conserving integral membrane protein in many photosynthetic and respiratory prokaryotic systems. It is suspected to be a functional replacement for Complex III or cytochrome bc_1/b_6f complex, but has no homology or relation to these complexes. In the Filamentous Anoxygenic Phototrophs, ACIII is being characterized in *Chloroflexus aurantiacus* and *Roseiflexus castenholzii*. Recent work on the *Chloroflexus* system has revealed new insights to the structure and function of the unique complex. A selectively denatured ACIII was reconstituted with its soluble monoheme subunit and shown to regain full enzyme activity. Also, the likely electron acceptor for the complex has been re-examined in light of recent findings about Auracyanin A and B expression. These results, in addition to a homology model of the complex with polysulfide reductase, have yielded potential mechanisms for this novel complex.



Possible Electron Transfer Pathways in ACIII based on known proteins in the same family.
Left Column: Known Electron Flow in CISM member.
Right Column: Possible electron flow in ACIII
A. DMSO Reductase. **B.** Nitrite Reductase **C.** Polysulfide Reductase.
Black arrows designate electron flow. Straight arrows show proton flow from the quinone site. Straight dashed arrows represent proton translocation across the membrane. QH_2 is quinol and Q is quinone. Cubes are iron-sulfur clusters. Black diamonds are hemes.

THE DYNAMICS OF THE NON-HEME IRON IN BACTERIAL REACTION CENTERS FROM *RHODOBACTER SPHAEROIDES*

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We investigate the dynamical properties of the non-heme iron (NHFe) in His-tagged photosynthetic bacterial reaction centers (RCs) isolated from *Rhodobacter (Rb.) sphaeroides*. Mössbauer spectroscopy and nuclear inelastic scattering of synchrotron radiation (NIS) were applied to monitor the arrangement and flexibility of the NHFe binding site. In His-tagged RCs, NHFe was stabilized only in a high spin ferrous state. Its hyperfine parameters (IS=1.06±0.01mm/s and QS=2.12±0.01mm/s), and Debye temperature (θD0~167K) are comparable to those detected for the high spin state of NHFe in non-His-tagged RCs. For the first time, pure vibrational modes characteristic of NHFe in a high spin ferrous state are revealed. The vibrational density of states (DOS) shows some maxima between 22 and 33meV, 33 and 42meV, and 53 and 60meV and a very sharp one at 44.5meV. In addition, we observe a large contribution of vibrational modes at low energies. This iron atom is directly connected to the protein matrix via all its ligands, and it is therefore extremely sensitive to the collective motions of the RC protein core. A comparison of the DOS spectra of His-tagged and non-His-tagged RCs from *Rb. sphaeroides* shows that in the latter case the spectrum was overlapped by the vibrations of the heme iron of residual cytochrome c2, and a low spin state of NHFe in addition to its high spin one. This enabled us to pin-point vibrations characteristic for the low spin state of NHFe.

Acknowledgements
This work was supported partially by the project operated within the Foundation for Polish Science MPD Program co-financed by the EU European Regional Development Fund.

INACTIVATION OF SUCCINATE DEHYDROGENASE AND IMPACT ON CYCLIC ELECTRON FLOW IN *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA

Sheriff Otun[1], Brandon Brummeyer[2]*, Anuradha Marathe[2], Matthew Nelson[2] and Toivo Kallas[2]*

