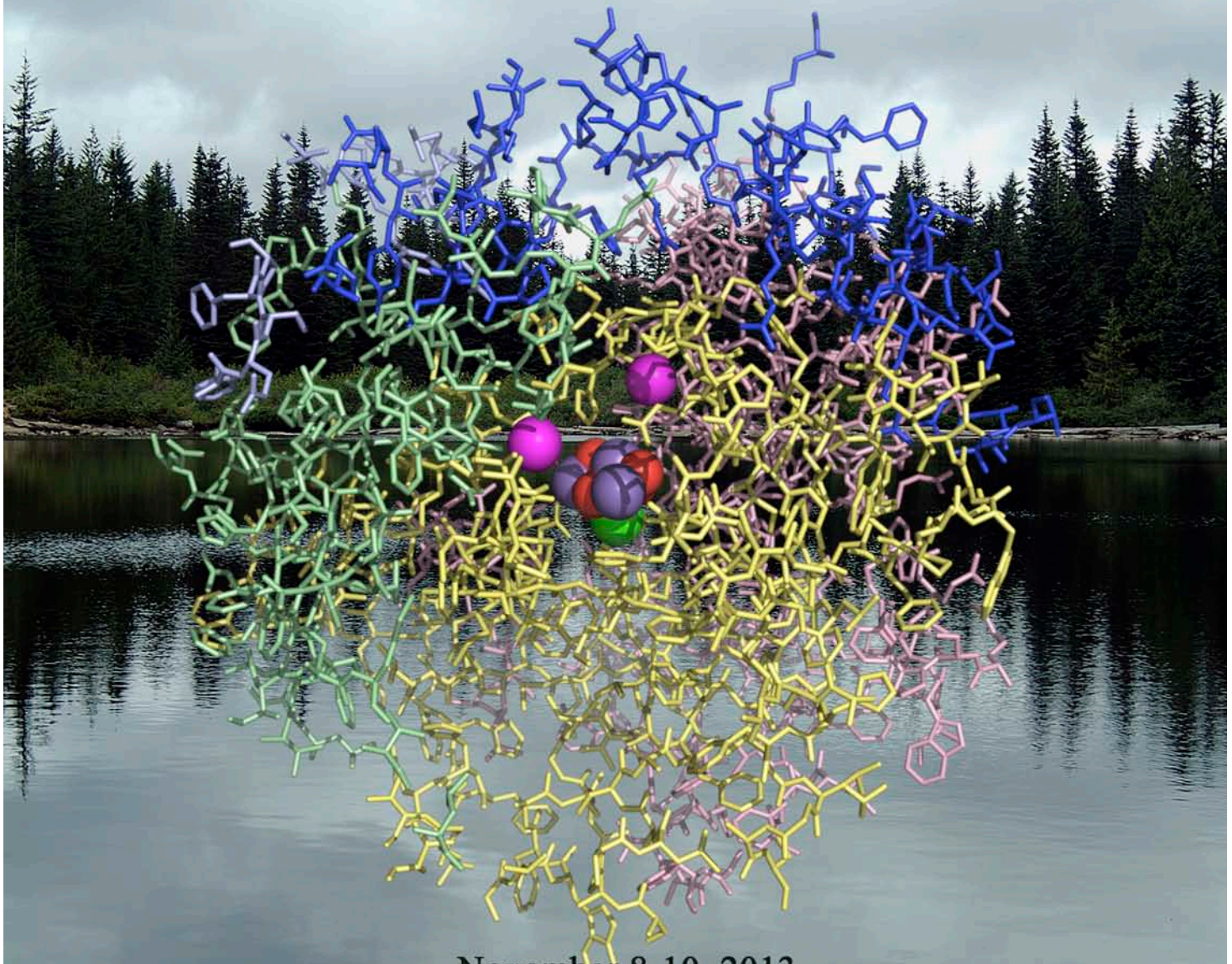
39th Annual Midwest/Southeast Photosynthesis Meeting Abstracts and Program



November 8-10, 2013
Turkey Run State Park
Marshall, IN

PROGRAM AND ABSTRACTS

39th Annual Midwest/Southeast Photosynthesis Meeting

Turkey Run State Park
Marshall, Indiana
November 8-10, 2013

Organizers:

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Please note the dates of next year's MW/SE Photosynthesis Meeting: October 24-26, 2014

On the cover: The Manganese Cluster of Photosystem II over Mirror Lake, Mt. Hood, OR

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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
10:20 AM Coffee Break
10:50 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
8:20 PM Mixer and Poster Viewing

Sunday, November 11
7:00 AM Breakfast and checkout
9:20 AM Session V: Contributed Papers
10:00 AM Coffee Break and Checkout
10:20 AM Award Ceremony
10:25 AM Best poster talks
10:45 AM Closing Remarks
11:00 AM Departure

Friday Evening, November 8

Session I. Keynote Lectures

Chairs: Terry Bricker & Rob Burnap

7:30 PM Opening Remarks and Welcome

7:40 PM Keynote Speaker: James V. Moroney, Louisiana State University
IDENTIFYING THE COMPONENTS OF THE CO_2 CONCENTRATING MECHANISM OF *CHLAMYDOMONAS REINHARDTII*

8:30 PM Keynote Speaker: Jan Kern, Lawrence Berkeley National Laboratory
TAKING SNAPSHOTS OF PHOTOSYNTHETIC WATER OXIDATION - SIMULTANEOUS FEMTOSECOND X-RAY SPECTROSCOPY AND DIFFRACTION AT ROOM TEMPERATURE USING A X-RAY FREE ELECTRON LASER

9:20 PM Mixer and Poster Viewing

Saturday Morning, November 9

Session II. Organisms

Chair: Himadri Pakrasi

9:00 AM Eliezer Schwarz, Jingcheng Huang, and David Kramer
ENGINEERING BIOPHOTOVOLTAIC ORGANISMS

9:20 AM Steven C. Holland, Anthony D. Kappell, Minquan Zhang, Robert L. Burnap
REDOX CHANGES ACCOMPANYING CARBON DEPRAVATION IN THE CYANOBACTERIUM *SYNECHOCYSTIS SP.* PCC 6803

9:40 AM Yong Xue and Qingfang He
CYANOBACTERIAL PLATFORM FOR PRODUCTION OF PLANT SECONDARY METABOLITES

10:00 AM Shawn ME Daley, Steven C Holland, Juliana Artier, and Robert L Burnap
REGULATION OF THE INORGANIC CARBON CONCENTRATING MECHANISM (CCM) IN CYANOBACTERIA

10:20 AM Coffee/tea Break

Session III. Structure and Assembly

Chair: Alice Haddy

10:50 AM Rachna Agarwal, Stanislav D. Zakharov, S. Saif Hasan, Julian P. Whitelegge & William A. Cramer
CHARACTERIZATION OF A CYANOBACTERIAL OUTER MEMBRANE PROTEIN: AN *E. COLI* TOLC HOMOLOGUE FROM *SYNECHOCYSTIS* SP. PCC 6803

11:10 AM Manjula P. Mummadisetti, Larry Sallans, Patrick A. Limbach, Laurie K. Frankel, Terry M. Bricker
CONFORMATION CHANGES IN PSBP UPON BINDING TO SPINACH PS II

11:30 AM Johnna L. Roose, Laurie K. Frankel, and Terry M. Bricker
THE PSBP-DOMAIN PROTEIN 1 FUNCTIONS IN THE ASSEMBLY OF LUMENAL DOMAINS IN PHOTOSYSTEM I

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. Excitation Energy and Electron Transfer

Chair: Greg Engel

7:00 PM Peter D. Dahlberg, Graham J. Norris, Subha Viswanathan, Gregory S. Engel

ENERGY TRANSFER OBSERVED IN LIVE CELLS USING TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY

7:20 PM Adrien Chauvet, Valentyn Stradnytskyi, Steven Romberger, John H. Golbeck, Sergei Savikhin

THE INTRINSIC CHARGE SEPARATION RATE IN THE REACTION CENTER FROM *CHLOROBIUM TEPIDUM*

7:40 PM Erica Wunderlich Majumder[1], Hao Zhang[1], Alastair T. Gardiner[2], Aleksander W. Roszak[2], Richard J. Cogdell[2] and Robert E. Blankenship[1]

STRUCTURE/FUNCTION INSIGHTS OF THE CYCLIC ELECTRON TRANSPORT CHAIN OF *ROSEIFLEXUS CASTENHOLZII*: THE LIGHT HARVESTING REACTION CENTER COMPLEX AND THE ALTERNATIVE COMPLEX III

8:00 PM Rodney L. Burton, Doreen Victoria, Amit Desai, PJ Kenis, AR Crofts

UNLOCKING THE GATE OF THE BC_1 COMPLEX; THE Q_O SITE MECHANISM

8:20 PM Mixer and Poster Viewing

Sunday Morning, November 11

Session V. Water, Oxygen and Proton Transport

Chair: Derrick Kolling

9:20 AM Han Bao, Preston L. Dilbeck, Curtis L. Neveu, and Robert L. Burnap
ROLE OF D1-VAL185 AS A SECOND SPHERE LIGAND OF THE MN CLUSTER IN CONTROL OF THE REACTIVITY OF WATER OXIDATION

9:40 AM Geoffry A. Davis, Atsuko Kanazawa, Kaori Kohzuma, Deepika Minhas, Amit Dhingra, Rong Jin, Jin Chen, David M. Kramer
EVIDENCE THAT THE TYLAKOID PROTON MOTIVE FORCE REGULATES PHOTOINHIBITION

10:00 AM Coffee/Tea Break

10:20 AM Session VI. Awards

Chair: Robert Burnap

10:25 AM Talk: Best Undergraduate Student Poster

10:35 AM Talk: Best Graduate Student Poster

10:45 AM Closing Notes and Announcements - Chair: Rob Burnap

11:00 AM Departure

Abstracts of Talks

(In Order of Appearance)

IDENTIFYING THE COMPONENTS OF THE CO_2 CONCENTRATING MECHANISM OF *CHLAMYDOMONAS REINHARDTII*

Bratati Mukherjee, Nadine Jungnick, Kelley G. Nunez, Yunbing Ma, Robert DiMario, Susan Laborde, Mary C. Machingura, John R. Battista and James V. Moroney
Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70808, USA

Photosynthetic microalgae optimize the utilization of inorganic carbon (CO_2 + HCO_3^- + CO_3^{2-}) by active uptake and concentration of inorganic carbon. This process, called the CO_2 Concentrating Mechanism (CCM), results in an increase in the concentration of CO_2 around the carbon-fixing enzyme Rubisco, which is localized to the chloroplast pyrenoid. Recently, there is interest in introducing algal CCM components into higher plants to improve photosynthesis. Two important components of the CCM of the green alga *Chlamydomonas reinhardtii* are inorganic carbon transporters and carbonic anhydrases. One carbonic anhydrase that is essential to the operation of the CCM is CAH3, which is located in the thylakoid lumen. The function of CAH3 is thought to be to convert bicarbonate accumulated in the chloroplast stroma to CO_2 in the thylakoid.

However, to reach the thylakoid lumen, inorganic carbon must cross three membranes: the plasma membrane, the chloroplast envelope and the chloroplast thylakoid membrane. While CO_2 is thought to be able to cross biological membranes by diffusion, the charged HCO_3^- would require a channel or transport protein to cross the membranes at fluxes high enough to support photosynthesis. A number of proteins have been identified as potential inorganic carbon transporters, including CCP1, CCP2, HLA3, LCI1 and NAR1.2 (LCIA). In addition to CAH3, other carbonic anhydrases are likely to be needed to interconvert the inorganic carbon species from CO_2 to HCO_3^- during the uptake process. Gaps in our understanding of how these components interact and work within the CCM is partly due to the absence of knockout lines for many of these genes.

In this presentation we will report on our work to characterize these putative inorganic carbon transporters and determine their location within the cell. We will also describe our efforts to identify new components of the CCM using insertional mutagenesis along with innovative techniques that will help determine which genes have been disrupted in the insertional mutants. We will show our initial efforts to determine whether inorganic carbon transporters from green algae have the potential to improve photosynthesis in green plants. This work is supported by NSF grant IOS-1146597 and a subcontract from the University of Illinois.

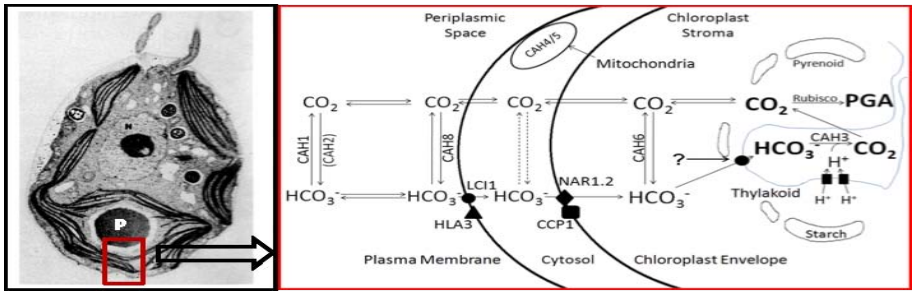


Figure 1. Working model of the CCM of Chlamydomonas

TAKING SNAPSHOTS OF PHOTOSYNTHETIC WATER OXIDATION - SIMULTANEOUS FEMTOSECOND X-RAY SPECTROSCOPY AND DIFFRACTION AT ROOM TEMPERATURE USING A X-RAY FREE ELECTRON LASER

Jan Kern[1,2]
[1]*Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA;*
[2]*LCLS, SLAC National Accelerator Laboratory, Menlo Park, CA 94025, USA.*

The start of operations at the first hard X-ray free electron laser, the LCLS at Stanford has spurred the development of new X-ray experiments on biological samples. The ultra short pulses in the 5 to 100 fs timescale coupled with the high brilliance opens up possibilities for novel kinds of experiments: e.g. 1) the measurement of X-ray data from radiation sensitive compounds at room temperature before the onset of radiation induced changes ("collect before destroy"); 2) the time resolved measurement of dynamics in biological samples under "native" conditions in the femtosecond to microsecond time scale.

We applied this novel method to the water oxidizing membrane protein complex Photosystem II (PSII) to further understand the structure and mechanism of PSII. The catalytic site of this multi-subunit membrane protein complex is a Mn_4CaO_5 cluster, located at the lumenal side of the complex. Upon light excitation it cycles through a series of states (S_0 to S_4) with S_1 being the dark stable state and water oxidation taking place in the S_3-S_0 transition. Despite a recent high resolution structure[1] many details of the reaction mechanism are still unknown, especially, as the Mn_4CaO_5 cluster is highly susceptible to radiation damage during X-ray diffraction (XRD) data collection[2]. We collected room temperature diffraction data sets from thousands of micro crystals of PSII in the dark stable S_1 state and in the first illuminated S_2 state at the CXI instrument of the LCLS, using sub-50 fs X-ray pulses[3,4]. Simultaneously, Mn Kβ X-ray emission spectra (sensitive to oxidation state changes of the Mn) of the same crystals were collected to monitor the intactness of the catalytic showing that the Mn_4CaO_5 cluster of PSII is not damaged within the time span (<50 fs) of the measurement[4]. Our studies open the possibility to follow changes at room temperature of the geometric and electronic structure of the catalytic site of PSII during the reaction cycle and further studies at different time points in the catalytic cycle are currently under way.

