

**36TH ANNUAL
MIDWEST/SOUTHEAST
PHOTOSYNTHESIS
MEETING**

**Turkey Run State Park
Marshall, Indiana**

October 29 - 31, 2010

PROGRAM AND ABSTRACTS

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Organizers:

**Greg Engel
Chemistry Department
The James Franck Institute
929 East 57th Street
Chicago, IL 60637
(773) 834-0818
gsengel@uchicago.edu**

**Toivo Kallas
Department of Biology &
Microbiology
University of Wisconsin
Oshkosh, WI 54901
(920) 424-7084
kallas@uwosh.edu**

**(Cover: Isabella River, Boundary Waters Canoe Area, Minnesota, June 2010,
photo by Toivo Kallas)**

Arrival

4:00 – 6:30 PM **Check-in**

6:30 – 7:15 **Dinner**

Opening Session: Friday Evening, October 29

7:20 PM Opening Remarks and Welcome: Greg Engel and Toivo Kallas

7:30 PM Keynote Speaker: Krishna K. Niyogi
University of California, Berkeley

REGULATION OF PHOTOSYNTHETIC LIGHT HARVESTING

8:30 PM Guest Lecture: Kevin Redding
Arizona State University

FUNCTION AND EVOLUTION OF TYPE I REACTION CENTERS

9:00 PM Robin S. Sibert, Mira Josowicz, and Bridgette A. Barry,
Georgia Institute of Technology

CONTROL OF PROTON AND ELECTRON TRANSFER IN DE NOVO DESIGNED, BIOMIMETIC BETA HAIRPINS

9:20 PM **MIXER, POSTER MOUNTING AND VIEWING**

Session II: Saturday Morning, October 30 Energy Transfer I

9:00 AM Warren F. Beck and Kevin L. Dillman
Michigan State University

**INTERMOLECULAR VIBRATIONAL COHERENCE IN
BACTERIOCHLOROPHYLL AND Zn(II)-PORPHYRIN SYSTEMS:
IMPLICATIONS FOR THE STRUCTURAL BASIS FOR QUANTUM
EFFICIENCY IN PHOTOSYNTHETIC REACTION CENTERS**

9:25 AM Nan Zhao and Gary Hastings
Georgia State University

**TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY FOR THE STUDY OF
A₁, THE SECONDARY ELECTRON ACCEPTOR IN PHOTOSYSTEM I**

9:50 AM Dugan Hayes and Gregory S. Engel
University of Chicago

**EXTRACTING THE EXCITONIC HAMILTONIAN OF THE FENNA-
MATTHEWS-OLSON COMPLEX USING THREE DIMENSIONAL
THIRD-ORDER ELECTRONIC SPECTROSCOPY**

10:15 AM Coffee/Tea Break

Session III: Saturday Morning, October 30

Regulation and Environmental Responses

10:45 AM April Nesbit, Andrian Gutu, Craig Whippo, David M. Kehoe
Indiana University

ROLE OF TRANSLATION INITIATION IN REGULATION OF PHYCOBILISOME BIOSYNTHESIS

11:10 AM George L. Weir, Kraig Short, Matthew Nelson, Alina Ott, David R.
Rivera Aponte, Justin Zangel and Toivo Kallas
University of Wisconsin-Oshkosh, & UW Stevens Point

REDOX SENSING-SIGNALING OF GENE EXPRESSION IN CYTOCHROME *bf* LOW- AND HIGH- POTENTIAL CHAIN MUTANTS OF *SYNECHOCOCCUS* SP. PCC 7002

11:35 AM Junlei Sun, Linda K. Vuorijoki, George L. Weir IV, Matthew Nelson,
Patrik R. Jones, John H. Golbeck and Toivo Kallas
Pennsylvania State University, University Turku, & UW-Oshkosh

IRON-STRESS IN *SYNECHOCOCCUS* SP. PCC 7002: 520 NM ELECTROCHROMIC SIGNAL ASSOCIATED WITH ISIA AND ROLES OF ISCA AND SUFA

12:00 LUNCH

SATURDAY AFTERNOON

1:00 PM LEISURE TIME

4:00 – 6:00 PM POSTER SESSION

6:30 PM DINNER

Session IV: Saturday Evening, October 30

Cofactors and Protein Complexes

7:30 PM Shulu Zhang, Laurie K. Frankel and Terry M. Bricker
Louisiana State University

**THE SII0606 PROTEIN IS REQUIRED FOR PHOTOSYSTEM II
ASSEMBLY/STABILITY IN THE CYANOBACTERIUM
SYNECHOCYSTIS sp. PCC 6803**

7:55 PM Paweł Zatwarnicki, Wojciech Białek, Matthew Nelson, Andrzej
Szczepaniak and Toivo Kallas
University of Wroclaw & University of Wisconsin-Oshkosh

**STRUCTURES, PROPERTIES AND POSSIBLE FUNCTIONS OF c_6 -LIKE
CYTOCHROMES OF THE CYANOBACTERIUM
SYNECHOCOCCUS SP. PCC 7002**

8:20 PM Haijun Liu, Johnna L. Roose, Jeffrey C. Cameron, Himadri B. Pakrasi
Washington University

**ISOLATION AND CHARACTERIZATION OF TWO CONSECUTIVE PSII
ASSEMBLY INTERMEDIATES IN *SYNECHOCYSTIS* SP. PCC 6803**

8:45 PM Tina M. Dreaden, Jun Chen, Sasha Rexroth, and Bridgette A. Barry
Georgia Institute of Technology