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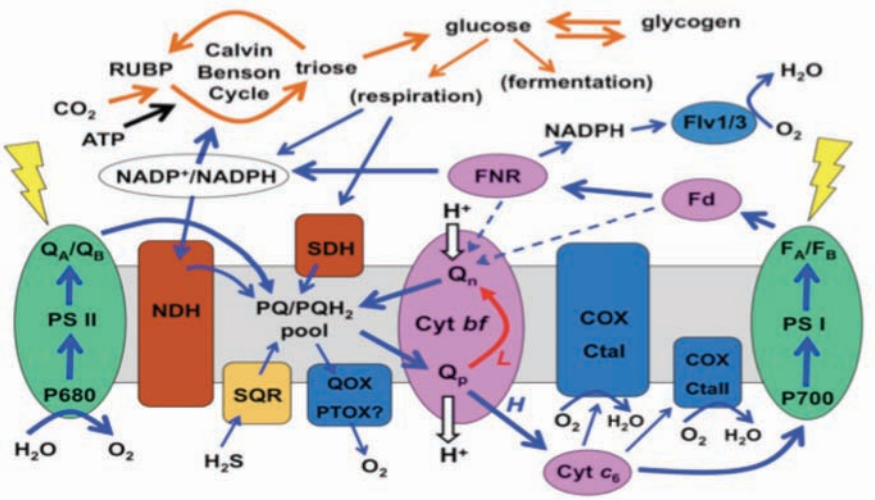
Cyclic electron transport (ET) around photosystem I (PSI) and the cytochrome (Cyt) *bf* complex provides 'extra' ATP for efficient CO_2 fixation and is implicated in defenses against photodamage. In chloroplasts, a Cyt *bf* – PSI supercomplex catalyzes cyclic flow (Iwai et al., 2010 *Nature*), but such a complex has not been identified in cyanobacteria. We have used a pump-probe kinetics spectrophotometer (BioLogic JTS-10) to re-examine cyclic ET in native *Synechococcus* 7002 and an NdhF mutant that lacks a functional NAD(P)H dehydrogenase (NDH-I), and thus the known cyclic ET pathway of cyanobacteria. At high light intensity (2000 µmol m^{-2} s^{-1}), cyclic ET increased dramatically accounting for 30% or more of linear flow in both the NdhF mutant and wild type. Cyclic ET in the NdhF strain presumably cannot occur via the NDH pathway and our findings thus suggest that it involves formation of a Cyt *bf* – PSI supercomplex that is important for adaptation and growth of *Synechococcus* 7002 at extreme, high-light intensities. However, other possible cyclic ET pathways might exist. A more circuitous cyclic ET route could involve succinate dehydrogenase (SDH), which is important for plastoquinone (PQ) pool reduction in *Synechocystis* PCC 6803 (Cooley & Vermaas, 2001 *J. Bacteriol.*). To test this, we have used a megaprimer PCR method to inactivate the catalytic, SdhB subunit of succinate dehydrogenase in *Synechococcus* PCC 7002. ΔSdhB segregants have been obtained and the impact of the *sdhB* mutation is being tested on growth, cyclic electron flow, and PQ pool reduction.



Electron and carbon flow pathways of cyanobacteria. NDH, NAD(P)H dehydrogenase; SDH, succinate dehydrogenase; SQR, sulfide-quinone oxidoreductase; QOX, quinol oxidase, PTOX, plastoquinone terminal oxidase; COX (Cta), cytochrome oxidase I or II; Fd, ferredoxin; FNR, ferredoxin-$NADP^+$-oxidoreductase. SDH provides a pathway for PQ pool reduction that might be involved in a possible cyclic ET route.

STABILITY, DENATURATION AND UNFOLDING STUDIES ON PHYCOCYANIN

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Phycocyanin (PC) is a complex assembly of protein subunits composed of apoproteins (polypeptides) and chromophores (tetrapyrroles or bilins). Hydrophobic interactions are believed to play a major role in PC stability and aggregation state. Conformational stability of phycocyanin has been investigated by fluorescence, fluorescence quenching and circular dichroism (CD) spectroscopy using urea & guanidine hydrochloride (GdHCL) as denaturants. The values of ΔG^o by both CD and fluorescence spectroscopy are comparable in both cases. Thermal unfolding of PC shows an endothermic process. Fluorescence quenching studies for analyzing protein conformational changes have so far been reported by monitoring tryptophan exposure to the solvent. Tryptophan exposure or burial in the case of PC is not an accurate reporter of the protein unfolding event. The tetrapyrrole, despite being a small chromophore localized to a small region of the protein, is a good reporter of the unfolding of the protein. We have shown that it is possible to use the quenching of the tetrapyrrole emission using small molecular weight quenchers as a reporter of protein conformational change. In other words, Quenching of the tetrapyrrole emission can be used as one of the technique for unfolding studies in PC.