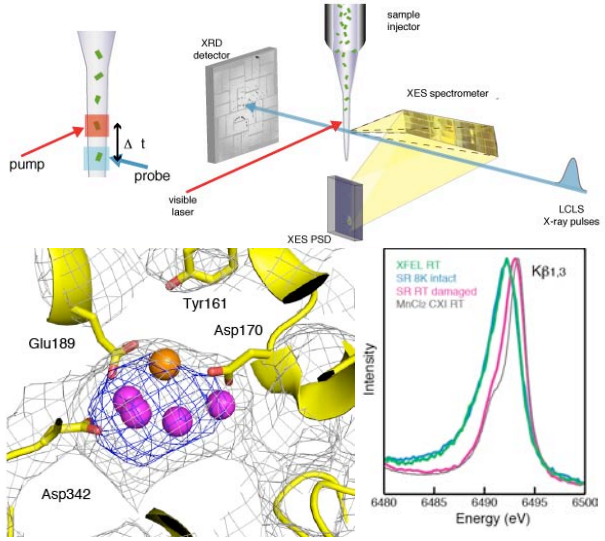


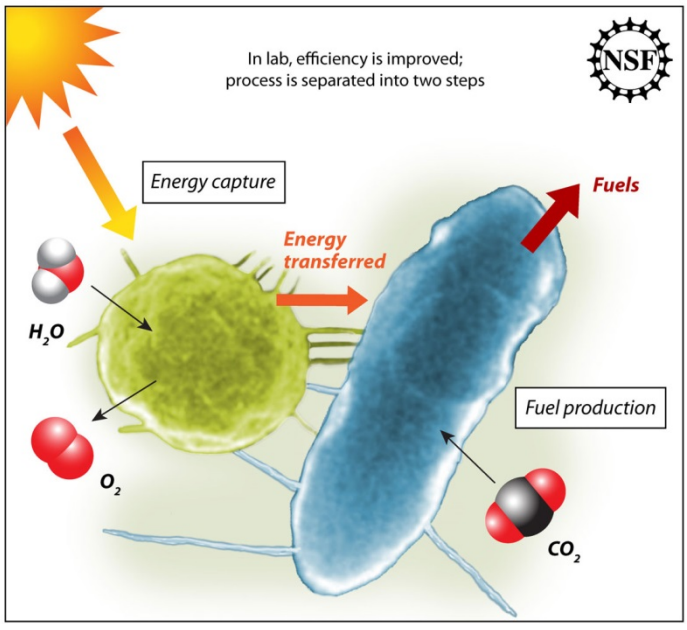
Fig. 1: Schematic setup for combined XES/XRD measurement at LCLS (top), obtained electron density in the region of the OEC (left) and comparison of XES from LCLS and synchrotron experiments (right).

1. Umena, Y *et al.* (2011) *Nature* **473**, 55-60.
2. Yano, J. *et al.* (2005) *Proc. Natl. Acad. Sci. USA* **102**, 12047-12052.
3. Kern, J. *et al.* (2012) *Proc. Natl. Acad. Sci. U. S. A.* **109**, 9721-9726.
4. Kern, J. *et al.* (2013) *Science* **340**, 491-495.

ENGINEERING BIOPHOTOVOLTAIC ORGANISMS

Eliezer Schwarz, Jingcheng Huang, and David M. Kramer
[1]Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan, USA

Due to the inefficiencies of carbon fixation the majority of light energy captured by a leaf in full sunlight can not be utilized for photosynthesis and is instead wasted as heat. If electron transport from PSI can be redirected from carbon fixation to other useful energy sinks this lost efficiency could potentially be recovered as fuel, or even directly as electricity. Engineering an alternative electron transport pathway presents a number of challenges that would need to be overcome; An ideal electron carrier would need to be both rapid and switchable. It would have a low midpoint potential to maintain energetic efficiency, yet would still resist donating electrons to molecular oxygen while traveling relatively long distances in an oxygenic environment. It is not currently clear what makes ferredoxin, the normal electron acceptor from PSI, ideal for its role, rather than e.g. cytochromes. To investigate the suitability of potential redox carriers as alternative electron transport proteins we have chosen as test subjects the annotated suite of redox proteins in *Shewanella oneidensis* MR-1, due to the fact that *Shewanella* already possesses a naturally extensive and versatile system of soluble redox carriers, mostly cytochromes. We have taken an in-vitro spectroscopic approach to characterize the reduction and reoxidation kinetics by thylakoids and molecular oxygen, respectively, of the entire suite of purified *Shewanella* cytochromes and ferredoxins, as well as their interactions with each other. Our studies have uncovered a surprising range of specificity, both in the interactions between PSI and soluble carriers as well as between carriers themselves. These results will hopefully provide us with principles needed to design an electron transport system with properties appropriate to our needs. Current work focuses on designing mechanism, based on findings to date, for phosphorylation-based induction and control of switchable electron transport circuits.



Postdoctoral Presenter

REDOX CHANGES ACCOMPANYING CARBON DEPRAVATION IN THE CYANOBACTERIUM *SYNECHOCYSTIS SP.* PCC 6803

Steven C. Holland, Anthony D. Kappell, Minquan Zhang, Robert L. Burnap
Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078

Oxygenic photosynthesis by cyanobacteria and plants is a primary source of CO_2 fixation in the biome. Cyanobacteria employ a system of carbon transporters to bring inorganic carbon (C_i) into the cell. These transporters are part of the carbon concentrating mechanism (CCM) that saturates the active site of Rubisco with CO_2 and out-competes the energetically wasteful oxygenase reaction. While some of these C_i transporters are constitutively expressed, others are induced when a cell experiences a lower carbon environment. This research explores the metabolic changes that accompany carbon limitation in order to further understand changes in cellular physiology and explore a model of metabolite control of CCM induction. Using chlorophyll fluorescence sampling of cells experiencing carbon limitation, changes in the redox state of the plastoquinone pool can be observed. It has long been known that C_i-limitation leads to an over-reduced plastoquinone pool. (Miller and Canvin 1989) However, the present experimental design allows for comparison of chlorophyll fluorescence and blue-green fluorescence (NADPH) simultaneously, allowing an examination of the light and dark photosynthetic reactions. Upon switching from carbon replete to carbon limited environment, intracellular concentrations of $NADP^+$ are lowered. This is seen using *in vivo* blue-green fluorescence as well as through biochemical analysis of cell extracts. It is hypothesized that a carbon-limited Calvin cycle slows the utilization of NADPH causing an increase in intracellular $NADP^+$. Carbonic anhydrase inhibitor ethoxyzolamide (EZ) was used to simulate carbon deprivation and resulted in a large increase in blue-green fluorescence during actinic light exposure. Biochemical analysis shows nearly complete reduction of the NADP pool. These findings point to a potential use of EZ as a tool in analyzing NADPH using fluorometry. These results provide evidence for a model of cyanobacterial sensing intracellular metabolites in order to alter gene transcription. Previous evidence has shown that the LysR-type transcriptional regulator CcmR (NdhR) has oxidized NADP and α-ketoglutarate as cofactor molecules. (Daley *et al.* 2012) This research elucidates a number of physiological changes that accompany carbon limitation and can be used to create a model of carbon limitation lowering internal $NADP^+$ concentrations. This change is sensed by CcmR, leading to a de-repression of CCM genes and an increase in carbon transporter expression. These results can be important for future research into cell and systems biology and may provide preliminary evidence for metabolite control of CCM induction. Future experiments will be aimed towards biochemical analysis of CcmR interaction with its oxidized NADP ligand and its effect on transcription. Additionally, further understanding of NADPH fluorescence transients, which are still not well characterized, will be explored.

This work was supported by the United States Department of Energy, Office of Basic Energy Sciences, DE-FG02-08ER15968.

Graduate Student Presenter

CYANOBACTERIAL PLATFORM FOR PRODUCTION OF PLANT SECONDARY METABOLITES

Yong Xue and Qingfang He
Department of Applied Science, University of Arkansas at Little Rock, United States

Cyanobacteria are being actively used for bioproduction in recent years. However, the application of them for enhancing production of plant secondary metabolites is essentially lacking. Under current technology level (both growth and processing), we believe, this is a particularly attractive field because of the existence of extensive multifunctional membranes and high content of reductants for biosynthesis of plant secondary metabolites in the cells. Our laboratory has been exploring the suitability of using *Synechocystis* PCC 6803 for production of coumaric acid, caffeic acid and other plant secondary metabolites that exhibit beneficial effects on human health as anticancer, antioxidant, anti-virus and anti-inflammatory agents.

Production of caffeic acid by bacterial systems is technically challenging due to difficulties in functionally expressing p-coumarate-3-hydroxylase (C3H), a cytochrome P450 enzyme that converts *p*-coumaric acid into caffeic acid. Here, we report for the first time that the cyanobacterium *Synechocystis* PCC 6803 is able to produce caffeic acid from *p*-coumaric acid upon heterologous expression of C3H. The *Arabidopsis thaliana ref8* gene, which encodes a C3H, was synthesized and codon-optimized for enhanced expression in *Synechocystis*. Expression of the synthetic *ref8* gene was driven by a native *psbA2* promoter and confirmed at the transcriptional and translational levels. This heterologous pathway enabled *Synechocystis* to produce caffeic acid at a concentration of 7.2 mg/L from *p*-coumaric acid under oxygenic photosynthetic growth conditions.

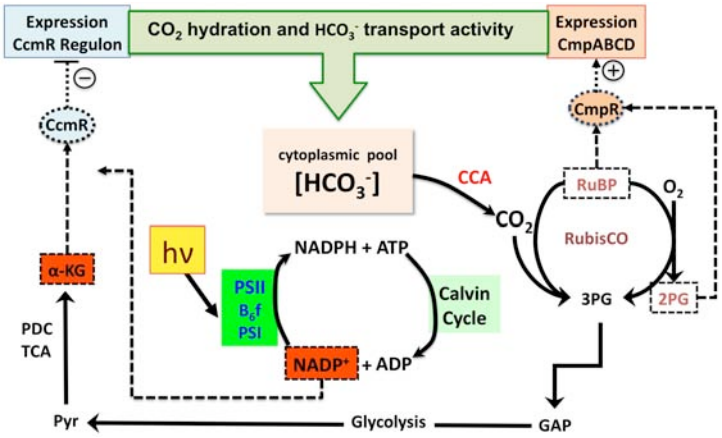
The *sam8* gene, coding for a tyrosine ammonia-lyase was genetically engineered into *Synechocystis* sp. PCC 6803 and the strain was found to accumulate no or trace amounts of *p*-coumaric acid. Upon deletion of a putative degrading enzyme from the genome of the strain, the accumulation of *p*-coumaric acid was detected using LC/MS and the maximum titer reached ~ 82.6 mg/L.

These studies demonstrated that cyanobacteria are well suited for the bioproduction of plant secondary metabolites that are difficult to produce in other bacterial systems.

REGULATION OF THE INORGANIC CARBON CONCENTRATING MECHANISM (CCM) IN CYANOBACTERIA

Shawn ME Daley, Steven C Holland, Juliana Artier, and Robert L Burnap
Department of Microbiology & Molecular Genetics, Oklahoma State University, Stillwater OK 74078

Inorganic carbon is the major macronutrient required by organisms utilizing oxygenic photosynthesis for autotrophic growth. Aquatic photoautotrophic organisms are dependent upon a CO_2 concentrating mechanism (CCM) to overcome the poor CO_2-affinity of the major carbon-fixing enzyme, ribulose-bisphosphate carboxylase/oxygenase (Rubisco). The CCM involves active transport of inorganic forms of carbon (C_i) into the cell to increase the CO_2 concentration around the active site of Rubisco[1]. Here the inducible CCM is being studied to understand how it regulated. The basic hypothesis was that a tight integration is achieved through specific regulatory interactions between photosynthetic metabolites and the transcriptional regulatory proteins that control the expression of the structural genes for the inducible CCM[2]. While such interactions had been hypothesized, the actual mechanisms had remained unresolved. Using surface plasmon resonance analysis to test whether small candidate metabolites interact with the known transcriptional repressor called CcmR (encoded by *Synechocystis* orf sll1594) we observed the two metabolites indeed enhance the binding of CcmR to the operator regions of the controlled CCM genes[3]. Specifically, we found that $NADP^+$ and α-ketoglutarate (α-KG) act as allosteric effectors of CcmR, providing an explanation for how the CCM genes are regulated. Regulatory molecules and interactions are indicated with in the figure dashed lines, metabolic fluxes indicated with solid arrows. The depletion of $NADP^+$ and α-KG results in the de-repression CCM transcriptional expression to alleviate the scarcity of inorganic carbon (Ci). The results leading to these conclusions and model shown above will be discussed.



This work was supported by the United States Department of Energy, Office of Basic Energy Sciences, DE-FG02-08ER15968.

1. Price, G. D., Badger, M. R., Woodger, F. J., and Long, B. M. (2008) Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants, *J. Exp. Bot. 59*, 1441-1461.
2. 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.
3. 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.

THE PSBP-DOMAIN PROTEIN 1 FUNCTIONS IN THE ASSEMBLY OF LUMENAL DOMAINS IN PHOTOSYSTEM I

Johnna L. Roose, Laurie K. Frankel, and Terry M. Bricker
Department of Biological Sciences, Biochemistry and Molecular Biology Section, Louisiana State University, Baton Rouge, LA 70803, USA

Photosystem I (PS I) is a multisubunit membrane protein complex that functions as a light-driven plastocyanin-ferredoxin oxidoreductase during oxygenic photosynthetic electron transport. The PsbP-domain Protein 1 (PPD1, At4g15510) is located in the thylakoid lumen of plant chloroplasts and is essential for photoautotrophy as a PS I assembly factor. In this work, RNAi was used to suppress *PPD1* expression, yielding mutants displaying a range of phenotypes with respect to PS I accumulation and function. These PPD1 RNAi mutants showed a loss of assembled PS I which was correlated to loss of the PPD1 protein. In the most severely affected PPD1 RNAi lines, the accumulated PS I complexes had defects in electron transfer from plastocyanin to the oxidized reaction center P_{700}^{+}. The defects in PS I assembly in the PPD1 RNAi mutants also had secondary effects with respect to the association of light-harvesting antenna complexes to PS I. Due to the imbalance in photosystem function in the PPD1 RNAi mutants, light-harvesting complex II (LHC II) associated with and acted as an antenna for the PS I complexes. These results provide new evidence for the role of PPD1 in PS I biogenesis, particularly as a factor essential for proper assembly of the lumenal portion of the complex.

This work was supported by the United States Department of Energy, Office of Basic Energy Sciences DE-FG02-09ER20310 to T.M.B and L.K.F.