**A NEW CHROMOPHORE IN THE CP43 SUBUNIT OF
PHOTOSYSTEM II: *N*-FORMYLKYNURENINE**

9:10 PM **MIXER, POSTER VIEWING**

Session V: Sunday Morning, October 31 Energy Transfer II

9:00 AM Hari P. Lamichhane and Gary Hastings
Georgia State University

ONIOM METHOD FOR THE EXPLORATION OF DOUBLE DIFFERENCE SPECTRA OF UBIQUINONE IN THE QA BINDING SITE OF RB. SPHAEROIDES

9:25 AM Kristin L. M. Lewis, Jeffrey A. Myers, Franklin Fuller, Patrick F.
Tekavec, Charles F. Yocum, Jennifer P. Ogilvie
University of Michigan

TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY OF THE D1-D2- CYT B559 REACTION CENTER COMPLEX: EXPERIMENTS AND MODELING

9:50 AM Elad Harel, Phil Long, Andrew Fidler, and Gregory S. Engel
University of Chicago

GRAPE SPECTROSCOPY: A NEW APPROACH TO REAL-TIME FEMTOSECOND 2D SPECTROSCOPY OF PHOTOSYNTHETIC LIGHT HARVESTING COMPLEXES

10:15 AM **Coffee/Tea Break**

Session VI: Sunday Morning, October 31

Electron and Proton Transfer

10:45 AM Adam R. Offenbacher, Jun Chen, Bridgette A. Barry
Georgia Institute of Technology

PROTEIN STRUCTURAL DYNAMICS LINKED WITH REDOX CHANGES AND METAL ASSEMBLY IN RIBONUCLEOTIDE REDUCTASE

11:10 AM Saurabh Ranade, Kenneth Toson, Matthew Nelson and Toivo Kallas
University of Wisconsin-Oshkosh

KINETICALLY COUPLED CYTOCHROME b_6 IN AN UNUSUAL CYTOCHROME- f DEFICIENT PETC2-RIESKE CYTOCHROME bf COMPLEX OF *SYNECHOCYSTIS* SP. PCC 6803

11:35 AM Xinliu Gao, Yueyong Xin, Jianzhong Wen, Patrick D. Bell
and Robert E. Blankenship
Washington University

ALTERNATIVE COMPLEX III IN THE ELECTRON TRANSFER CHAIN OF THE PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

12:00 **PRESENTATION OF AWARDS AND CLOSING REMARKS**

Oral presentation Abstracts

(In order of presentation in the program)

OPENING SESSION: FRIDAY, October 29

Keynote Lecture

REGULATION OF PHOTOSYNTHETIC LIGHT HARVESTING

Krishna K. Niyogi

Department of Plant and Microbial Biology, 111 Koshland Hall, University of California,
Berkeley, CA 94720-3102, USA

Plants and algae need light for photosynthesis, but the intensity of sunlight can vary over several orders of magnitude and on timescales ranging from seconds to seasons. To optimize utilization of available light in different environments, photosynthetic organisms have evolved various ways of sensing and responding to changes in light intensity. In response to rapid fluctuations in light intensity, a nonphotochemical quenching (qE) mechanism that regulates photosynthetic light harvesting is induced rapidly by changes in thylakoid lumen pH. qE dissipates excess absorbed light energy via a mechanism that involves chlorophyll and xanthophyll pigments. In plants, the PsbS protein associated with photosystem II appears to sense lumen pH and turn on qE in specific antenna proteins. In the green alga *Chlamydomonas reinhardtii*, qE occurs by a similar biophysical mechanism but involves different antenna proteins.

Guest Lecture

FUNCTION AND EVOLUTION OF TYPE I REACTION CENTERS

Kevin Redding

Arizona State University, 1711 S. Rural Rd., Tempe, Arizona 85287-1604, U.S.A.

All photosynthetic reaction centres (RCs) share a common structural theme, in which two membrane-embedded polypeptides come together to form a cage of 10 transmembrane α -helices (5 from each subunit) around the cofactors involved in charge separation and electron transfer. Because of this dimeric structure, there are 2 branches of cofactors; on the donor side is a pair of chlorins (bound symmetrically by both polypeptides), followed by two branches arranged symmetrically about a C₂ axis, consisting of a pair of chlorins and a quinone. All type 2 RCs are heterodimeric and use one of the quinones as a terminal electron acceptor; the other branch is specialized for charge separation. There are now 4 known type I RCs: Photosystem I (PSI), the Heliobacterial RC, the Chlorobial RC, and the Chloroacidobacterial RC. Despite PSI being considered the ‘progenitor’ of the type I RCs, it is decidedly distinct from them, and may in fact be even more modified, in terms of the basic charge separation mechanism, than Photosystem II is from the bacterial type II RCs. For example, all the anoxygenic type I RCs are homodimeric. Moreover, recent evidence strongly suggests that, despite the presence of quinones in these RCs, the quinone is not used as an obligatory ET intermediate, as it is in PSI.

In this talk, I will summarize some basic similarities and differences between PSI with the anoxygenic type I RCs. These similarities include: (1) the use of the 13² stereoisomer in the special pair, (2) use of a Chl *a*-like molecule as the primary acceptor, (3) inclusion of naphthoquinones, (4) the F_X Fe₄S₄ cluster at the dimer interface, (5) the terminal F_A/F_B clusters bound by an extrinsic subunit, and (6) the similar kinetics for the initial charge separation reactions. Differences between PSI and the other type I RCs include: (1) very different kinetics for the secondary electron transfer reactions, (2) different antenna sizes and use of different pigments, and (3) less tightly bound quinones and terminal FeS cluster proteins in the anoxygenic RCs. Based partially on some of our recent research on PSI and the heliobacterial RC, I will discuss potential reasons for underlying mechanistic differences between the type I RCs, and a scheme for how the type I RCs may have evolved.