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/gene sets for xylose namely *xylE*, *xylFGH*, *galP* and *glf* and three transporter genes/ gene sets for arabinose namely *araE*, *araFGH* and *araJ*. These seven transporters, *xylE*, *xylFGH*, *galP*, *glf, araE, araFGH* and *araJ* were inserted into *Synechocystis* genome to generate seven individual recombinants. Reverse transcriptase-polymerase chain reactions (hereafter RT-PCR) revealed expression of the genes/gene sets in the host. This was followed by generation of double-recombinants by insertion of *xylAB* (gene set for xylose utilization) and *araABD* (gene set for arabinose utilization) into genomes of xylose and arabinose transporter containing strains respectively. Expression of *xylAB* and *araABD* gene sets into respective strains was confirmed by RT-PCR. Growth curve studies showed successful utilization of xylose by all of the double transformants for xylose while arabinose related double transformants failed to utilize arabinose. Work is underway to modify *araFGH* and *araABD* gene sets with *Synechocystis* specific ribosome binding sites in between the genes. Using C-14 labeled xylose, activities of xylose transporters were compared. Enzymatic activities of XylA and XylB proteins were shown. Work is underway to show amount of xylose utilization against optical densities.

Except gene *glf* which is sourced from *Zymomonas mobilis* ZM4; all other transporter as well as utilization genes/gene sets source from *Escherichia coli* K-12. 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.

OPTIMIZATION OF ETHYLENE BIOPRODUCTION IN *SYNECHOCYSTIS* SP. PCC 6803

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Ethylene is the most widely produced petrochemical feedstock. It is currently produced exclusively from fossil fuels through petroleum fractionation, the largest CO_2-emitting process in the chemical industry. Thus, alternatives to petroleum based production are desired and some success has been achieved in bioproduction of ethanol. In this study, the *efe* gene encoding an ethylene-forming enzyme was expressed in *Synechocystis* sp. PCC 6803, leading to continuous photosynthetic production of ethylene. By increasing or decreasing key nutrients in the media of *Synechocystis* 5-fold, we saw as in figure 1 below that reduction of any single nutrient in the standard media attenuated growth in *Synechocystis*. Using response-surface methodology, we determined that the major nutrients found in the standard *Synechocystis* media, which include NO_3^-, PO_4^{3-}, SO_4^{2-}, Ca^{2+}, Mg^{2+}, and HCO_3^- are required for optimal growth, suggesting that general growth and ethylene production are linked.