Postdoctoral presenter

CONFORMATION CHANGES IN PSBP UPON BINDING TO SPINACH PS II

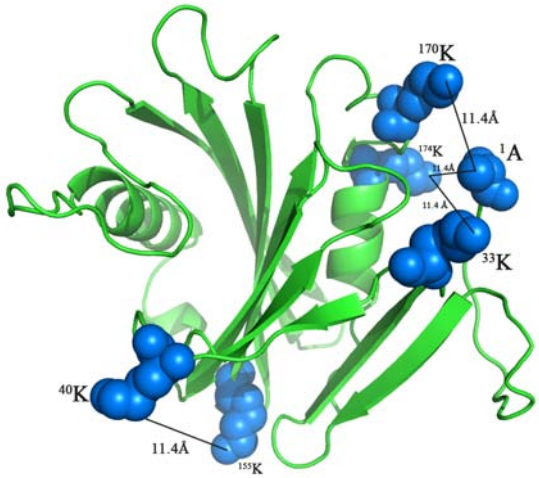
[1]Manjula P. Mummadisetti, [2]Larry Sallans, [2]Patrick A. Limbach, [1]Laurie K. Frankel, [1]Terry M. Bricker, [1]Department of Biological Sciences, Division of Biochemistry and Molecular Biology, Louisiana State University, Baton Rouge, Louisiana 70803, USA; [2]The Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221.

The extrinsic proteins (PsbO, PsbP and PsbQ) located on the lumenal side of higher plant Photosystem II are critically important for oxygen evolution at physiological calcium and chloride concentrations. While protein crosslinking has been used to demonstrate a close interaction between these components and the intrinsic proteins of the photosystem, the interacting domains on these proteins have not been established. The overall goal of our research focuses on determining how the PsbP and PsbQ proteins interact with each other and with the other components of Photosystem II.

In this communication, we have examined the internal crosslinking pattern of PsbP using the homobifunctional crosslinker BS3 (spacer arm length 11.4 Å). PS II membranes (BBYs) were treated with 5 mM BS3, the extrinsic PsbP and PsbQ proteins were isolated by salt-washing, and the proteins were resolved by LiDS-PAGE. A 18 kDa band which appears to contain only the PsbP protein was then examined. Tryptic fragments of this component were analysed using ESI-MS/MS spectrometry and the crosslinked peptides were identified using the MassMatrix and pLink programs. The crosslinked residues were mapped onto the 1.98 Å crystal structure of the isolated PsbP protein. A number of crosslinked residues were identified, some of which mapped to the unresolved N-terminus of PsbP (^{1}A-^{15}N). It had been shown previously that the N-terminal region plays a crucial role in the ability of PsbP to bind to the photosystem and to support oxygen evolution at low calcium concentrations.

The following residues were crosslinked with high confidence ($p \leq 1 \times 10^{-3}$): ^{1}A-^{174}K, ^{1}A-^{170}K, ^{13}K-^{174}K, ^{14}K-^{174}K, ^{33}K-^{174}K and ^{40}K-^{155}K. These results indicate that the N-terminus of PsbP and the ε amino groups of ^{13}K, ^{14}K and ^{33}K are all located within 11.4 Å of ^{174}K. The ^{40}K-^{155}K crosslink is particularly interesting. In the crystal structure the ε amino groups of these residues are separated by 16.2 Å, which could not be bridged with the 11.4 Å spacer arm of BS3. This result may indicate that a significant conformational change occurs in PsbP upon its binding to PS II.

This work was supported by the United States Department of Energy, Office of Basic Energy Sciences DE-FG02-09ER20310 to T.M.B and L.K.F. and the National Institutes of Health RR019900 and GM58843 to P.A.L.



Graduate Student Presenter

CHARACTERIZATION OF A CYANOBACTERIAL OUTER MEMBRANE PROTEIN: AN *E. COLI* TOLC HOMOLOGUE FROM *SYNECHOCYSTIS* SP. PCC 6803

Rachna Agarwal[1,2], Stanislav D. Zakharov[1,3], S. Saif Hasan[1], Julian P. Whitelegge[1,4] & William A. Cramer[1]

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E. coli TolC (**tol**erance to **c**olicins) represents a unique class of outer membrane (OM) proteins, as it has an α-helical periplasmic tunnel and β-barrel membrane region, providing a conduit for export of metabolites and xenobiotics from cell interior to exterior, and for import of colicin E1. A TolC homologue, Slr1270 from *Synechocystis* 6803, cloned and expressed in E. coli, has ~ 40% similarity and ~16% identity to E. coli and *Pseudomonas* counterparts, and has a similar domain organization. Homology modeling using *Pseudomonas* OprM as template modeled 93% of Slr1270 sequence. The 1581bp slr1270 gene was cloned and overexpressed in *E. coli*. Protein from inclusion bodies, refolded through step-wise dialysis, showed major bands at ~55kDa and > 150 kDa on SDS-PAGE corresponding to the monomer and trimer respectively. Purifed protein displays a far-UV CD spectrum characteristic of *E. coli* TolC with > 50% α-helix, and formed channels in planar lipid bilayers with a characteristic single channel conductance of ~50 pS in 0.1M NaCl and ~ 140 pS in 0.1M KCl. The intact protein mass spectrum (LC-MS) with a major peak at 54,489 probably represents a mixture of two species, the TolC product with 1-40 removed and an intact 6-His tag (calculated mass 54,457.1 Da), and a product with 1-38 removed and a 5-His tag (calculated mass 54,490.2 Da) after a single carboxypeptidase event. The small peak at 54,638 Da probably corresponds to a TolC product with 1-38 removed and an intact 6-His tag (calculated mass 54,627.3 Da). Peptides 39-76 and 41-76 were recovered from trypsin digests confirming N-terminal heterogeneity. This study assigns function to a previously known hypothetical protein and adds to the knowledge base of cyanobacterial outer membrane proteins, for which there is not yet one crystal structure of an intact protein.

Postdoctoral Presenter

STRUCTURE/FUNCTION INSIGHTS OF THE CYCLIC ELECTRON TRANSPORT CHAIN OF *ROSEIFLEXUS CASTENHOLZII*: THE LIGHT HARVESTING REACTION CENTER COMPLEX AND THE ALTERNATIVE COMPLEX III

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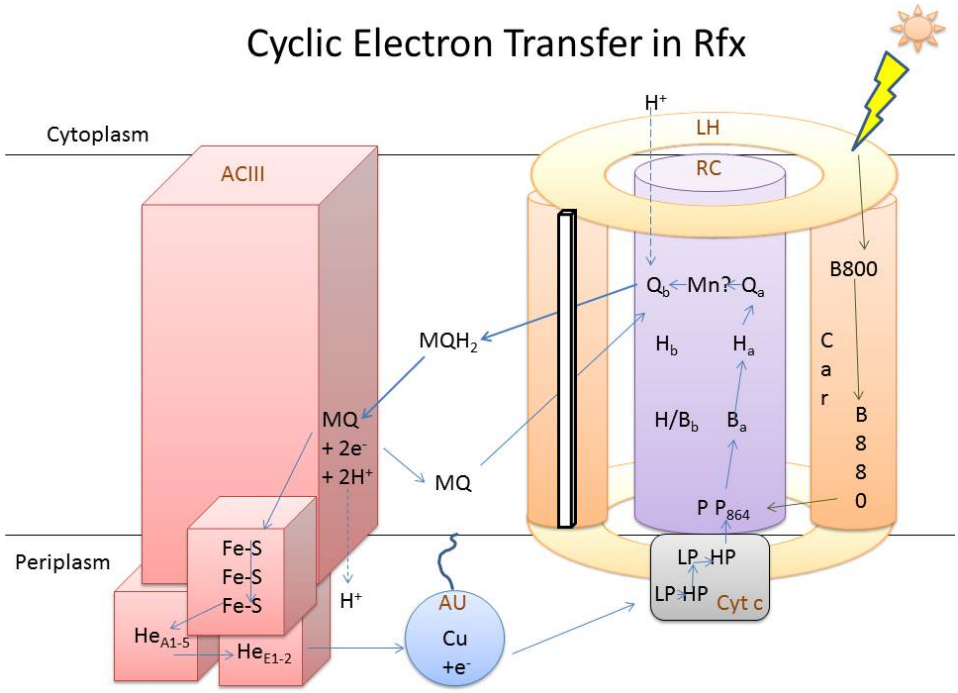
The structure of the Light Harvesting Reaction Center complex (LHRC) from the chlorosome-lacking Filamentous Anoxygenic Phototroph (FAP) *Roseiflexus castenholzii* is being studied by native mass spectrometry and X-ray crystallography. The LHRC in FAP's contains a type II reaction center similar to those in purple bacteria (without the H subunit), and is surrounded by a light-harvesting ring similar to LH1, but has spectral similarity to LH2. In this work, the exact mass and cross-sectional area were determined by native spray ion-mobility mass spectrometry. The M/L subunit was investigated by HPLC and PCR to reveal if the fused gene is expressed as one subunit or two. These experiments provide constraints for generating a model from X-ray crystallography. The complex has been crystallized and preliminary diffraction data to 8 Å resolution obtained. Additionally, the unique cytochrome *bc* type complex, the Alternative Complex III is being isolated and characterized. The novel protein components contribute to distinct mechanisms for photosynthetic electron transport.



Graduate Student Presenter

ENERGY TRANSFER OBSERVED IN LIVE CELLS USING TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY

Peter D. Dahlberg,[1] Graham J. Norris,[2] Subha Viswanathan,[2] Gregory S. Engel[2]
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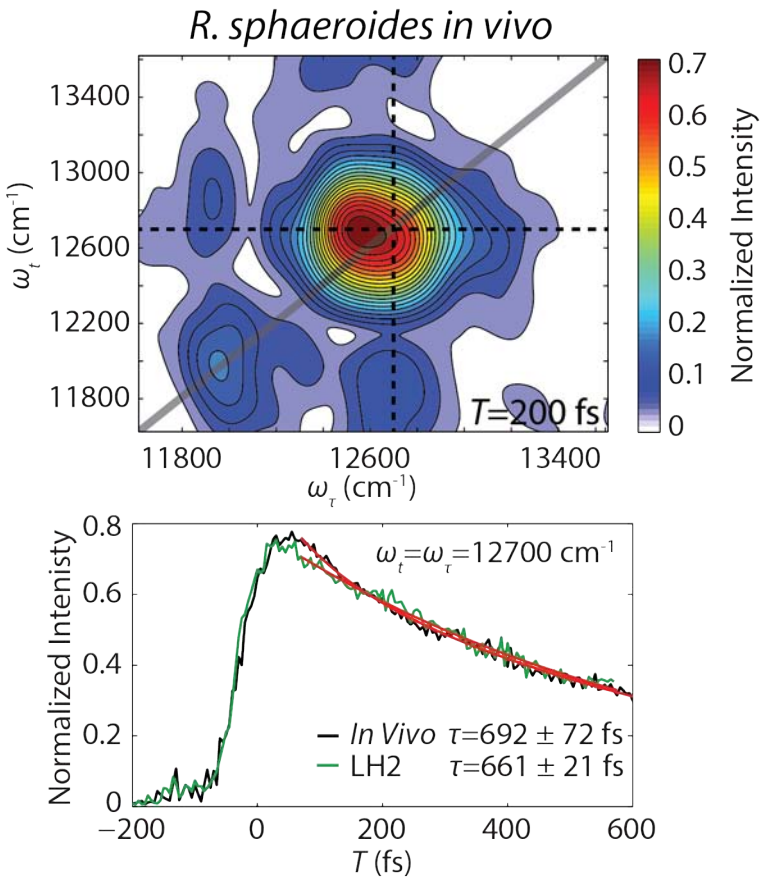
Two-dimensional electronic spectroscopy (2DES) elucidates electronic structure and dynamics on a femtosecond time scale and has proven to be an incisive tool for probing congested linear spectra of biological systems. However, samples that intensely scatter light frustrate 2DES analysis, necessitating the use of isolated protein chromophore complexes when studying photosynthetic energy transfer. We present a method for conducting 2DES experiments that takes only seconds to acquire thousands of 2DES spectra and permits analysis of highly scattering samples. We apply this technique to whole cells of the purple bacterium *Rhodobacter sphaeroides*. These *in vivo* 2DES experiments reveal similar timescales for energy transfer within the antennae complex (light harvesting complex 2, LH2) both in the native photosynthetic membrane environment and in isolated detergent micelles.



(Top) 2DES spectra of whole cells of *Rhodobacter sphaeroides* acquired at a waiting time of T=200 fs. The two diagonal peaks correspond to the B850 and B800 absorption features of LH2 from left to right. The cross-peaks indicate energetic coupling and transfer between B800 and B850. *(Bottom)* Waiting Time traces extracted from a time series of 2DES spectra of whole cells and isolated LH2. The traces were extracted from the B800 peak at ω_τ=ω_t=12700 cm^{-1} designated by the cross hairs in the top figure. The decay of B800 is dominated by energy transfer to B850 and the recovered lifetimes agree with previous measurements of energy transfer rates from B800 to B850, ~700 fs.

Graduate Student Presenter

UNLOCKING THE GATE OF THE BC_1 COMPLEX; THE Q_O SITE MECHANISM

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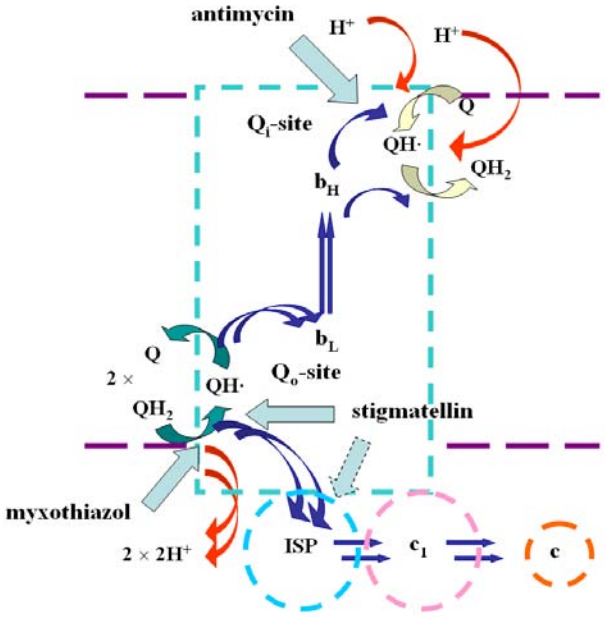
With photosynthesis only using a very small percent of the available energy from sunlight, it is imperative that we understand the key regulatory steps limiting this energy being transferred into biologically useful forms. The bc_1 complex of the electron transport chain is critical to production of the proton gradient through quinone oxidation, while simultaneously minimizing toxic ROS formation from semiquinone intermediates. Structural and kinetic analysis of the bc_1 complex suggests a proton/electron gating mechanism in the bifurcated Q_o site based on E295 rotating to allow for semiquinone migration towards the low potential chain. Flash-activated kinetics of wild type and E295 mutant bc_1 in *R. sphaeroides* chromatophores shows a dramatic qualitiative and quantitative difference in activity across a pH gradient; suggesting a rate-limiting second electron transfer in E295 mutants. CW EPR experiments were conducted on freeze-quenched bc1 activity with reduced decylubiquinol as substrate. This CW EPR shows semiquinone intermediate occupancies in E295W at steady state conditions to be 1000-fold higher than expected for a non-migratory concerted model. For further time-resolved EPR analysis, instrumentation has been developed allowing for freeze-quenching of purified isolated bc_1 within as little as 56µs of initiation of reaction with quinol. In preliminary experiments we have observed semiquinone Q_o-site intermediate. All observed data continues to support a migratory model of the Qo-site semiquinone with E295 as a critical gate to ensure proper proton/electron channeling.



bc_1 complex basic Q cycle bifurcated reaction:
1. QH_2 from reaction center enters Q_o site, and Q enters Q_i site 2. First round of Qo site oxidation passes one H^+/e^- to high and low potential chains (Q_i semiquinone) 3. Second QH_2 from reaction center enters Q_o site 4. Second round of Qo site oxidation passes one H^+/e^- to high and low potential chains (Q_i quinol). Stigmatellin or Ascochlorin are inhibitors to the Q_o site, and Antimycin-A is the inhibitor of the Q_i site.