CONTROL OF PROTON AND ELECTRON TRANSFER IN DE NOVO DESIGNED, BIOMIMETIC BETA HAIRPINS

^{1,2}Robin S. Sibert, ¹Mira Josowicz, and ^{1,2}Bridgette A. Barry

¹Department of Chemistry and Biochemistry and the ²Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332

Redox active tyrosine residues catalyze coupled proton and electron transfer (PCET) in several enzymes, including photosystem II (PSII). PSII contains two redox active tyrosines, TyrD and TyrE, with different midpoint potentials and roles in catalysis. To serve as an experimentally tractable model for investigating the effect of the protein environment on PCET, we designed a novel 18-amino acid beta hairpin peptide (Peptide A, IMDRYRVRNGDRIHRLR (Sibert et al. *J. Am. Chem. Soc.* **2007**, 129, 4393). Peptide A contains one tyrosine residue. The NMR structure shows a cross-strand aromatic interaction between Tyr 5 and His 14, a hydrogen bond between Tyr 5 and Arg 16, and a possible pi-cation interaction between Tyr 5 and Arg 12. In our previous study, electrochemical titration showed that Tyr 5 and His 14 are involved in PCET reactions. Yet, the role of the Tyr 5-Arg 16 hydrogen bond and the Tyr 5-Arg 12 pi-cation remained unclear. Comparison of the protein environments of TyrZ and TyrD suggested that Tyr-Arg pi-cation interactions may distinguish the two sites. Therefore, we designed three additional beta hairpin peptides to examine the role of arginine in tyrosine oxidation (Sibert et al. *ACS Chem. Bio.* **2010**, in press). In these peptides, the cross-strand histidine and/or the pi-cation arginine residue is replaced with a neutral, hydrophobic amino acid side chain. In a fourth peptide, we replaced the hydrogen bonded arginine with a neutral hydrophobic amino acid side chain. Electrochemical titration of peptides C, D, E, and F supported our previous conclusion that the Tyr 5-His 14 interaction is involved in PCET reactions. Substitution of Arg 12 or Arg 16 by a hydrophobic residue increased the redox potential of Tyr 5. The increase in redox potential caused by substitution of Arg 12 is consistent with a pi-cation interaction and with the midpoint potential difference observed for TyrZ and TyrD in PSII. Optical titrations showed that removal of the Tyr 5-Arg 16 hydrogen bond altered the pK of tyrosine. Our results demonstrate that



PCET reactions, hydrogen bonding, and a pi-cation interaction alter redox-active tyrosine function. These interactions can contribute equally to the control of the redox potential of tyrosine. Supported by GM43273.

Figure. *NMR structures of peptide A at pH 5.*

(Top) Overlap of four of the lowest energy conformations. Only tyrosine and four of its vicinal amino acid side chains are shown in purple.

(Bottom) Interactions with the tyrosine, Y5, in the averaged, minimized NMR structure. Hydrogen atoms are omitted for clarity.

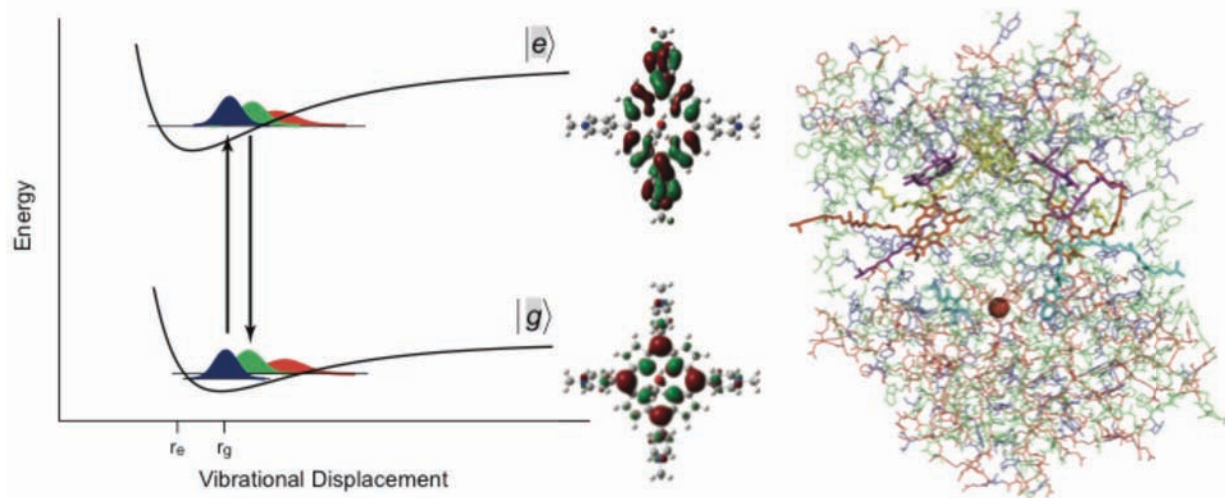
Session II: Saturday Morning, October 30

Energy Transfer

INTERMOLECULAR VIBRATIONAL COHERENCE IN BACTERIOCHLOROPHYLL AND Zn(II)-PORPHYRIN SYSTEMS: IMPLICATIONS FOR THE STRUCTURAL BASIS FOR QUANTUM EFFICIENCY IN PHOTOSYNTHETIC REACTION CENTERS