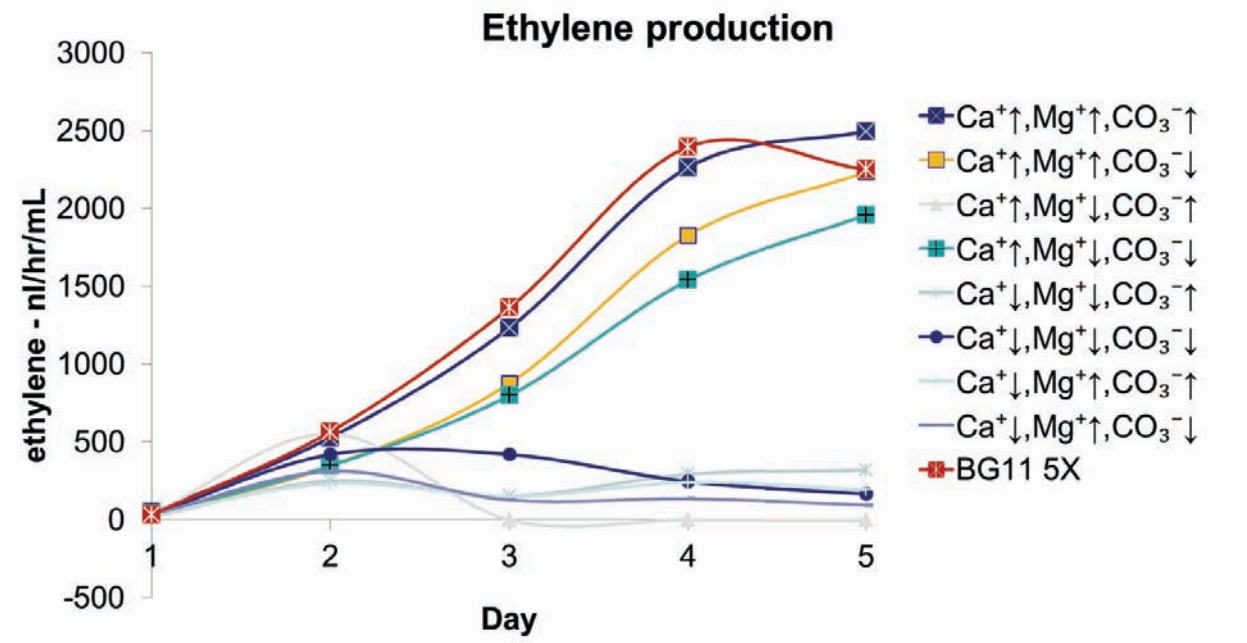


Fig. 1 — Graph of volumetric production of ethylene over 5 days. BG11 (5X) increases ethylene production beyond levels seen when nutrients are limiting. By day 5 BG11 (5X) has become limiting.

REGULATION OF TYPE IV CHROMATIC ACCLIMATION IN MARINE *SYNECHOCOCCUS* BY TWO PUTATIVE TRANSCRIPTION FACTORS

Joseph Sanfilippo, Animesh Shukla, and David Kehoe

Department of Biology, Indiana University, Bloomington, Indiana, 47405

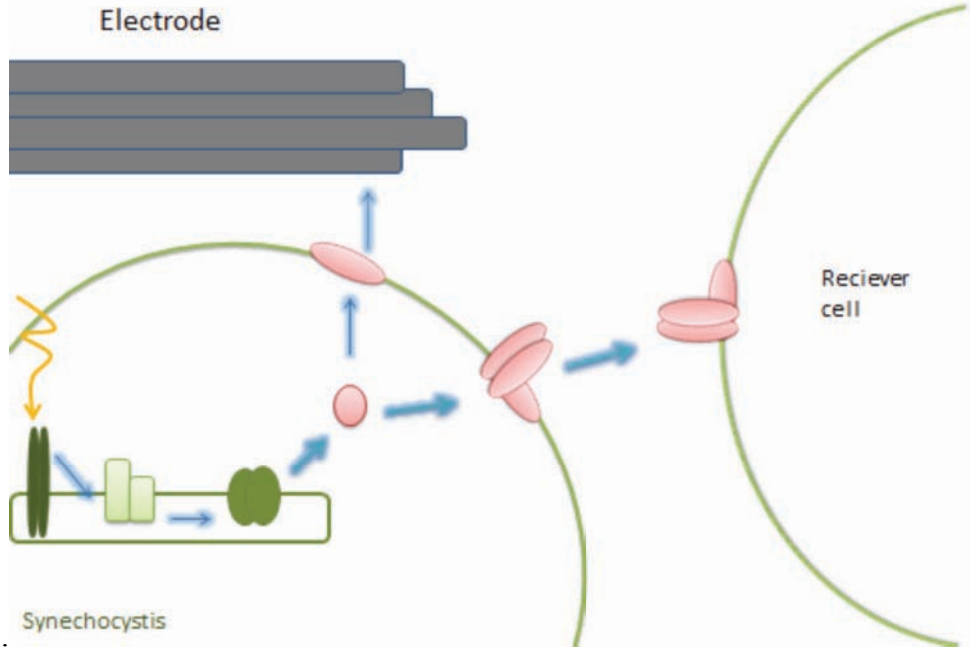
Cyanobacteria of the genus *Synechococcus* are ubiquitous in the world's oceans and are responsible for a significant portion of primary production on earth. These organisms not only have the ability to perform photosynthesis, but they also have a unique ability to utilize a wide range of light wavelengths. Some marine *Synechococcus* cells can undergo a blue-green acclimation known as "Type IV Chromatic Acclimation" (CA4), which allows them to adjust their photosynthetic machinery to optimize the efficiency of photosynthesis in different light conditions. During CA4, cells reversibly attach different chromophores to the phycobiliproteins phycoerythrin I and II. In blue light, the chromophore phycourobilin (PUB) is attached, and in green light, the chromophore phycoerthyrobilin (PEB) is attached. Research has shown that this process is controlled by the lyase-isomerase MpeZ, which converts PEB into PUB and attaches it to phycoerythrin II. The transcript levels of *mpeZ* are six times higher in blue light than green light and this regulation is necessary for normal CA4. Using a reverse genetic approach, we have identified two transcription factors that, when interrupted, have a strong mutant phenotype. Interruptions of these genes lead to a mutant CA4 phenotype and appear to lose the proper regulation of *mpeZ*. Interestingly, interruptions of these two genes appear to have opposite effects which suggest complicated regulation of *mpeZ*. This preliminary data provides valuable information regarding the signal transduction pathway of CA4.