Graduate Student Presenter

THE INTRINSIC CHARGE SEPARATION RATE IN THE REACTION CENTER FROM *CHLOROBIUM TEPIDUM*

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The reaction center (RC) complex from green sulfur bacterium *Chlorobium tepidum* contains a minimum of 16 bacteriochlorophyll (BChl) *a* and 4 Chlorophyll (Chl) *a* pigments [1, 2]. While its structure is still unknown, biochemical analyses suggest that it is a type I RC, similar to the RC of photosystem I (PS I) [2]. The core of the protein is comprised of two BChl *a* pigments forming a special pair P_{840} and four Chl *a* are believed to serve as pairs of accessory (A) and primary electron acceptor (A_0) pigments in analogy with the PS I RC. The remaining BChl *a* pigments serve as an immediate RC antenna and mediate excitation energy transfer to RC. This is a dramatic simplification compared to PS I, which contains about 100 Chl *a* pigments. The resulting spectral congestion in PS I has prevented direct visualization of ultrafast electron transfer processes within PS I RC. In the case of *Chlorobium tepidum* RC, the accessory and A_0 pigments absorb at ~665 nm (Chl *a*), while the special pair and antenna pigments absorb at >790 nm (BChl *a*). The presence of two types of pigments in RC complex from *Chlorobium tepidum* removes the spectral congestion and opens a way to directly visualize the intrinsic electron transfer steps in type I RC using ultrafast pump-probe spectroscopy. At low temperatures, the special pair absorption appears as a separate band at 840 nm, enabling its direct excitation by an ultrashort laser pulse (Fig 1, left). The formation of the charge separated state involves the reduction of one of the Chl *a* molecules (A or A_0) and can be monitored by a second ultrashort probe pulse as a photobleaching of Chl *a* band at ~665 nm (Fig. 2, right). Using this approach, we found that the Chl *a* band photobleaching occurs within <200 fs after excitation of the special pair at 840 nm, while upon exciting into RC antenna this band bleaches much slower and its kinetics can be described with two lifetimes of 1 ps (40 %) and ~9 ps (60 %). These data suggest that the intrinsic primary charge separation in this RC may occur in ~200 fs or even faster.

The authors acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE- FG02-09ER16084 (to SS) and Grant DE-FG02-08ER15989 (to JHG) for funding these studies.

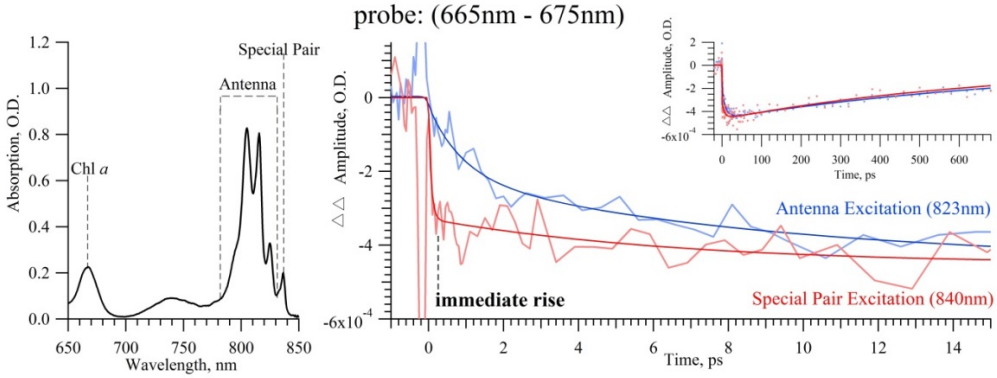

Figure 1. The absorption spectrum of RC from *Chlorobium tepidum* at 20 K (left) and the kinetics of the photobleaching band at 665 nm after exciting at 840 nm and at 823 nm (right)

1. Permentier, H., et al., *Composition and optical properties of reaction centre core complexes from the green sulfur bacteria Prosthecochloris aestuarii and Chlorobium tepidum.* Photosynthesis Research, 2000. **64**(1): p. 27-39.
2. Oh-oka, H., *Type 1 Reaction Center of Photosynthetic Heliobacteria†.* Photochemistry and Photobiology, 2007. **83**(1): p. 177-186.

Graduate Student Presenter

EVIDENCE THAT THE TYLAKOID PROTON MOTIVE FORCE REGULATES PHOTOINHIBITION

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The proton motive force (*pmf*) generated across the thylakoid membrane during photosynthesis drives light-driven ATP; it is also a central regulatory intermediate, controlling both light capture and electron transfer. The pH of the thylakoid lumen controls the photoprotective qE response, the xanthophyll cycle, and downregulation of cytochrome b_6f. It has also been shown *in vitro* to destabilize the oxygen evolving complex of photosystem II (PSII). A large scale phenometrics experiment measuring the effects of fluctuating light on a library of Arabidopsis mutants defective in chloroplast-targeted proteins turned up a strong positive relationship between qE and photoinhibition, suggesting that low lumen pH may trigger photoinhibition *in vivo*. To test this possibility, we generated a series of site-directed mutants in the *Arabidopsis thaliana* chloroplast ATP synthase g-subunit. *In vivo* spectroscopy revealed that these mutants have a range of ATP synthase activities, resulting in a wide range of steady-state extents of *pmf*, qE responses and linear electron flow (LEF) rates. Increased *pmf* was strongly correlated with increased photoinhibition. The effects were not correlated with Q_A redox state, suggesting that *pmf* directly triggers PSII photoinhibition on the donor side, rather than indirectly through buildup of electrons on Q_A. The effects of lincomycin demonstrated that increased photoinhibition was not caused by decreased rates of PSII repair. Thus we conclude that high *pmf* or low lumen pH directly sensitizes PSII to photoinhibition. The continuous relationship between *pmf* and photoinhibition includes (and extends beyond) the wild type, implying that this mechanism is physiologically important, i.e. it happens in wild type plants under growth conditions. We propose a model in which the lumen pH regulates photosynthesis at several levels: 1) controlling electron transfer through the cytochrome b_6f complex to protect photosystem I from photodamage; 2) initiating the qE response by activating the xanthophyll cycle and protonation of PsbS; 3) activating long-term photoinhibition of PSII. All of these processes may be viewed as regulatory, preventing the buildup of deleterious reactive oxygen species.

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Graduate Student Presenter

Abstracts of Posters

(Alphabetical by First Author)

FUNCTION OF CUPA IN THE NDH1$_3$ COMPLEX OF *SYNECHOCYSTIS* PCC 6803

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Cyanobacteria have evolved different mechanisms to survive and adapt to adverse conditions. The CO_2 concentrating mechanisms (CCM) are responsible for increase on inorganic carbon (C_i) to be used in the Calvin Cycle. *Synechocystis* have five Ci uptake mechanisms: three HCO_3^- transporters and two CO_2 uptake mechanisms. CO_2 uptake involves specialized NDH-1 complexes, NDH-1$_3$ (NdhF3/NdhD3/CupA/CupS) and NDH-1$_4$ (NdhF4/NdhD4/CupB). Mutants of these subunits presented impaired CO_2 uptake (1,2). It was suggested that CupA and CupB are possible carbonic anhydrases (1,3,4), however expression of soluble CupA and CupB and detection of CO_2 hydration activity were until now unsuccessful. These complexes appear to utilize redox energy of either ferredoxin or NADPH, but the mechanism of coupling this energy to CO_2 hydration and how the complexes fit into the overall patterns of cyclic electron transport (CET) is debated (1,2). CcmR is a protein that regulates the expression of the NDH-1 complex involved in C_i transport, by sensing changes in [α-ketoglutarate] and [$NADP^+$] that act as co-repressors of CcmR (5). We are studying the integration of the CCM with the PSET producing ATP and NADPH. Mutants affecting NDH complexes, CET and the CCM are being compared using analysis of chlorophyll fluorescence as an indicator of CET and CO_2 uptake and P700 oxidation-rereduction as a measure of CET. The results will be presented here. Also our group was able to express CupA, using a maltose binding protein fused with CupA from *Synechocystis*. We are performing biochemical analysis on the protein to elucidate structure-function relationships implied by its putative CO_2-hydration activity.

Supported by US Department of Energy, Office of Basic Energy Sciences DE-FG02-08ER15968

1. Maeda, S., Badger, M. R., and Price, G. D. (2002) *Mol Microbiol* **43**, 425-435
2. Shibata, M., Ohkawa, H., Kaneko, T., Fukuzawa, H., Tabata, S., Kaplan, A., and Ogawa, T. (2001) *Proc Natl Acad Sci U S A* **98**, 11789-11794.
3. Ohkawa, H., Price, G. D., Badger, M. R., and Ogawa, T. (2000) *J of Bacteriol* **182**, 2591-2596
4. Kaplan, A., and Reinhold, L. (1999) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 539-570
5. Daley, S., Kappell, A., Carrick, M., and Burnap, R. (2012) *PloS one* **7**, 41286-41286

Graduate Student Presenter

DEEP SEQUENCING OF THE SINUS MICROBIOME REVEALS ABUNDANT LEVELS OF CYANOBACTERIAL SPECIES

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To gain further insights into the etiology of chronic rhinosinusitis (CRS), a persistent inflammation of the nasal and paranasal mucosa, we analyzed the microbiome of patients with CRS. A lavage of middle meatus, the first mucosal surface encountered in the inhaled breath, was performed on 30 CRS patients and 12 healthy controls. Bacterial 16S rRNA genes were amplified from genomic DNA isolated from the lavage, and the amplicons were analyzed by deep sequencing. The most abundant bacterial species present, with 43% of the 800,000 sequence-reads belonged to the phylum Cyanobacteria. We identified 127 different uncultured species (at the 16S rRNA sequence or operational taxanomic units) in the lavage samples. No statistical difference in the abundance or species of Cyanobacteria was observed between CRS patients and the healthy controls. To confirm the presence of Cyanobacteria in the lavage samples the samples were analyzed by flow-cytometry to detect presence of phycoerythrin (PE) and allophycocyanin (APC). Our results indicate readily detectable presence of PE and APC double-positive cells that are quite large (~5 μM). Cyanobacteria are common in marine and aquatic environments. Consistent with their need to capture light for photosynthesis, Cyanobacteria are typically found close to the surface of the water column. Therefore, it is intriguing why these Cyanobacteria colonize a relatively dark environment like the middle meatus of the sinus. We will also present the results of the comparison of the sinus microbiome to other sites of human microbiome, where Cyanobacteria have been identified.

PHOTOHETEROTROPHIC GROWTH OF *PHYSCOMITRELLA PATENS*

[a]Terry M. Bricker, [a]Adam J. Bell, [b]Lan Tran, [a]Laurie K. Frankel and [b]Steven M. Theg,
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Physcomitrella patens is a model bryophyte representing an early land plant in the green plant lineage. This organism possesses many advantages as a model organism. Its genome has been sequenced, its predominant life cycle stage is the haploid gametophyte, it is readily transformable and it can integrate transformed DNA into its genome by homologous recombination. One limitation for the use of *P. patens* in photosynthesis research is its reported inability to grow photoheterotrophically, in the presence of sucrose and the Photosystem II inhibitor DCMU, which prevents linear photosynthetic electron transport. In this poster we describe the facile isolation of a *P. patens* strain which can grow photoheterotrophically. Additionally, we have examined a number of photosynthetic parameters for this strain grown under photoautotrophic, mixotrophic (in the presence of sucrose) and photoheterotrophic conditions, as well as the DCMU-inhibited state.

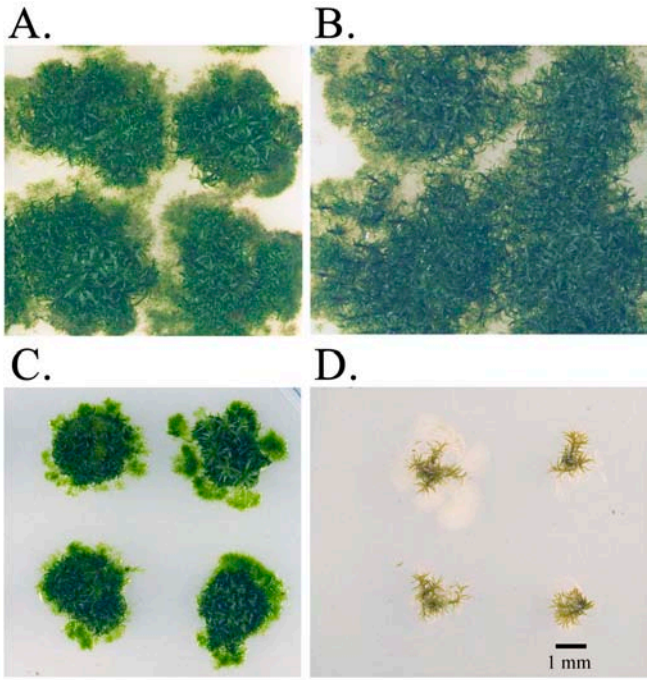

Figure 1. Illustrated is the growth of 1.5-2 mm gametophytic explants after 8 weeks, all panels are at the same magnification. A. Photoautotrophic growth, B. Mixotrophic growth, C. Photoheterotrophic growth, and, D. PS II-inhibited gametophytes. At 8 weeks, the DCMU-inhibited gametophytes have fully bleached and are apparently dead. Clearly, photoheterotrophic growth is slower than photoautotrophic or mixotrophic growth conditions. This was fully expected and is similar to that observed in *Synechocystis* 6803 and *Chlamydomonas* grown under analogous growth conditions.

CRITICAL ROLES OF PFSR IN REGULATION OF IRON HOMEOSTASIS IN *SYNECHOCYSTIS* PCC 6803

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Iron is an essential cofactor in a multitude of cellular processes. Although iron is abundant on earth, its availability is normally limited because of its low solubility in aerobic ecosystems. Cyanobacteria have evolved complex regulatory networks to tightly control iron homeostasis, ensuring the essential function of iron yet avoiding cellular damage. In this study, we investigated in depth the function of PfsR (photosynthesis, Fe homeostasis and stress-response regulator) in iron homeostasis. The *pfsR* deletion mutant showed stronger tolerance to iron starvation and accumulated chlorophyll a, carotenoid and phycocyanin to a significantly higher level than wild-type under iron limitation conditions. Western Blot results, consistent with 77K fluorescence emission data, revealed that the *pfsR* mutant assembled more photosystem Ⅰ and photosystem Ⅱ compared with wild-type after iron step-down. In addition, the measurements of oxygen evolution capacity and yields of chlorophyll fluorescence indicated that both photosystem Ⅰ and photosystem Ⅱ activities were obviously higher in *pfsR* mutant than in wild-type cells under iron-starved conditions. Moreover, transcripts of the *fut* genes (encoding ferric iron transporter), the *feoB* gene (encoding ferrous iron transporter), the *bfr* genes (encoding bacterioferritin), and the *isiA* gene, were up-regulated, while transcripts of the *ho* genes (encoding heme oxygenase) were down-regulated in the *pfsR* mutant, especially upon iron deprivation. The electrophoretic mobility shift assay showed that PfsR was auto-regulated by binding to its own promoter. These results suggest a critical regulatory role of PfsR in iron homeostasis.