Warren F. Beck and Kevin L. Dillman, Department of Chemistry, Michigan State University, East Lansing, Michigan 48824 USA

The nature of the intermolecular vibrational modes between the redox-active chromophores and the protein medium in the photosynthetic reaction center is central to an understanding of the structural origin of the quantum efficiency of the light-driven charge-separation reactions that result in storage of solar energy. In recent work on this issue, we have characterized the low-frequency vibrational coherence of Zn^{II} *meso*-tetrakis(N-methyl-pyridyl)-porphyrin (ZnTMPyP) and compared it to that from bacteriochlorophyll *a* in polar solution and in the small light-harvesting subunits B820 and B777 from LH1 in *Rhodospirillum rubrum* G9. The charge-transfer character of ZnTMPyP's π^* excited states afford us the opportunity to characterize how the intermolecular vibrational modes and potential with the surrounding medium are affected by the charge on the porphyrin macrocycle. The results show that charge-dependent terms in the intermolecular potential contribute to a significant stabilization of the equilibrium geometry of the porphyrin-solvent complex in the excited state. In the photosynthetic reaction center, these terms will play an important role in trapping the charged products of the forward, charge-separation reactions, and the location of the bacteriopheophytin acceptor in a nonpolar region of the structure enhances the rate of the secondary charge-separation reaction.



Ground-state and excited-state wavepacket motion is controlled in ZnTMPyP by intermolecular potentials with first-shell solvent molecules. The charge-dependent terms in the intermolecular potential play a dominant role in the forces that stabilize the charge-separated intermediates in the photosynthetic reaction center.

TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY FOR THE STUDY OF A₁, THE SECONDARY ELECTRON ACCEPTOR IN PHOTOSYSTEM I

Nan Zhao and Gary Hastings

Department of Physics and Astronomy, Georgia State University, Atlanta, GA 30303

The A₁ binding site in photosystem I (PSI) particles from *Synechocystis* sp. 6803 is occupied by a phylloquinone (PhQ) molecule (2-methyl, 3-phytyl, 1, 4-naphthoquinone). In *menB* mutant strains, the biosynthetic pathway of PhQ is blocked and a plastoquinone-9 (PQ9) molecule occupies the A₁ binding site instead of PhQ, or the binding site is empty. By incubating *menB* mutant PSI particles in a large molar excess of PhQ (or PhQ analogues), we have shown that it is possible to incorporate fully functional PhQ into the A₁ binding site. To probe the molecular properties of the introduced PhQ (or analogue) in both the neutral and anion radical states, we have been using time-resolved FTIR difference spectroscopy. These difference spectra are somewhat convoluted, with protein bands and pigment bands overlapping. To distinguish bands associated with neutral and reduced PhQ from protein bands in time-resolved FTIR difference spectra we have adopted a variety of isotope labeling strategies. Here we describe the reconstitution of unlabeled (¹²C) PhQ into the A₁ binding site in fully ¹³C labeled *menB* mutant PS I particles. We show that by comparison of FTIR difference spectra obtained for PhQ reconstituted unlabeled and ¹³C labeled *menB* PSI particles we can distinguish bands of neutral and reduced PhQ from bands of the protein. In particular we propose that negative bands at 1661/1632 cm⁻¹ are due to the non H-bonded (C₁=O) and H-bonded (C₄=O) C=O modes of neutral PhQ. A negative band at 1593 cm⁻¹ is due to a C=C mode of neutral PhQ, and a band at 1494 cm⁻¹ is due to a C=O mode of the PhQ anion. We also propose that a positive band at 1413 cm⁻¹ is due to another C=O mode of PhQ⁻.

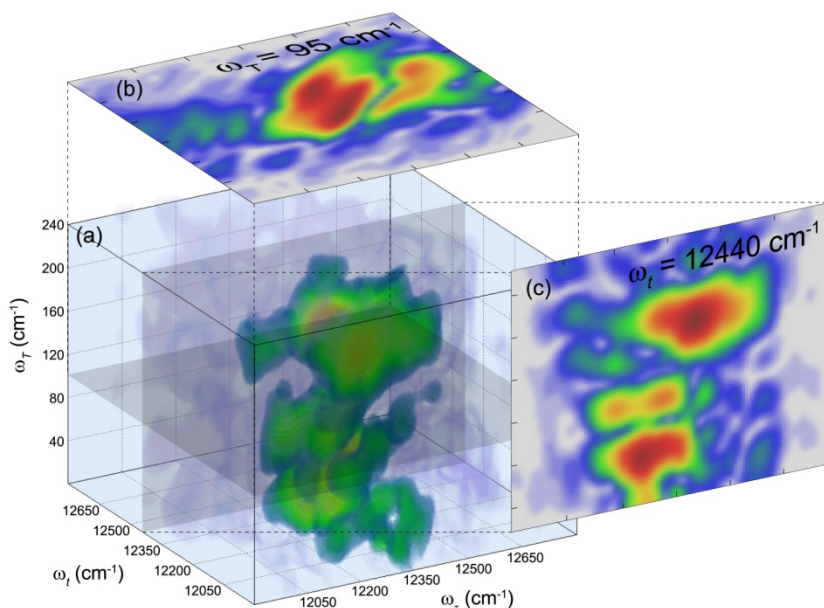
EXTRACTING THE EXCITONIC HAMILTONIAN OF THE FENNA-MATTHEWS-OLSON COMPLEX USING THREE-DIMENSIONAL THIRD-ORDER ELECTRONIC SPECTROSCOPY