CYTOCHROMES AS ALTERNATIVE ELECTRON SHUTTLES FROM PSI

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 CO_2 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. For this reason a great deal of synthetic biology research aims to take advantage of the excess reductive capacity of the electron transport chain to directly run biosynthetic processes for fuel production. Our strategy ultimately seeks to export this excess capacity to the cell surface directly as electricity, making use of existing cytochrome-based pathways from Shewanella oneidensis MR-1. The beginning of any such alternative electron pathway starts with electron transfer from PSI, which is normally accomplished by ferredoxin. The current research investigates the properties of various cytochrome protein constructs as substrates for electron transfer from PSI. Special attention is paid to transfer of electrons from reduced cytochromes to molecular oxygen, a competing reaction of any single electron transfer process and a defining characteristic for any potential alternative electron transport pathway from photosynthesis.



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.

THE TEMPERATURE DEPENDANCE OF PHOTOASSEMBLY IN *CHLAMYDOMONAS REINHARDTII* BBY PARTICLES

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This work was focused on developing a better understanding of the light-driven assembly (photoassembly) of the photosystem II (PSII) oxygen-evolving complex (OEC). Oxygen evolution measurements were performed with whole cells of *Chlamydomonas reinhardtii* at temperatures ranging from 5–45°C with and without 100-μM 2,6-dicholoro-p-benzoquinone (DCBQ). The temperature maximum without DCBQ was determined to be 38 °C, while the temperature maximum in the presence of DCBQ shifted to 28 °C. Potential reasons for these differences in temperature maxima will be discussed. Difficulties with generating OEC-depleted PSII in whole cells led us to restructure our approach using PSII-enriched membranes (BBY particles) from *C. reinhardtii* using an established protocol from the Govindjee lab [1]. The oxygen evolution of these BBY particles were measured from 10–35 °C with and without 100 μM DCBQ. The temperature maximum for both measurements was determined to be 28 °C, which is consistent with literature values for BBY particles from other organisms [2]. Generation of OEC-depleted PSII-enriched particles (apo-BBY) was successful, but measuring the yield of photoassembly was hampered by a small quinone pool and the fact that DCBQ cannot be used. Future experiments with *C. reinhardtii* BBY particles will be discussed.

[1] Hyunsuk Shim, Jiancheng Cao, Govindjee & Peter G. Debrunner. 1990. Purification of highly active oxygen-evolving photosystem II from *Chlamydomonas reinhardtii. Photosynthesis Research.* 26: 223-228

[2] Schiller, H.; Dau, H. 2000. Preparation protocols for high activity Photosystem II membrane particles of green algae and in higher plants, pH dependence of oxygen evolution and comparison of the S_2-state multiline signal by X-band EPR spectroscopy. *Journal of Photochemistry and Photobiology.* 55:138–144.