Postdoctoral Presenter

Inducible Carotenoid Binding Protein Complex From *Synechocystis 6803*

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Photosynthetic organisms provide food and oxygen that are essential for life on earth by harvesting solar energy and converting it into chemical energy. However, Changes in global environment might lead to stress conditions in photosynthetic organisms and limit the efficiency of photosynthesis. The damages can be largely contributed to oxygen-dependent destruction of photosynthetic apparatus and other cellular components. 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 photo protective mechanisms. In this study we have isolated a novel high light and iron stress inducible carotenoid-binding protein complex from the thylakoid membranes of *Synechocystis PCC 6803* cells that are exposed to high light intensity and long term iron deprivation. Pigment of the complex, were extracted in acetone—methanol (7/2) volume and separated by HPLC. The majority of the pigments in the complex are myxoxanthophyll and zeaxanthin, with a neglectable amount of Chl a and β carotene. We hypothesize that the complex may protects thylakoid membranes from extensive photoxidative damages, iron stress by direct or indirect scavenging of reactive oxygen species and most likely involve in state transition. Experiments are underway to determine the protein composition.

Graduate Student Presenter

CAROTENOID VARIATION IN *ROSEIFLEXUS CASTENHOLZII* UNDER NATIVE-LIKE GROWTH CONDITIONS

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Roseiflexus castenholzii (RFX) is a filamentous anoxygenic phototroph naturally found in hot springs. RFX contains a unique photosystem with components that have structural similarities to purple bacteria, and contains the novel Alternative Complex III instead of a cytochrome *bc* type complex. In nature, RFX grows in microbial mats in a matrix with cyanobacteria such as *Arthrospira platensis* (Spirulina). In the laboratory, RFX is grown on standard O2YE media, which contains yeast extract as the carbon source. In this project, RFX was grown in media made using Spirulina powder instead of yeast extract in order to mimic the native-like growth conditions. The cells and pigment expression were monitored approximately every twelve hours for seven days to see how the change of carbon source affects the overall growth of the cells. The resulting experimental cell yield was comparable to the yield of the control culture grown on standard media. However, the experimental cells showed significant variation in color from the control cells. To understand this result, the pigments were extracted from the cells and high-pressure liquid chromatography (HPLC) was used to identify and compare the pigment content. The cells were found to contain the same bacteriochlorophyll *a*, but a different composition of carotenoid pigments. Therefore, growth media ingredients that more closely mimic the natural system should be chosen to produce cells with more native-like light harvesting systems.

Undergraduate Presenter

RE-EXAMINATION OF THE DARK NADPH-LINKED CHLOROPHYLL FLUORESCENCE RISE THOUGHT TO INDICATE CYCLIC ELECTRON FLOW IN HIGHER PLANT CHLOROPLASTS

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Addition of NADPH and ferredoxin (Fd) to broken chloroplast preparations in the dark results in a characteristic slow increase in chlorophyll fluorescence with a half-time measured in tens of seconds which plateaus at approximately 15% of the fluorescence observed during a saturating actinic flash. This phenomenology, which is sensitive to antimycin A and quenched by far red light, has been ascribed to the reduction of the plastoquinone pool via cryptic ferredoxin:plastoquinone reductase activity (Mills JD *et al* Biochim. Biophys. Acta 547: 127, 1979; Munekage Y *et al* Nature 429: 579, 2004). It is frequently used as an assay for investigating processes postulated to involve cyclic electron flow around photosystem I (CEF1).

We have systematically investigated the basis of this dark NADPH-associated fluorescence rise in spinach chloroplast preparations, observing a similar antimycin A-sensitive phenomenology under conditions where Q_A-associated PSII variable fluorescence has been eliminated due to the presence of hydroxylamine and DCMU. Therefore the signal does not reflect PQ reduction associated with CEF as previously supposed, but another process. This fluorescence rise is abolished in the presence of diphenyleneiodonium (DPI), an FNR inhibitor. Examination of the fluorescence induction kinetics indicates reduction of the bulk plastoquinone pool by NADPH, although we note that the fluorescence rise is unaffected by saturating concentrations of tridecylstigmatellin (an inhibitor of quinol oxidation in the cytochrome b6f complex) and the kinetics of intersystem chain oxidation by $P700^+$ in the presence of NADPH and Fd are slower than those observed in the fluorescence rise assay. From this, we conclude that the fluorescence quenching species participating in the dark NADPH rise is not plastoquinone or a component linked to the plastoquinone pool.

Room temperature fluorescence emission spectroscopy shows no evidence for Photosystem I-associated variable fluorescence in the dark NADPH-associated rise. Redox titration of the rise suggests the presence of a component with a midpoint potential of approximately -340 mV. The fluorescence rise is abolished under conditions where the grana are likely to be unstacked (i.e. chloroplasts prepared in the absence of exogenous Mg^{2+}), but is immediately restored upon addition of Mg^{2+}. Although the identity of the quenching species removed during the dark NADPH-linked fluorescence rise is currently unknown, we suggest that it is photosystem II (or antenna)-associated, and may represent a low potential electron carrier within a subpopulation of this enzyme or a conformational change associated with a PSII or antenna redox carrier. Small changes in 77K fluorescence emission spectra suggest possible regulation of PSII-associated antenna. As such, the dark NADPH fluorescence rise may not be directly associated with CEF1, but may represent the activation of a redox switch for regulation of CEF1 or other processes.

Supported by Grant DE-FG02-11ER16220 from the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the US Department of Energy.

Postdoctoral Presenter

CHARACTERIZING SYNTHETIC LIGHT HARVESTING COMPLEXES TO RESOLVE PROTEIN-CHROMOPHORE AND CHROMOPHORE-CHROMOPHORE INTERACTIONS

Moira Flanagan[*], Daniel Finley[†], Justin Caram[‡], Andrew Fidler[‡], Matthew B. Francis[†], Gregory S. Engel[*‡]

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In photosynthesis, pigment-protein antenna complexes capture light, and efficiently transfer energy to the reaction center. To do this, the protein environment of the complex coordinates and directs energy transfer via chromophore arrangement, coupling, energetic disorder and fluctuations. To study these effects, we probe a virus-templated synthetic light harvesting complex using femtosecond spectroscopy and modeling. Using Tobacco Mosaic Virus (TMV) capsid proteins as a scaffold, we assemble a pigment-proteins into a double-disc structure, similar to Light Harvesting Complex 2 (LH2). Using this model system, we can isolate the effects of coupling by probing chromophores conjugated to either one capsid monomer or an entire ring of capsid monomers. We study 4 specific model systems using linear and nonlinear electronic spectroscopy, shown in figure 1: a disc protein complex with only one chromophore attached, a disc with a ring of chromophores separated by about 17 Å, a disc with a ring of chromophores separated by 12 Å, and the free chromophore in solution. By isolating the protein environment from coupling strength experimentally, the complexity of photosynthetic energy transfer can be elucidated. Using linear absorption, two-dimensional electronic spectroscopy, and models based on spectral density, we present a characterization of these TMV-based light harvesting analogs.

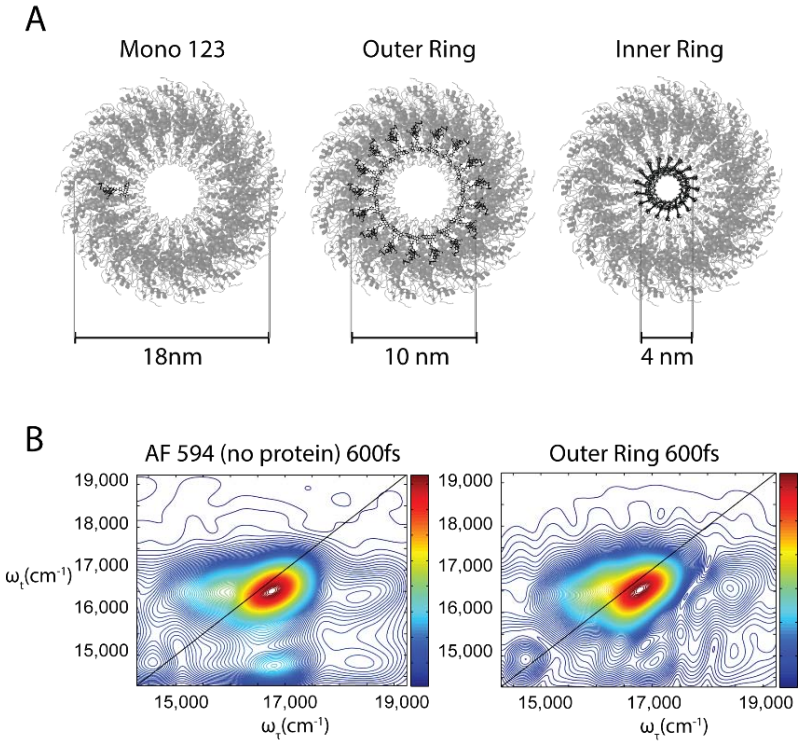

Fig. 1. TMV capsid protein based light harvesting complex. (A) The predicted crystal structures of the different proteins as viewed from above. The diameters of the disc protein, ring of chromophores in the outer ring and for the inner ring are given. (B) Sample 2D absolute value spectra of chromophore Alexa Fluor 594 nm in solution and in the outer ring complex with Alexa Fluor 594 nm at 600 fs. The main peak in the outer ring is slightly inhomogeneously elongated than the free chromophore. The vibrational shoulder becomes more distinctly separated.

Graduate Student Presenter

KINETIC ANALYSIS OF THE pH AND CHLORIDE DEPENDENCE OF OXYGEN EVOLUTION BY PHOTOSYSTEM II: AN UPDATED MODEL

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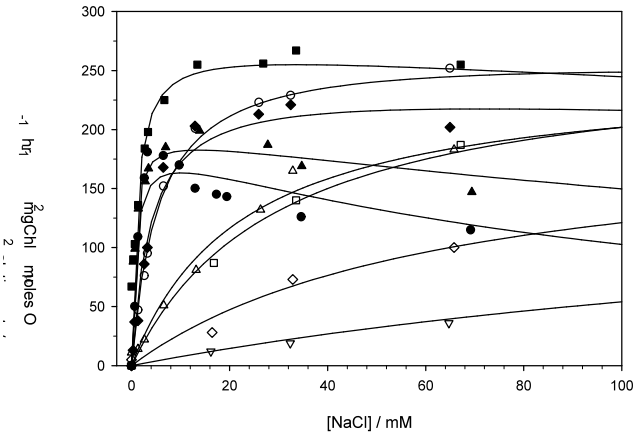
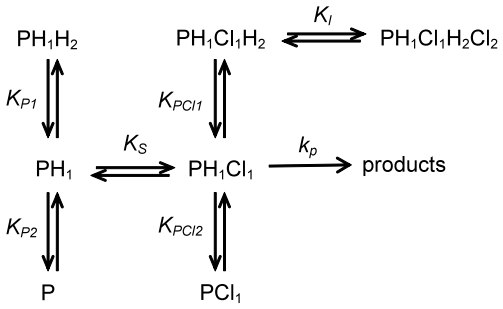
Oxygen evolution by photosystem II (PSII) involves activation by Cl^- ions, which is regulated by extrinsic subunits PsbQ (17 kDa) and PsbP (23 kDa). In this study, the kinetics of chloride activation of oxygen evolution was studied over the pH range from 5.3 to 8.1 in PSII depleted of the PsbQ and PsbP subunits using two methods: treatment with 2 M NaCl and EDTA; and incubation with 50 mM Na_2SO_4 at pH 7.5. Both preparations showed activation and inhibitory effects of added Cl^-, with both effects more pronounced at lower pH. The data were analyzed using a model involving four protonation steps for the pH dependence of Cl^- activation plus a binding step for inhibitory Cl^-. This model is based on the classic pH dependence model of enzyme kinetics, but was extended to include a substrate inhibition step, and represents a more complete model than that developed previously in the seminal work by Homann (1985, *Biochim Biophys Acta 809*, 311). Two alternative version of the model are considered. For the simpler of the two, it was found that the pH-independent dissociation constant for Cl^- activation, K_S, was 0.9 ± 0.2 mM for both preparations and the pH-independent inhibition constant, K_I, was 64 ± 2 mM and 103 ± 7 mM for the NaCl-washed and Na_2SO_4/pH7.5-treated preparations, respectively. In addition, three of the four pK_as for protonation were determined, while the fourth one was found to be outside the pH range studied. The two sites are considered in relation to the Cl^- binding sites found in recent X-ray diffraction studies. The enzyme kinetics analysis used in this study provides a more complete characterization of chloride and pH dependence of O_2 evolution activity than has been previously presented. In addition it allows characterization of the inhibitory effect of chloride for the first time.





Left: Chloride dependence of the rate of oxygen evolution by NaCl-washed PSII at various pHs: 5.36 (●), 5.50 (▲), 6.19 (■), 6.43 (♦), 6.71 (○), 7.39 (open triangle), 7.65 (□), 7.92 (◊), and 8.01 (inverted open triangle).

Right: Model of pH dependence of activation by chloride based on the classic pH dependence model, including binding of a second inhibitory chloride (Alternative 1).

MAPPING TRIPLET STATES OF (BACTERIO)CHLOROPHYLLS

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(Bacterio)Chlorophyll ((B)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. In this work we measured the triplet-state energies for ten monomeric (B)Chls and one bacteriopheophytin in similar conditions via phosphorescence. For several of them the energies of triplet states were measured for the first time.

The phosphorescence signal is extremely weak, with a quantum yield 10^{-5} to 10^{-6} times lower 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 one to two orders of magnitude stronger than the phosphorescence at its peak. Fortunately, the fluorescence lifetime is ~5 ns while the phosphorescence lifetime is ~100 μs to ~1 ms so the fluorescence can be additionally gated in the time domain.