Dugan Hayes and Gregory S. Engel

Department of Chemistry and The James Franck Institute, The University of Chicago,
929 E. 57th Street, Chicago, IL

Photosynthetic antennae absorb sunlight and transfer this energy to the reaction center with near-perfect quantum efficiency. The mechanism by which these antenna complexes operate with such high efficiency remains unclear, and a large body of recent work has sought to understand and model energy transfer dynamics in biological systems. The Fenna-Matthews-Olson complex (FMO) from green sulfur bacteria serves as a model complex for these studies because it contains only seven electronically coupled bacteriochlorophyll-a chromophores and therefore only seven delocalized exciton states. The energies of these excitons correspond to transitions observable in a linear absorption spectrum, but unfortunately only three peaks can be resolved in the linear absorption spectrum of FMO at 77 K due to line broadening effects. Consequently, the energies of the seven excitons have never been experimentally measured, and simulations of energy transfer within FMO therefore rely upon approximate values obtained by fitting theoretical Hamiltonians to experimental linear and two-dimensional (2D) spectra. To address this problem, we have extended traditional 2D electronic spectroscopy into a third Fourier dimension without the use of additional optical interactions. By acquiring a set of 2D spectra evenly spaced in waiting time and dividing the area of the spectra into voxels, we eliminate population dynamics from the data and transform the waiting time dimension into frequency space. The resultant 3D spectrum resolves quantum beating signals arising from excitonic coherences along the waiting frequency dimension, thereby yielding up to seven-fold redundancy in the set of frequencies necessary to construct a complete set of excitonic transition energies.

Using this technique, we have obtained the first fully-experimental excitonic Hamiltonian for FMO, which can be used to improve theoretical simulations of energy transfer within this protein. These results also enable calculation of the Hamiltonian of FMO in the site basis by fitting to the experimental linear absorption spectrum.



Session III: Saturday Morning, October 30

Regulation and Environmental Responses

ROLE OF TRANSLATION INITIATION IN REGULATION OF PHYCOBILISOME BIOSYNTHESIS

April Nesbit, Andrian Gutu, Craig Whippo, David M. Kehoe
Department of Biology, Indiana University, Bloomington, IN 47405

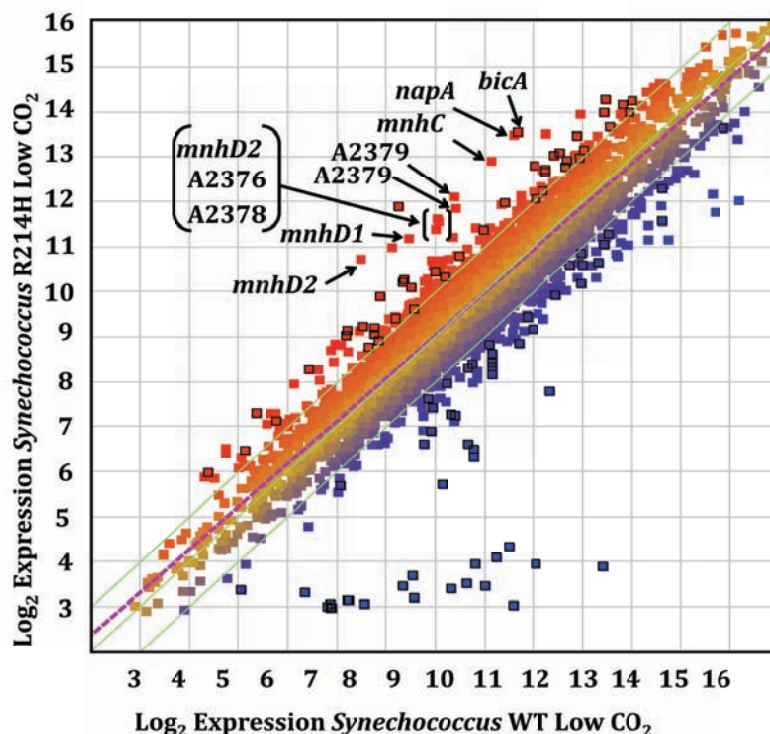
Examination of sequenced cyanobacterial and plant genomes shows that many species encode multiple homologs of translation initiation factor 3 (IF3). However, the roles of these are as yet unknown. IF3 is an essential component for bacterial translation initiation, and this bacterial paradigm is also thought to hold true for chloroplast translation. Our laboratory has discovered that the loss of one of the two IF3s in the cyanobacterium *Fremyella diplosiphon*, IF3a, resulted in an increase in phycoerythrin during growth in red light. In contrast, a *F. diplosiphon* strain lacking IF3b contained wild-type levels of phycoerythrin, suggesting distinct roles for IF3a and IF3b in this species. Both IF3a and IF3b from *F. diplosiphon* can complement an *Escherichia coli* mutant lacking IF3, indicating that they are *bona fide* IF3s. Another species that contains multiple putative IF3s is *Arabidopsis thaliana*. The three putative IF3s in *A. thaliana* are encoded on chromosome 1 (IF3-1), chromosome 2 (IF3-2), and chromosome 4 (IF3-4), and all are predicted to localize to the chloroplast. Microarray studies by other groups show that these putative IF3s are differentially expressed between organs and throughout development, suggesting that these IF3s may have unique roles in chloroplast translation. We have shown that *A. thaliana* seedlings with homozygous mutations in either IF3-2 or IF3-4 were resistance to the translation inhibitor spectinomycin. The study of IF3 function in species with multiple homologs will provide us the opportunity to gain a better understanding of the mechanism of translation in cyanobacteria and plant chloroplasts.