REGULATION OF LIGHT-HARVESTING PROTEINS BY TWO SYSTEMS IN THE AQUATIC CYANOBACTERIAUM, *FREMYELLA DIPLOSIPHON*

Lisa Wiltbank, Andrian Gutu, and David Kehoe

Indiana University, Department of Biology, Bloomington, IN 47405

To best utilize the wavelength of light available in aquatic environments, cyanobacteria restructure their light-harvesting structures in response to changing light colors. This acclimation gives them a competitive advantage and is accomplished by changes in gene expression of genes that encode the phycobiliproteins contained in the light-harvesting structures called phycobilisomes (PBS). In the freshwater species *Fremyella diplosiphon,* cells express the genes for production of PBS containing the phycobiliprotein phycocyanin (PC) in red light conditions and express the genes for production of PBS containing phycoerythrin (PE) in green light conditions. This gene regulation is conferred by the well-characterized Rca signal transduction system, but another system also regulates the levels of PE in the absence of the Rca system. We are interested in discovering more about what other factors affect PE expression.

Through a transposon mutant screen, we have identified two genes that, when disrupted, consistently lead to increased levels of PE. These genes encode for translation initiation factor (IF) 3 and a sensor histidine kinase. The two disruptions have remarkably similar phenotypes. I am working to determine if these two factors function in the same system and how each may be involved in regulating PE production. We are also creating deletions of each of the genes encoding the Rca system components in the *F. diplosiphon* genome to gain a more reliable background for our studies of other systems that regulate PE.

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

Yong Xue, Yan Zhang, 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. Synechocystis mutant strain harboring codon optimized *ref8* gene (*sref8*) was constructed. After codon optimization, the expression level of C3H was enhanced and the caffeic acid production yield was increased from 5.2 mg/L to 7.3 mg/L.

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. Upon feeding of p-coumaric acid , the immediate precusor for resveratrol biosynthesis, the maxium yield of resveratrol reached 120mg/L.

ELECTROSTATICALLY CONSTRAINED PATHWAY OF INTRA-MONOMER ELECTRON TRANSFER IN THE CYTOCHROME B_6F COMPLEX

Stanislav D. Zakharov[1,3], S. Saif Hasan*[1], Adrien Chauvet[2], Sergei Savikhin[2] and Willam A. Cramer[1]*

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The preferred pathway of trans-membrane electron transfer in the symmetric dimeric cytochrome b_6f complex, involving four *b*-type hemes organized as pairs in two symmetric monomeric units, was studied by simultaneous measurement of the time course of heme redox state and of heme-heme exciton interaction through differential circular dichroism spectra. The rate of heme reduction, in the dimeric or monomeric complex, coincides with an increase in amplitude of a split CD spectrum having a node at the absorbance maximum. Utilizing crystal structure information on inter-heme orientation and separation, it is inferred that reduction of the trans-membrane *b*-type hemes, whose individual reduction cannot be temporally separated on the time scale of the measurements, occurs cooperatively and preferentially through the heme monomer unit of the dimeric complex. The relatively small contribution of inter-monomer exciton interactions to the split CD spectra is a consequence of the dependence on inter-heme distance and orientation. The limited rate of heme reduction is attributed to intra-protein electrostatic repulsion caused by injection of uncompensated electronic charge into the apolar interior of the protein complex. This is inferred from the pH dependence of the heme reduction rate, a kinetic deuterium isotope effect, and a red shift in the Q_y-band absorbance maximum (Stark Effect) of the chlorophyll-*a* that is integral to the complex. Intra-complex structure changes associated with heme reduction are inferred from considerations of electrostatic interactions that imply the direction of the observed Stark Effect wavelength shift is opposite to that experimentally observed.

Financial Support: WAC (NIH 038323); SSH (Purdue University Fellowship); SS (DOE DEFG02-09ER16084)

SSL2148, A STRESS INDUCIBLE PROTEIN WITH ONE HELIX IN *SYNECHOCYSTIS* PCC 6803

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There are four high light-inducible polypeptides (HLIPs) with single trans-membrane helix in *Synechocystis* PCC 6803; these polypeptides are important for survival under high light conditions. One ORF, *ssl2148*, in *Synechocystis* PCC 6803 encodes a conserved hypothetical protein with one trans-membrane helix which is similar to HLIP. *ssl2148* has been shown to be up-regulated under high light conditions (HL) in *Synechococcus* OS-B' (Kilian, 2007).