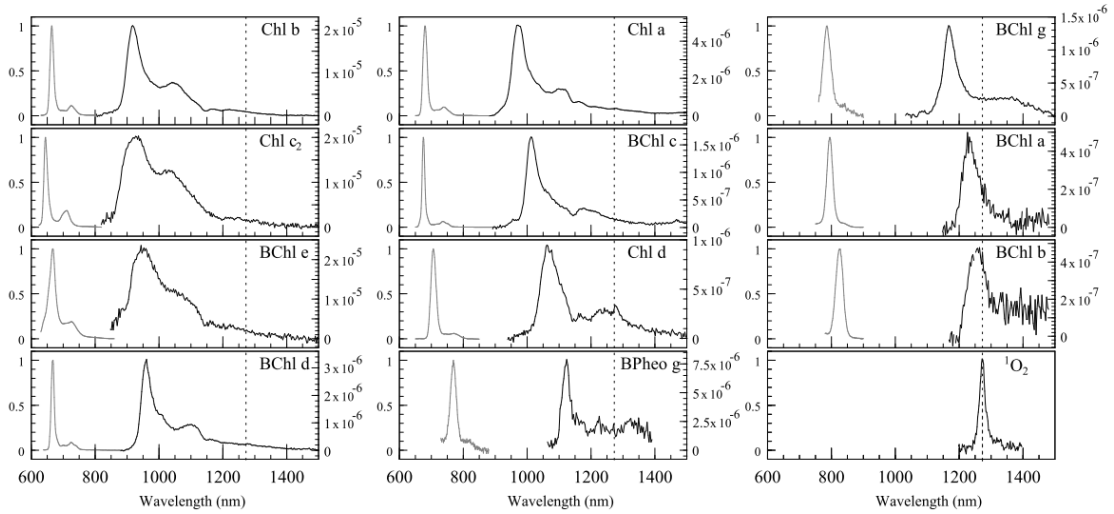

Figure 1: Fluorescence (grey, arbitrary scale) and phosphorescence (black, scale relative to fluorescence intensity) emission spectra of ten (B)Chls, BPheo *g* and singlet oxygen.

The ultimate goal of this project is to catalog the triplet-state energies of all known (B)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*, *c*2, *d* and bacteriochlorophylls *a*, *b, c, d, e, g* and bacteriopheophytin *g* have been measured. The next step will be to investigate (B)Chl dimers and aggregates for evidence of triplet exciton formation and their effect on the triplet excited state energy as well as the triplet state energies of photosynthetic pigment protein complexes such as the Fenna-Matthew-Olson complex.

The authors acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE- FG02-09ER16084 for funding these studies.

Graduate Student Presenter

COMPARISON OF CALCULATED AND EXPERIMENTAL ISOTOPE EDITED FTIR DIFFERENCE SPECTRA FOR PURPLE BACTERIAL PHOTOSYNTHETIC REACTION CENTERS WITH DIFFERENT QUINONES INCORPORATED INTO THE Q_A BINDING SITE

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ONIOM (QM/MM) calculations were undertaken in order to simulate Q_A^-/Q_A isotope edited FTIR difference spectra obtained using *Rhodobacter sphaeroides* photosynthetic reaction centers with a variety of unlabeled and ^{18}O labeled quinones incorporated into the Q_A binding site. Isotope edited FTIR difference spectra were calculated for reaction centers with 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone (MQ_0), 2,3,5,6-tetramethyl-1,4-benzoquinone (duroquinone, DQ), and 2,3-dimethyl-l,4-naphthoquinone (DMNQ) incorporated, and compared to corresponding experimental spectra. The calculated and experimental spectra agree remarkably well. Good agreement between calculated and experimental spectra could not be obtained by considering quinone molecules in the gas phase. Calculations including the protein environment are a necessity.

The normal modes that contribute to the bands in the spectra, their composition, frequency and intensity, and how these quantities are modified upon ^{18}O labeling, are presented. It is shown that the ^{18}O isotope induced changes for normal modes of DMNQ are very different to that found for normal modes of DQ or MQ_0. The calculations lead to a detailed interpretation of ^{18}O isotope induced changes in the experimental spectra. The calculated interpretation does not agree with previous interpretations that were suggested based on a simple analysis of the experimental spectra.

From experimental spectra it has been suggested that one of the carbonyl groups of the ubiquinone in the Q_A binding site is very strongly hydrogen bonded. Previous calculations refuted this claim (Lamichhane and Hastings (2011) *Proc. Nat. Acad. Sci.* 108, 10526-10531), suggesting that both ubiquinone carbonyl groups are relatively weakly hydrogen bonded. Here we show that hydrogen bonding is also relatively weak for all of the incorporated quinones. It is also shown that the protein environment does provide an asymmetric hydrogen bonding environment for all of the incorporated quinones. However, these protein interactions are calculated to lead to only a 10-13 cm-1 separation in the frequencies of the carbonyl vibrational modes.

The above listed quinones are “tail-less” (and symmetric). Isotope edited FTIR difference spectra were also calculated for reaction centers with corresponding “tail” containing quinones incorporated into the Q_A binding site. It is found that replacement of the quinone methyl group by a phytyl or prenyl chain does not alter ONIOM calculated spectra. The quinone “tail” does not significantly modify the asymmetrical bonding of the quinone head-group in the QA binding site, although calculations suggest that the “tail” may impose a small geometrical constraint on the orientation of the quinone ring.

STRUCTURAL ANALYSIS OF A HOMODIMERIC REACTION CENTER COMPLEX FROM *CHLOROBACULUM TEPIDUM*

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In the green sulfur bacterium *Chlorobaculum tepidum*, light energy collected by chlorosome antenna complexes is transferred to the reaction center (RC) through a bacteriochlorophyll-containing protein called the Fenna-Matthews-Olson (FMO) protein. We purified the intact FMO/RC complex, which is composed of FMO, PscA, PscB, PscC, and PscD subunits. The spatial interaction between FMO and the rest of the RC was studied by chemically crosslinking the purified sample. We used three different cross-linkers: BS_3, DSS and DTSSP. The complex was cross-linked and processed in the absence of reducing reagents, such as β- mercaptoethanol, which will break the sulfur-sulfur bond of DTSSP. The interaction sites of the cross-linked complex were studied using LC/MS/MS. ^{79}K of FMO, which is located on the side of the FMO trimer is found to be linked with ^{45}K of PscA, ^{36}K of PscB, ^{60}K of PscB and ^{107}K of PscD. All those sites should be on the cytoplasmic side and close to each other. Our results confirm that the PscB and PscD should be the knob sitting on PscA. We found that ^{93}K of FMO, which is located on a loop on the top of FMO, is linked to ^{30}K of PscD. We also found that ^{132}K and ^{338}K of PscA are linked to the periplasmic soluble domain of PscC. Therefore, ^{132}K and ^{338}K of PscA should also be located on the periplasmic side of the membrane and the soluble domain of PscC should be close to PscA. Accordingly, a structural model for the FMO/RC complex is proposed as shown in Figure 1.

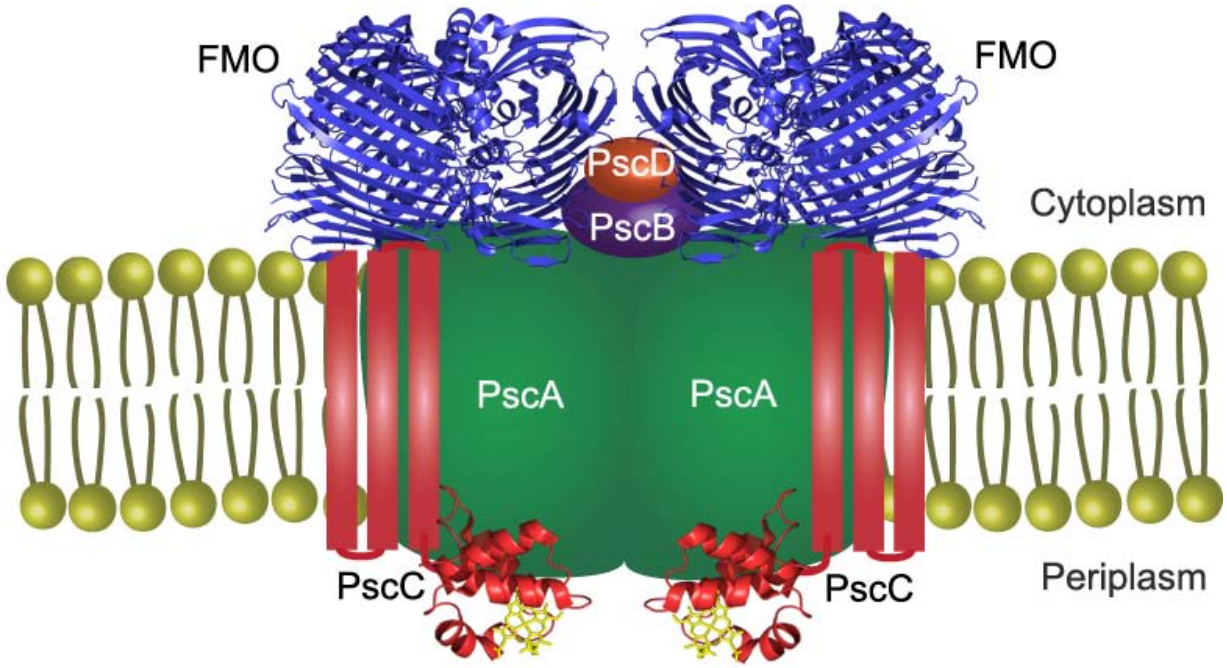

Figure 1 Proposed structural model of FMO/RC complex from *Cb. tepidum*.

Graduate Student Presenter

EXPRESSION OF FLAVO-CYTOCHROMES FOR PHOTOSYNTHETIC ENERGY TRANSDUCTION

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Michigan State University 612 Wilson East Lansing, MI PRL/MMG

Light harvested by a leaf cannot be fully utilized by photosynthesis. Instead, up to 90% of the light absorbed is dissipated as heat through non-photochemical quenching. This is problematic inasmuch as a potential energy resource is wasted. To optimize the efficiency of photosynthesis, we are pursuing the feasibility of alternative energy transduction pathways that can function in the electron transport chain following photosystem one. Shewanella Oneidensis is capable of anaerobic respiration using a multitude of different terminal electron acceptors. This capability is possible due to a plethora of cytochromes found in the Shewanella genome, amongst which are flavo-cytochromes. These flavo-cytochromes have not yet been characterized and are potentially interesting for accepting electrons from photosynthesis and converting them into electric potential outside the membrane. In this portion of the project, we will be expressing and purifying these flavo-cytochromes in hopes that they will serve as electron transducants, and ultimately aid in photosynthetic optimization.

Undergraduate Student Presenter

A SULFUR-SPARING FORM OF PHYCOCYANIN GIVES *FREMYELLA DIPLOSIPHON* A FITNESS ADVANTAGE IN LOW SULFUR CONDITIONS

LaDonna Jones and David M. Kehoe. Department of Biology Indiana University, Bloomington, Indiana

Fremyella diplosiphon is a freshwater cyanobacterium that has an interesting response to changes in sulfur concentrations in its environment. At sulfate levels below 150 uM, *F. 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. Phycobiliproteins are found in a light harvesting complex called the phycobilisome. Phycobilisomes are attached to the thylakoid membrane and channel light energy to the photosynthetic reaction centers. Changing from a PC1/PC2 to a PC3-containing phycobilisome is a significant undertaking because phycobilisomes are the most abundant protein structure in the cell. The average PC1 and PC2 containing phycobilisomes have around 1000 sulfur containing amino acids more than PC3-containing phycobilisomes. By remodeling the most abundant protein structure in the cell, there is a significant savings of sulfur containing amino acids that can be used for other cellular processes.

It has not been previously investigated whether the sulfur sparing response gives *F. diplosiphon* a growth advantage in sulfur-limited conditions. I performed a competition experiment between wild type and mutant cells incapable of undergoing the sulfur sparing response and determined the ratio of wild type to mutant cells when they are grown in sulfur-replete and sulfur-deficient conditions. Wild type cells almost completely replace the mutant cells by day 20 in sulfur-deficient media in red light. They comprise 92% of a mixed population. There is no advantage for wild type cells in sulfur-replete growing conditions. Therefore, PC3-containing phycobilisomes in low sulfur conditions provides *F. diplosiphon* with a fitness advantage in red light. PE and PC3 accumulate in low sulfate and green light in wild type cells even though PC3 absorbs red light. PC3 abundance, unlike PC2, does not decrease in green light. I have also performed a competition experiment between wild type and mutant cells in green light. In green light, PC3 does not provide wild type cells with the same fitness advantage in low sulfate conditions as it does in red light.

Graduate Student Presenter

LIMITATIONS IN BIOLOGICAL SOLAR ENERGY CONVERSION DUE TO THE TEMPRETURE DEPENDENCE OF PHOTOSYSTEM II CATALYZED WATER OXIDATION AND OXYGEN-EVOLVING COMPLEX

James Board, Hope Cook, Benjamin Blodgett, and Derrick R. J. Kolling
Marshall University, Department of Chemistry, One John Marshall Drive, 490 Science Hall, Huntington, WV 25701

Transduction of solar energy into chemical energy by photosynthetic organisms occurs via several stages with the eventual production of adenosine triphosphate and a fuel in the form of carbohydrates $(CH_2O)_n$. Each step results in a loss of energy: some notable examples are the color blindness of the reaction centers—all charge separations events require only the energy equivalent to a 700-nm photon—and the damage accumulated in the reaction centers due to the strongly oxidizing intermediates produced during charge separation. Our lab studies several other phenomena that limit efficient energy transduction in oxygen phototrophs. The first phenomenon is assembly of the oxygen-evolving complex (OEC, Mn_4CaO_5) of photosystem II (PSII), the site of water oxidation. The assembly of the OEC occurs during de novo synthesis of PSII or following repair of photodamaged PSII. (Damage accumulated during peak light conditions results in turnover of the D1 subunit of PSII with a $t_{1/2}$ of 60 min.[1]) Most steps of photoassembly are light dependent, but there is one dark dependent step that likely involves a rearrangement of the metal atoms or a protein conformational change.[3,4] Using oximetry and fluorometry, we are working to understand the nature of this compulsory dark step. Interestingly, while this process can occur under dim light, it cannot proceed under high light conditions.[3] Based on our findings and these high light observations, we have proposed a light-sensitive intermediate with a low quantum yield. This will be discussed in light of a recent OEC photoassembly study that favors the low-oxidation-state model of the OEC catalytic cycle. Two other phenomena studied by our group are the temperature dependence of PSII-catalyzed water oxidation and OEC photoassembly. Using oximetry, we have mapped the temperature dependence of PSII-catalyzed water splitting and have shown that in intact organisms and chloroplasts, maximum activity occurs at 38°C, while in PSII-enriched membranes or damaged thylakoid membranes, the maximum shifts to 28°C. Using fluorometry, we showed that damage to the membrane is the likely source of the shift. Studies of the temperature-dependence of OEC photoassembly resulted in a similar temperature-dependence profile to PSII-catalyzed water oxidation and both share a temperature maximum.

1. Neidhardt, J., Benemenn, J.R., Zhang, L., and Melis, A. (1998) Photosystem II repair and chloroplast recovery from irradiance stress: relationship between chronic photoinhibition, light-harvesting chlorophyll antenna size and photosynthetic productivity in *Dunaliella salina* (green algae), Photosynthesis Research. 56, 175–184.
2. Burnap, R.L. 2004. D1 protein processing and Mn cluster assembly in light of the emerging Photosystem II structure. Journal of Physical Chemistry. 6:4803-4809.
3. Cheniae, G.M., Martin, J.F. 1972. Effects of Hydroxylamine on Photosystem II. Plant Physiology. 50:87-94.
4. Kolling, D.R.J., Cox, N. Ananyev, G.M., Pace, R.J., Dismukes, G.C. 2012. What Are the Oxidation States of Manganese Required To Catalyze Photosynthetic Water Oxidation? Biophysical Journal. 103:1-10.