REDOX SENSING-SIGNALING OF GENE EXPRESSION IN CYTOCHROME *bf* LOW- AND HIGH- POTENTIAL CHAIN MUTANTS OF *SYNECHOCOCCUS* SP. PCC 7002

George L. Weir¹, Kraig Short¹, Matthew Nelson¹, Alina Ott², David R. Rivera Aponte³, Justin Zangel¹ and Toivo Kallas¹

¹Univ. Wisconsin-Oshkosh, ²Univ. Wisconsin-Stevens Point, ³Univ. Puerto Rico, Bayamon

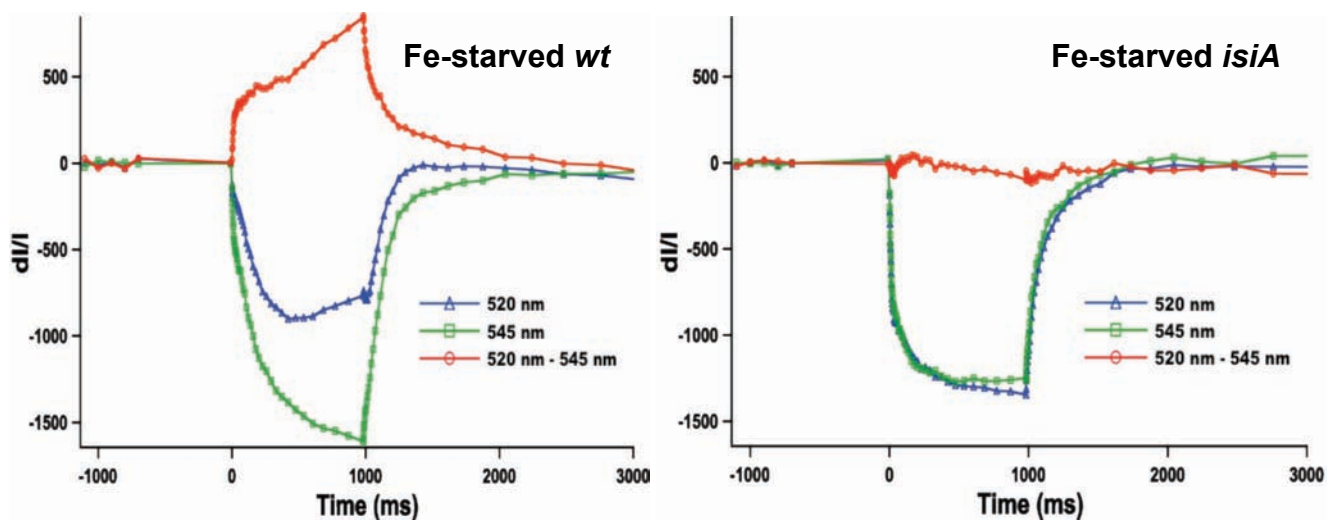
The cytochrome (Cyt) *bf* complex is central to photosynthetic electron transport and redox sensing of the plastoquinone (PQ) pool and signaling of adaptive state transitions (light-harvesting protein redistribution) and gene expression. Mutation PetB-R214H (Nelson *et al.* 2005 *JBC*) in the marine cyanobacterium, *Synechococcus* PCC 7002 impedes electron flow in the Cyt *bf* low-potential chain resulting in slower turnover of the *bf* complex and increased oxygen radical production. Mutation PetC1-Δ2G (Yan & Cramer 2003 *JBC*) slows electron flow into the high-potential chain and similarly slows turnover of the *bf* complex. We have used high-density oligonucleotide microarrays to investigate the role of the Cyt *bf* high- and low-potential chains in redox signaling of gene expression in *Synechococcus* grown phototrophically in low and high CO₂. Numerous genes were differentially regulated comparably in both mutants relative to the control, implying signaling events at the level of the PQ pool. A smaller number of genes was differentially regulated in PetB-R214H relative to PetC1-Δ2G, implying specific signaling events related to the redox status or conformation of the low- and high-potential chains. Differentially regulated genes included ones for bicarbonate uptake, Na⁺/H⁺ antiporter subunits, and unknown or hypothetical proteins. Data analysis revealed a motif for a 'YrdC-like,' DNA-binding protein in genes up-regulated under low CO₂ (including one upstream of the *rbcR*, low-CO₂ inducible regulator, Hong *et al.*, 2004 *JBC*) and in some genes differentially expressed in PetB-R214H. The gene for a 'YrdC-like' protein was itself up-regulated under low CO₂. These findings will help identify components and targets of Cyt *bf*-mediated redox signaling.



IRON-STRESS IN *SYNECHOCOCCUS* SP. PCC 7002: 520 NM ELECTROCHROMIC SIGNAL ASSOCIATED WITH ISIA AND ROLES OF ISCA AND SUFA

Junlei Sun¹, Linda K. Vuorijoki³, George L. Weir IV⁴, Matthew Nelson⁴, Patrik R. Jones³, John H. Golbeck^{1,2} & Toivo Kallas⁴, ¹Department of Biochemistry & Molecular Biology, ²Department of Chemistry, Pennsylvania State University, University Park, PA, USA, ³Department of Biochemistry & Food Chemistry, University of Turku, Finland, ⁴Department of Biology & Microbiology, University of Wisconsin-Oshkosh, Oshkosh, WI, USA