In this study, we deleted *ssl2148* from *Synechocystis* PCC 6803 genome. The Δ*ssl2148* strain showed no significant differences in growth, chlorophyll and carotenoid content as compared to the *Synechocystis* PCC 6803 wild type strain (hereafter WT). The photosynthetic pigment complexes were isolated by sucrose gradient centrifugation from the thylakoid membranes of WT and *ssl2148* cells that were grown under normal light (NL, 40 μM m^{-2} s^{-1}) and HL (400 μM m^{-2} s^{-1}). The result showed that the chlorophyll content of F2 fraction (PS I monomers and PS II) of Δ*ssl2148* was much lower than that of WT under NL. In contrast, the chlorophyll content of F2 fraction of the mutant is higher than that of WT under HL. The PSI:PSII ratios in either the thylakoid membrane or the F2 fractions of the mutant (as evaluated by 77K fluorescence spectroscopy) were about 41% and 23%, respectively, higher than those in the WT strain under NL. But under HL, the PSI:PSII ratios were observed to be 20% or 10% lower in the thylakoid membrane or F2, respectively, than those in WT. These results suggest that Ssl2148 might be involved in regulation of PSI trimer to monomer ratio or involved in assembly/disassembly of PS I trimers.

Late Abstracts

(Listed alphabetically by first author)

THE SUN AS A SOURCE OF LOW ENTROPY AND THE EFFECT OF CLIMATE CHANGE ON LOW ENTROPY PRODUCTION

Charles H. Ernst

University of Colorado at Colorado Springs

A discussion of the sun as a source of low entropy, first proposed by Erwin Schrodinger in his book, What is Life, published in 1947. Recently, Roger Penrose expanded this idea in his new book, Cycles of Time, published in 2010, illustrated in Fig. 2.9 below.

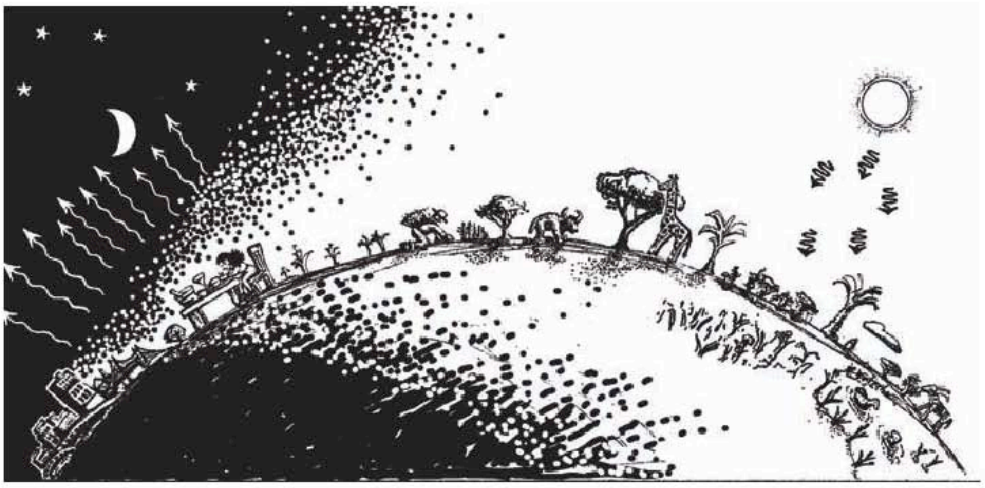

Fig. 2.9 Photons arriving at the Earth's surface from the Sun have higher energy (shorter wavelength) than those returned to space by the Earth. Given an overall energy balance (the Earth does not get hotter over time), there must be more photons leaving than arriving; that is, the energy arriving has lower entropy than that departing.

I propose further questions of how climate conditions like black carbon and sulfate particles could affect this low entropy production through changes in the solar spectrum, at lower frequencies. I will also discuss how greenhouse gases could change this low entropy production due to changes at higher frequencies of the solar spectrum. Finally, I will ask for input and propose ways we might obtain data to study this problem.

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