LIGHT HARVESTING AND ENERGY TRANSDUCTION MODULE OF THE CPCG2-PHYCOBILISOME AND PHOTOSYSTEM I

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[b]Photosynthetic Antenna Research Center, Washington University in St. Louis, St. Louis, MO 63130
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Phycobilisomes (PBS) capture light energy and transmit it into reaction centers in cyanobacteria and red algae. Previous studies indicated that two distinct forms of PBS are assembled with different CpcG copies, which have been referred to as the "rod-core linker", in the cyanobacterium *Synechocystis* sp. PCC 6803. CpcG1-PBS is considered to be the conventional PBS supercomplex, while CpcG2-PBS is a rod-like phycobilisome that is hypothesized to connect to the reaction center and/or thylakoid membrane directly through CpcG2 linker protein. Although the energy transfer from CpcG2-PBS to reaction centers has been established recently, little is known about the structural interface between this type of PBS and the thylakoid membrane and reaction centers. We have isolated a complex composed of CpcG2-PBS with its energy acceptors in a PBS core mutant (DeltaAB) by using *in vivo* chemical cross-linking. Steady-state fluorescence spectroscopy analysis indicated efficient excitation energy transfer from CpcG2-PBS to photosystem I (PSI), but not to photosystem II (PSII). Mass spectrometry analysis identified only rod components, CpcG2, and ferredoxin $NADP^+$ oxidoreductase (FNR) in isolated CpcG2-PBS. Detailed mass spectrometry analysis is in progress with the aim of elucidating the structural interface between CpcG2 and PSI. Phycobilisome-dependent state transitions in the core mutant and wild type will be compared and discussed.

Acknowledgements: We thank Dr. G. Ajlani for the DeltaAB strain. The research was supported by the Photosynthetic Antenna Research Center (PARC), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Basic Energy Science (Grant No. DE-SC 0001035 to R.E.B) and National Institute of General Medical Science (Grant No. 8 P41 GM103422-35 to M.L.G).

Postdoctoral Presenter

REGULATION OF CYCLIC ELECTRON FLOW IN *CHLAMYDOMONAS REINHARDTII* UNDER FLUCTUATING CARBON AVAILABILITY

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The algal CO_2 concentrating mechanisms (CCM) actively accumulates CO_2 in the form of bicarbonate, increasing the ATP/NADPH ratio required to fix CO_2, but overcoming CO_2 diffusional limitations. To be efficient, the CCM must be dynamically regulated to meet rapidly changing C availability. In addition, the photosynthetic reactions must be regulated to accommodate the resulting alteration in demands for ATP:NADPH. Exactly how much extra energy CCM costs and how the photosynthetic apparatus is regulated to provide it are unknown. Complicating matters, algae have the capacity to utilize exogenous sources of organic carbon for energy, potentially altering cellular energy balance. How this dynamic balancing of the energy budget is achieved is unknown. To approach this problem, we adapted non-invasive spectroscopic techniques developed for higher plants to assess the impact of inorganic and organic carbon availability on the photosynthetic energy budget in the green algae *Chlamydomonas reinhardtii,* under rapidly changing carbon. We showed that cyclic electron flow (CEF), which is thought to augment the production of ATP by shuttling electrons around Photosystem I, can account for the additional ATP production required for CEF. Our measurements point to a CCM-related increase in ATP production through CEF of approximately one additional ATP per CO_2 fixed, consistent with recent models for CCM. It was previously proposed that CEF is regulated by antenna state transitions, but our results show that state transitions are far too slow to account for regulation of CEF. Instead, our results are consistent with a rapid redox-switch to regulated CEF.

Measurements and algal cultivation were supported by U.S. Department of Energy Office of Biomass Program grant DE-EE0003046 and the development of the spectrophotometer and related techniques by Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the US Department of Energy Grant DE-FG02-91ER20021.

Postdoctoral Presenter

NANOSECOND TIME-RESOLVED ABSORPTION SPECTROSCOPY FOR THE STUDY OF LIGHT INDUCED ELECTRON TRANSFER PROCESSES IN *MENB* PHOTOSYSTEM I

Hiroki Makita and Gary Hastings
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Wild type and *menB* mutant photosystem I (PSI) particles from *Synechocystis* sp. 6803 have been studied using nanosecond time-resolved absorption spectroscopy at room temperature. In WT/*menB* PSI the secondary electron acceptor, A_1, is a phylloquinone/plastoquinone species, respectively. Following laser excitation of *menB* PSI a ~50 ns decay phase is observed (at λ_p = 703 and 800 nm). Such a decay phase is not observed in wild type PSI particles. Upon consideration of absorption changes at 510, 703 and 800 nm, on a nanosecond to millisecond timescale, as well as the absorption changes observed in measurements under several sets of conditions (mediator concentration, excitation laser repetition rate), the most likely origin of the ~50 ns decay phase in *menB* mutant PSI is that it is due to the formation of ^{3}P700, which then decays in a few microseconds. This conclusion suggests some inability of plastoquinone to function effectively in forward electron transfer in *menB* PSI.

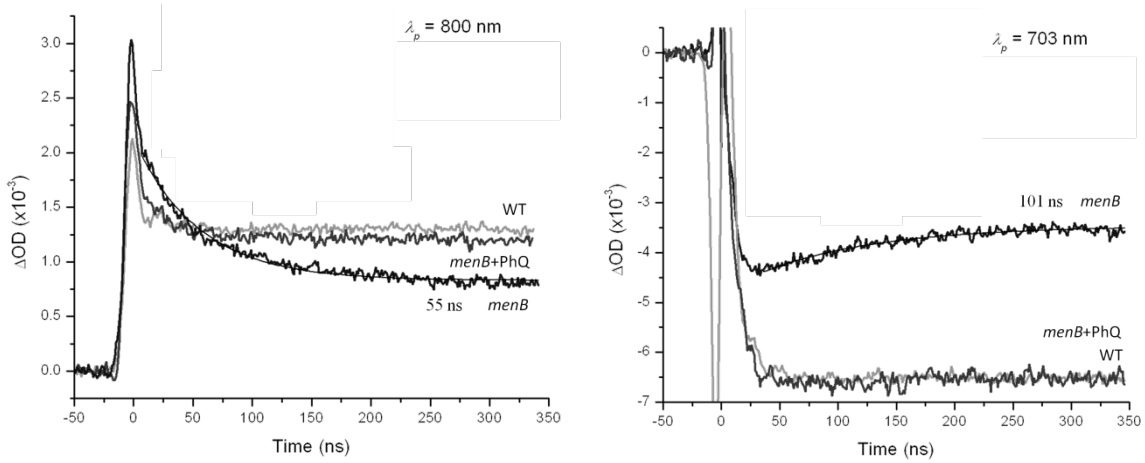


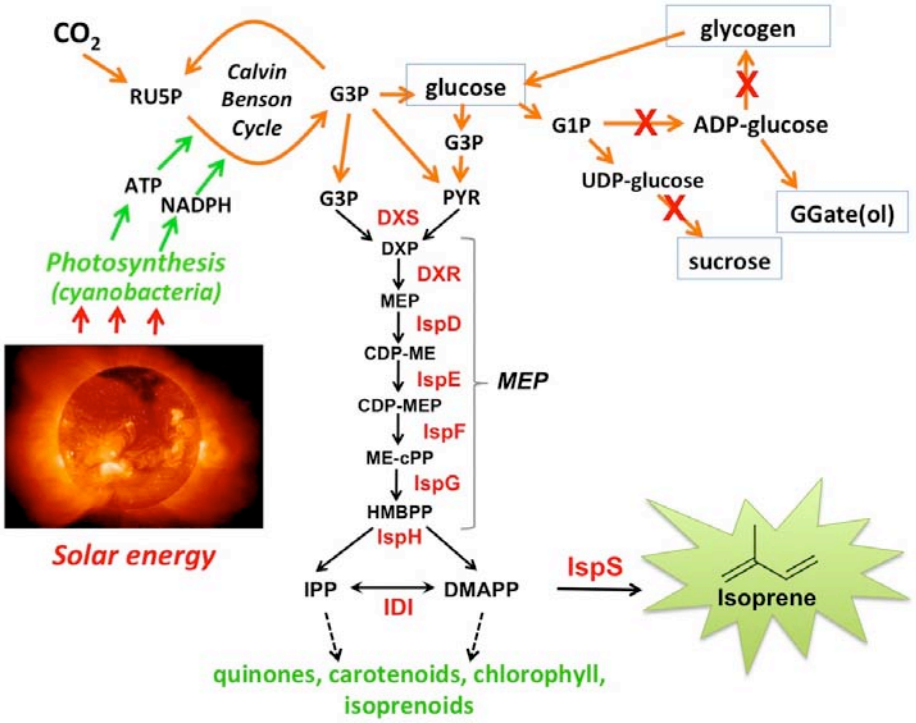
Figure: Absorbance change at 800 (*left*) and 703 (*right*) nm. At both wavelengths *menB* PSI particles exhibit a nanosecond decay phase that is not present in WT.

Graduate Student Presenter

ENHANCED PRODUCTION OF ISOPRENOID HYDROCARBONS IN *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA

Matthew E. Nelson[1,3], Olalekan Aremu[1], Brandon Thomas[1], Andrea Felton[1], Brandon Brummeyer[1], Rhiannon Carr[1], Shona Duncan[2], Eric Singsaas[2,3] and Toivo Kallas[1,3]
[1]Department Biology, Univ. Wisconsin Oshkosh, [2]Wisconsin Institute for Sustainable Technology, and UW-Stevens Point, [3]Algoma Algal Biotechnology LLC, Oshkosh, WI

Development of carbon-neutral bioproducts and biofuels is imperative for sustainable economic development, global ecology, and national security. We have introduced optimized isoprene synthase and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway genes into fast-growing, *Synechococcus* PCC 7002 cyanobacteria and produced isoprene (a C_5H_8 precursor for synthetic rubber and advanced biofuels) at rates ~80 times higher than those previously published. A MEP pathway isopentenyl isomerase gene increased isoprene production by ~15-fold, demonstrating that modifications of this pathway can substantially increase yields. Toward further improvement, we have 1) synthesized additional MEP pathway genes, 2) inactivated genes for glycogen synthesis, and are 3) down-regulating light-harvesting capacity for increased cell density, and 2) pursuing regulated gene expression to mitigate inhibitory effects. Inactivation of glycogen synthesis resulted in only a modest increase in isoprene, suggesting that carbon may be diverted to soluble sugars. Thus further modifications will be needed to funnel additional carbon toward isoprenoids. *Synechococcus* PCC 7002 grows well under 100% CO_2, full sunlight, and in varied salt concentrations, indicating that wastewaters and industrial gas effluents may be used as nutrient and CO_2 sources. A Phenometrix photobioreactor and real-time isoprene sensor are being used to optimize culture conditions for isoprene productivity. A patent application (13/952,071) has been filed on the cyanobacterial isoprene work.



Methyl-D-erythritol-4-phosphate (MEP) -- isoprene synthesis and competing carbon pathways.

BLUE-GREEN CHROMATIC ACCLIMATION IN MARINE *SYNECHOCOCCUS* IS CONTROLLED BY TWO PUTATIVE ARAC-CLASS TRANSCRIPTION FACTORS

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[c]CNRS, UMR 7144 Adaptation et Diversité en Milieu Marin, Groupe Plancton Océanique, 29680 Roscoff, France

Cyanobacteria of the genus *Synechococcus* are ubiquitous in oceans and are responsible for a significant portion of the primary productivity and oxygen production on Earth. These organisms contain phycobilisomes, which contain phycobiliproteins to which light harvesting molecules, known as chromophores, are attached. Some marine *Synechococcus* undergo blue-green chromatic acclimation, adjusting the composition of their phycobilisomes to optimize photon capture to drive photosynthesis in different light conditions.

During this type of chromatic acclimation, different chromophores are reversibly attached to three different positions on phycobiliproteins. Blue light absorbing chromophores and green light absorbing chromophores are attached in their respective light conditions. Part of this process is controlled by the lyase-isomerase MpeZ, which converts a green light absorbing chromophore into a blue light absorbing chromophore and attaches it to one specific phycobiliprotein site. The transcript levels of *mpeZ* are higher in blue light than green light.

Using a reverse genetic approach, we have identified two genes encoding putative AraC-family transcription factors whose presence in a number of *Synechococcus* strains is strongly correlated with the ability to undergo blue-green acclimation. These genes are contiguous in all of the genomes in which they occur and appear to form an operon. The interruption of each of these genes leads to a complete loss of this response, as judged by changes in phycobilisome composition. However, the phenotypes of two mutant classes are completely opposite from each other. An interruption in the first of these two genes results in mutant cells that behave as if they are always in green light, regardless of the ambient light color. Interruption of the second gene results in mutant cells that act as if they are always in blue light. Our results provide important genetic and biochemical information about the signal transduction pathway controlling the globally important process of blue-green chromatic acclimation in marine *Synechococcus*.

Graduate Student Presenter

CHARACTERIZATION OF 4(1H)-QUINOLONE (4-1HQ) AND 2-NONYL-1-HYDROXY-4(1H)-QUINOLONE (NQNO) AS INHIBITORS OF PHOTOSYNTHETIC ELECTRON TRANSFER AND SIGNALING

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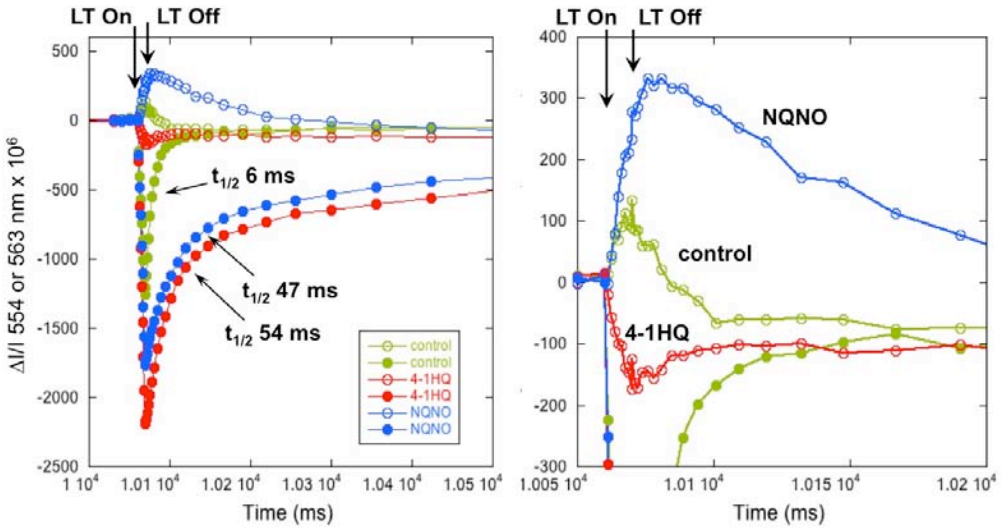
Energy conversion by the cytochrome *bf* complex involves two binding sites for quinones (Q_p and Q_n), on the positive and negative sides of the membrane, respectively, with corresponding high- and low-potential electron transfer chains. Inhibitors are important for investigating such reactions but few are available for cytochrome *bf* complexes. 2-nonyl-1-hydroxy-4(1H)-quinolone (NQNO) binds the quinone-reductase (Q_n) site and slows electron flow through the low-potential chain, but is not available commercially. We have synthesized NQNO and a related precursor, 4(1H)-quinolone (4-1HQ), and used a BioLogic JTS-10 kinetics spectrophotometer to investigate the their impacts on electron transfer in *Synechococcus* sp. PCC 7002 cyanobacteria. In contrast to NQNO, 4-1HQ resulted initially (within ~50 ms of illumination) in oxidation, rather than reduction, of the low-potential chain. Both NQNO and 4-1HQ slowed electron flow through the *bf* complex (measured as cytochrome f/c_6 re-reduction) after short (9 ms) illumination. However, after longer illumination (e.g. 10 sec) only 4-1HQ remained effective as an inhibitor of cytochrome *bf* turnover. In contrast, NQNO became ineffective as an inhibitor of steady-state electron flow although the low-potential chain (*b*-hemes) remained reduced. These data indicate that 4-1HQ binds the cytochrome *bf* Q_p-site and inhibits quinol oxidation at this site rather than quinone reduction at the Q_n site. The initial inhibition by NQNO of electron flow through the *bf* complex, and subsequent loss of inhibition, suggests that NQNO binding to the Q_n-site, or reduction of the low-potential chain, elicits a conformational change that renders NQNO ineffective. This has interesting implications for electron transfer and signaling reactions mediated by the cytochrome *bf* complex, and for formation of supercomplexes with photosystem I or other proteins.