There are three known systems for iron-sulfur clusters assembly, *nif* (nitrogen fixation), *isc* (iron sulfur cluster) and *suf* (sulfur utilization factor). The *suf* system predominates in cyanobacteria and SufA and IscA are implicated in regulation. In this work, iron-limiting growth conditions were established for *Synechococcus* sp. PCC 7002 wild type, *iscA*, *sufA* and *sufR* strains (carrying knockout mutations of these genes) to investigate their impacts on global gene expression. Time-resolved optical spectroscopy of these Fe-starved whole cells revealed a light-induced 520 nm spectral signal that may represent an electrochromic shift of pigment proteins that reflects trans-membrane charge separation. We propose that the IsiA protein, a pigment-binding and light-harvesting protein that associates with photosystem I in Fe-limiting conditions, produces this electrochromic signal. The *isiA* and *isiB* genes in the *isi* operon were inactivated, respectively, to generate *isiA* and *isiB* mutants. Only the *isiA* mutant showed differences in growth rate and chlorophyll content relative to the wild type when grown in Fe-depleted medium. Further, the 520 nm spectral signal did not appear in Fe-starved *isiA* mutant cells. We conclude that the 520 nm signal serves as marker for Fe-starvation and is associated with the IsiA protein.



Kinetic traces at 520 nm (triangle) and 546 nm (square) of Fe-starved *wt* (A) and *isiA* (B) cells during and after a 1 second actinic illumination of green light (520 nm, $300 \mu\text{mol m}^{-2} \text{s}^{-1}$), and the difference spectra (circle).

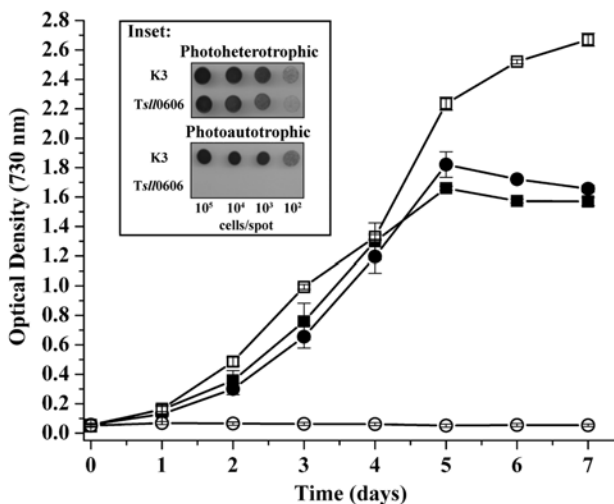
Session IV: Saturday Evening, October 30

Cofactors and Protein Complexes

THE *SII0606* PROTEIN IS REQUIRED FOR PHOTOSYSTEM II ASSEMBLY-STABILITY IN THE CYANOBACTERIUM *SYNECHOCYSTIS* sp. PCC 6803

Shulu Zhang, Laurie K. Frankel and Terry M. Bricker, Division of Biochemistry and Molecular Biology, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.

An insertional transposon mutation in the *sII0606* gene was found to lead to a loss of photoautotrophy, but not photoheterotrophy, in the cyanobacterium *Synechocystis* sp. PCC 6803. Complementation analysis of this mutant (*TsII0606*) indicated that an intact *sII0606* gene could fully restore photoautotrophic growth. Gene organization in the vicinity of *sII0606* indicates that it is not contained in an operon. No electron transport activity was detected in *TsII0606* using water as an electron donor and 2,6 dichlorobenzoquinone as an electron acceptor, indicating that PS II was defective. Electron transport activity using dichlorophenol indolephenol plus ascorbate as an electron donor to methyl viologen, however, was the same as observed in the control strain. This indicated that electron flow through Photosystem I was normal. Fluorescence induction and decay parameters verified that Photosystem II was highly compromised. The quantum yield for energy trapping by Photosystem II (F_v/F_m) in the mutant was less than 10% of that observed in the control strain. The small variable fluorescence yield observed after a single saturating flash exhibited aberrant Q_A^- reoxidation kinetics which were insensitive to dichloromethylurea. Immunological analysis indicated that while the D2 and CP47 proteins were modestly affected, the D1 and CP43 components were dramatically reduced. Analysis of two-dimensional blue native/LiDS-PAGE gels indicated that no intact PS II monomer or dimers were observed in the mutant. The CP43-less PS II monomer did accumulate to detectable levels. Our results indicate that the *SII0606* protein is required for the assembly/stability of a functionally competent Photosystem II.

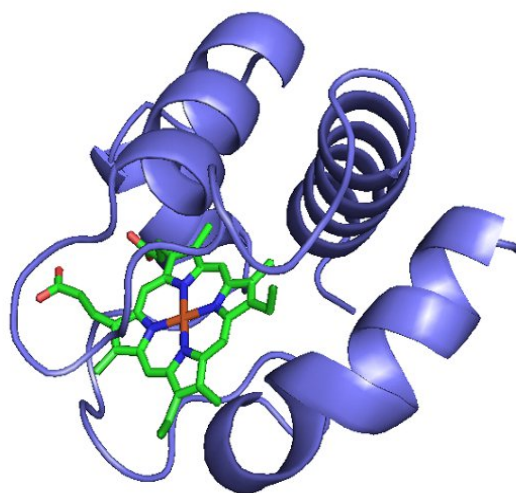


Growth of the Control Strain K3 and *TsII0606* Mutant Cells in Liquid BG-11 Medium and on Solid BG-11 Medium. The optical densities of the photoheterotrophically and photoautotrophically grown cultures were measured daily for 1 week. Symbols: Photoheterotrophically grown K3 (■) and *TsII0606* (●) cells; photoautotrophically grown K3 (□) and *TsII0606* (○) cells, $n \geq 3$, vertical bars ± 1.0 standard deviation. In some instances the standard deviation was smaller than the symbol. Inset, Growth of the Control Strain K3 and the Mutant *TsII0606* on Solid BG-11 Medium. The cells were quantitatively spotted onto the media and grown for two weeks before the results were recorded.