NQNO and 4-1HQ – Impacts on cytochrome *bf* redox kinetics – 9 ms illumination.

Undergraduate Student Presenter

ENGINEERING QUANTUM COHERENCE IN SYNTHETIC HETEROTRIMERIC CHROMOPHORE SYSTEMS

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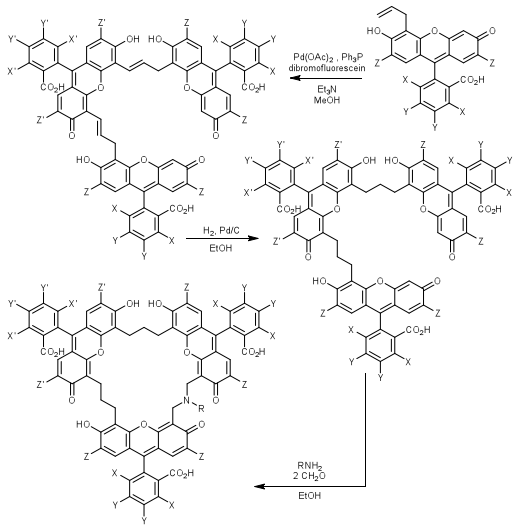
Long-lived quantum coherences in photosynthetic antenna complexes have been the subject of many experimental and theoretical studies seeking to examine their roles in energy transfer processes. However, the delicate structures of antenna proteins, their multifaceted energy transfer dynamics and complex spectral features complicate efforts to isolate the variables that contribute to energy transfer in these systems. Recent research efforts have turned to synthetic systems with relatively simple and controllable structures. Electronic coherences lasting for at least 300 fs have been observed in two-dimensional spectra of man-made fluorescein-based heterodimer systems, proving that quantum coherence can be engineered. However, to observe coherent energy transfer, a three state system is needed. Synthetic heterotrimeric fluorescein compounds have the advantage of energy tunability across the visible spectrum and can be extended to complex chromophore networks. By using 2D electronic spectroscopy to investigate their dynamics, they can provide a platform for understanding photosynthetic energy transfer and quantum information. We will explain the strategy for isolating coherent dynamics inspired by photosynthesis in small molecular systems.



Synthetic route of cyclic fluorescein heterotrimer. X, Y, Z and X', Y', Z' represent different substituents.

Graduate Student Presenter

REGULATION OF PHYCOERYTHRIN AND PHYCOCYANIN BY A CONSERVED BLUE-GREEN LIGHT RESPONSIVE CYANOBACTERIOCHROME IN A RED-GREEN ACCLIMATING CYANOBACTERIUM.

Lisa Wiltbank and David M. Kehoe. Department of Biology Indiana University, Bloomington, Indiana

The important light-harvesting phycobilisomes of the cyanobacterium, *Fremyella diplosiphon*, can change depending upon environmental conditions, such as nutrient deprivation, cellular differentiation, and light availability. One dramatic example is type III chromatic acclimation, during which cells are able to optimize light absorption in response to changes in available red and green light by changing levels of phycoerythrin (PE) and phycocyanin (PC). RcaE is a red-green light responsive photoreceptor that has a significant role in controlling this light color switch. Here we present data showing that a blue-green light responsive cyanobacteriochrome also affects expression of PE and PC, adding a new piece to the already-complex puzzle of light acclimation in *Fremyella diplosiphon*.

This cyanobacteriochrome, DpxA, contains a GAF domain and a kinase domain and is in an operon with genes encoding for a putative single domain response regulator and a putative histidine kinase with two receiver domains and two PAS domains. We have named this the dpx operon for its role in the decrease in phycoerythrin expression. Homologues of the dpx operon are present in at least 15 other cyanobacterial species, including several that do not produce PE, suggesting that this group of regulatory genes controls multiple processes in cyanobacteria. To this end, we are investigating a change in cell morphology that may be controlled by the dpx operon.

We have demonstrated that the GAF domain of DpxA binds a chromophore and switches between a green-absorbing form with a maximum absorbance of 567 nm and a blue-absorbing form with a maximum absorbance of 494 nm. An in-frame, clean deletion of *dpxA* results in a mutant with increased levels of PE and decreased PC. Current work will determine the role of DpxA's blue-green light sensing capability in its ability to repress PE synthesis. We are also interested in discovering the role of DpxB and DpxC, which are also in this conserved operon and have a similar color phenotype to DpxA.

Graduate Student Presenter

CONSTRUCTION OF A CHASSIS STRAIN OF *SYNECHOCOCCUS* SP. PCC 7002 FOR STRUCTURE-FUNCTION STUDIES OF PHOTOSYSTEM II

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Some strains of Cyanobacteria have long been used as models both for basic research and application-based research. While *Synechocystis* sp. PCC 6803 has been more used, less explored *Synechococcus* sp. PCC 7002 may be a better alternative for several reasons. Here, we use similar strategy as what have been done in *Synechocystis* sp. PCC 6803 to delete three *psbA* genes and generate His-tagged *psbB* gene in *Synechococcus* sp. PCC 7002, but instead of using one antibiotic for each gene manipulation, we tried to employ *sacB* counterselection to recycle antibiotics after confirming the completion of segregation. To do this, two plasmids, pRL277 and pRL278, which have Sp^R and Km^R cassette adjacent to s*acB* gene, respectively, are used. We also use Gibson Assembly to generate similar constructs for other antibiotics, and these fragments and upstream and downstream homologous fragments are assembled using Gibson Assembly or Fusion PCR. The full-length synthetic DNA fragments are introduced into *Synechococcus* sp. PCC 7002 by natural transformation. We have got individual mutants transformed with the four constructs, and physiological characterization indicates that SYNPCC7002_A1418 is the most abundantly expressed *psbA* gene under the conditions used. However, the *sacB* counterselection has been proved to not work for *Synechococcus* sp. PCC 7002. So, we have started to use a newly developed counterselection strategy to construct the strain. The resulting chassis strain with three *psbA* deletions and His-tagged *psbB* will play an important role in the elucidation of the mechanism of PSII.

References

[1] Debus, R.J., et al., *Directed mutagenesis indicates that the donor to $P680^+$ in Photosystem II is tyrosine-161 of the D1 polypeptide.* Biochemistry, 1988. **27**(26): p. 9071-9074.
[2] Begemann, M.B., et al., *An organic acid based counter selection system for cyanobacteria.* PLOS ONE, 2013. **8**(10): p. e76594.
[3] Ludwig, M. and D.A. Bryant, *Transcription profiling of the model cyanobacterium Synechococcus sp. strain PCC 7002 by Next-Gen (SOLiD) sequencing of cDNA.* Front Microbiol, 2011. **2**: p. 41.

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Graduate Student Presenter

EXPRESSION OF FENNA-MATTHEWS-OLSON PROTEIN IN *RHODOBACTER CAPSULATUS*

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The Fenna-Matthews-Olson (FMO) protein is an attractive subject for study as it transfers energy collected by the chlorosome complex to the reaction center in green sulfur bacteria with extreme efficiency. However, a detailed mechanism of this energy transfer still remains unclear due to difficulties with the mutagenesis of the *fmoA* gene. Thus, *Rhodobacter capsulatus* is an ideal candidate for heterologous expression of this protein since it contains the pigments required for the assembly of the FMO protein, does not require the protein for proper metabolism and is available for mutagenesis. Tri-parental mating was used to introduce the pUCA10 plasmid that contains *puc* promoter and C-terminally His-tagged *fmoA* gene to the *R. capsulatus* cells. Cells were grown non-photosynthetically and harvested after 48 hours. His-tagged FMO protein was purified through nickel affinity chromatography followed by gel filtration. The pure protein was identified with SDS-PAGE and western blot using anti-FMO antibodies. The SDS-PAGE showed single bands after gel-filtration, and the protein sequence was subsequently confirmed with mass spectrometry. From the protein sequence test, we know the correct protein is being synthesized in *R. capsulatus*, however determination of proper assembly of the protein with the pigment molecules is still required.

Graduate Student Presenter

PROTEASE INACTIVATION FOR ISOLATION OF A FUNCTIONAL CYTOCHROME *BF* COMPLEX FROM *SYNECHOCOCCUS* SP. PCC 7002 CYANOBACTERIA

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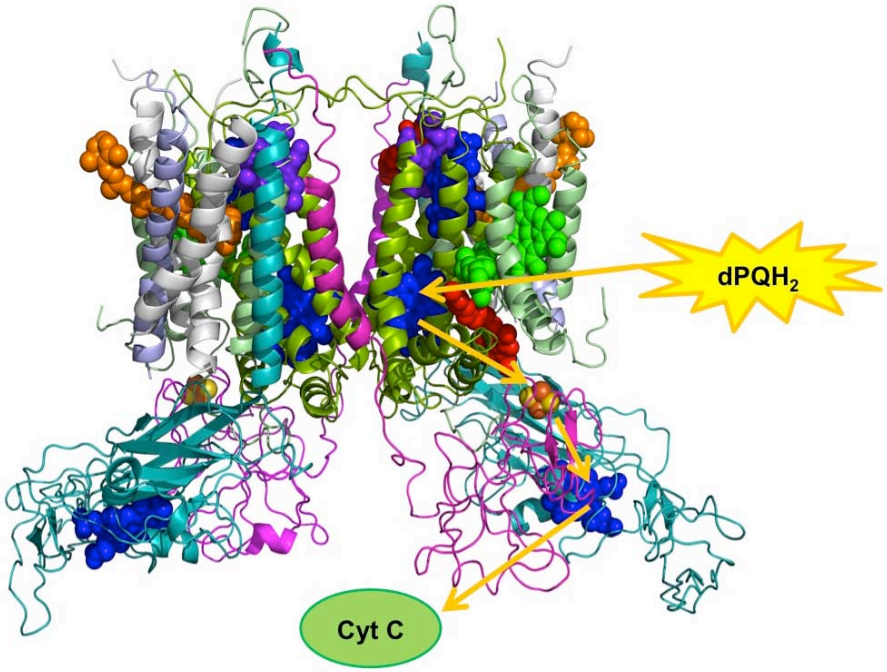
Photosynthesis is a vital biological process that produces nearly all of the organic matter and oxygen for life on earth. In this process, reaction center protein complexes harvest solar energy, exciting electrons to travel down an electron transport chain via oxidation-reduction reactions. A central component is the cytochrome (Cyt) *bf* complex, which is involved in linear and cyclic electron flow, and regulation of photosynthesis. A key strategy for investigating protein complexes is to isolate them from cells to study reaction mechanisms and structures in vitro. Functional Cyt *bf* complexes have not been isolated from the cyanobacterium *Synechococcus* PCC 7002, which is otherwise ideally suited for genetic and biochemical studies. This organism is thought to produce proteases that inactivate the dimeric, Cyt *bf* complex during isolation resulting in nonfunctional monomers. Thus, *Synechococcus* mutants were created that lack two putative proteases, ZDP and GlmS, that are absent from filamentous cyanobacteria such as *Mastigocladus laminosus*, from which active Cyt *bf* complexes can be isolated. The Cyt *bf* complex was isolated from these mutants and a simple kinetics assay used to determine activity. Inactivation of the proteases did not allow retention of Cyt *bf* activity. However, the activity assay that was developed that will aid future efforts to isolate functional Cyt *bf* complexes.



In vitro assay for activity of the cytochrome *bf* complex. Decyl-plastoquinol ($dPQH_2$) was the electron donor and *Synechococcus* 7002 cytochrome c_6 or horse-heart cytochrome *c* the electron acceptor. The structure shown is that of the *Mastigocladus laminosus* cytochrome *bf* complex based on structures 1VF5 (Kurisu et al., 2003) and 2E75 and 2E76 (Yamashita et al., 2007).

Graduate Student Presenter

AN ONIOM STUDY OF THE HYDROGEN BONDS TO UBIQUINONE IN THE Q_A BINDING SITE IN PURPLE BACTERIAL PHOTOSYNTHETIC REACTION CENTER

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The nature of hydrogen bonding to pigments in protein complexes is currently a topic of some debate. The debate centers on whether hydrogen bonds can be understood on purely electrostatic grounds, or whether they need to be considered quantum mechanically. This distinction is of current relevance, primarily because of the application of QM/MM computational methods to the study of biological problems. To address this problem we have used QM/MM methods to study the neutral state of the hydrogen bonded ubiquinone molecule termed Q_A that functions as an electron transfer cofactor in purple bacterial photosynthetic reaction centers. In these calculations we have treated the hydrogen bonding amino acids either quantum mechanically or using molecular mechanics methods. As a specific metric for comparing the different computational methods isotope edited FTIR difference spectra are calculated. The calculated spectra are in remarkable agreement with experimental spectra, and it is found that the calculated spectra are very similar when hydrogen bonding amino acids are treated using either QM or MM methods. The latter result suggests that hydrogen bonding to neutral ubiquinone in purple bacterial reaction centers can be considered in purely electrostatic terms, which is contrary to the widely held belief that the hydrogen bonding amino acids should be treated quantum mechanically. Natural bond orbital analysis is used to further verify that the hydrogen bonds are predominantly electrostatic in nature. Calculated bond lengths and vibrational frequencies of the N-H groups involved in hydrogen bonding are used to estimate the relative strengths of the hydrogen bonds to either UQ carbonyl group.

Graduate Student Presenter

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