STRUCTURES, PROPERTIES AND POSSIBLE FUNCTIONS OF C_6 -LIKE CYTOCHROMES OF THE CYANOBACTERIUM *SYNECHOCOCCUS* SP. PCC 7002

Paweł Zatwarnicki¹, Wojciech Bialek¹, Mathew Nelson², Andrzej Szczepaniak¹ and Toivo Kallas². ¹Department of Biophysics, University of Wrocław, Wrocław, Poland, ²Department of Biology-Microbiology, University of Wisconsin, Oshkosh, U.S.A.

Among known cyanobacteria, *Synechococcus* sp. PCC 7002 is one of the deeply studied and well-characterized strains. It carries two genes – *petJ1* and *petJ2* – that both encode soluble cytochrome c_6 proteins. These cytochromes are similar (36.9% amino acid identity) but differences in crucial amino residues result in huge differences in their properties. PetJ1 is acidic with a midpoint potential ($E_{m,7}$) of +319 mV whereas PetJ2 is basic with a potential of +148 mV and thus not an effective electron acceptor for the cytochrome *bc*₁ complex. Critical differences occur in the heme-binding pocket. PetJ1 carries the positively charged glutamine-57 in proximity to the heme. Mutation of Glu57 to valine (Q57V) in the PetJ1 cytochrome, which removes the stabilizing positive charge, lowers its midpoint potential to +231 mV. PetJ2 carries leucine in the same region and this seems to be a major determinant of its much lower redox potential. A second difference in the *Synechococcus* PetJ1 is a KDGSKSL insertion between its second and third α -helices, which makes this PetJ1 unique among other c_6 and c_6 -like cytochromes. This insertion seems to be essential for function. The function of PetJ2 and related c_{6C} cytochromes (Bialek et al. 2008 *Biochemistry*) remains unknown. Recently solved structures of *Synechococcus* PCC 7002 PetJ1 (Bialek et al. 2009 *FEBS J.*), PetJ Q57V, and PetJ2 will be presented and possible functions discussed. These structures illustrate the crucial importance of single amino acid changes in determining cytochrome properties and function.



Structure of cytochrome PetJ2 from *Synechococcus* sp. PCC 7002

ISOLATION AND CHARACTERIZATION OF TWO CONSECUTIVE PSII ASSEMBLY INTERMEDIATES IN *SYNECHOCYSTIS* SP. PCC 6803

Haijun Liu^a, Johnna L. Roose^b, Jeffrey C. Cameron^a, Himadri B. Pakrasi^{a,1}

^aDepartment of Biology, Washington University, St. Louis, MO 63130; and ^bDepartment of Biological Science, Louisiana State University, Baton Rouge, LA, 73803.

¹To whom correspondence should be addressed. E-mail: Pakrasi@wustl.edu

Keywords: Membrane protein complex, PS II assembly intermediate, *Synechocystis*.

Abstract

Photosystem II is a multisubunit membrane protein complex containing many cofactors. These cofactors must be accurately oriented within the assembled protein matrix to facilitate light harvesting and electron transfer reactions. Additionally, PSII constantly undergoes assembly and disassembly due to the unavoidable damage that results from its normal chemistry. Thus, in addition to the active PSII complexes present, there are always PSII subpopulations that are incompetent for oxygen-evolution, but are in the process of undergoing elaborate biogenesis and repair. Without these repair processes the photosynthetic apparatus will not remain active. These transient complexes are especially difficult to characterize due to their low abundance, structural heterogeneity, and thermodynamic instability. In this study, we show that the Psb27 protein of the PSII complex is involved in the formation of two consecutive and important PSII assembly intermediates leading to functional PSII complexes. Immunological analysis showed that in the presence of the processing protease CtpA, the PSII assembly intermediate containing the unprocessed pD1 protein is not readily captured. In the following donor side assembly, the PsbO protein is the first extrinsic protein binding to the donor side of newly processed D1 containing PSII intermediate. Atomic absorption spectroscopy and variable Chlorophyll *a* fluorescence relaxation kinetics indicate that the donor side is not fully assembled in these two complexes. D1 processing is required for manganese cluster assembly and extrinsic protein assembly into PSII (Roose and Pakrasi, 2004); however the processing does not produce a fully functional PSII due to the presence of the Psb27 protein. We propose that the Psb27 protein has a negative regulatory role for the (Mn₄-Ca) center assembly. Our identification and characterization of these novel Psb27 protein containing PSII assembly intermediates may also lead to new insights into the mechanism of photoactivation and photoprotection by PSII reaction center quenching. Experiments on the structural location of the Psb27 protein within PSII assembly intermediate complexes are also reported and discussed.

Acknowledgement, we thank Maitrayee [Bhattacharyya](#) for her time of critical reading and discussions. Funding of this research is provided by NSF-MCB0745611 and NSF-FIBR EF0425749

A NEW CHROMOPHORE IN THE CP43 SUBUNIT OF PHOTOSYSTEM II: N-FORMYLKYNURENINE

Tina M. Dreaden, Jun Chen, Sasha Rexroth, and Bridgette A. Barry

School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and Bioscience,
Georgia Institute of Technology, Atlanta, GA 30332

Water oxidation to form O₂ and protons occurs at the Mn₄Ca cluster of the Photosystem II (PSII) membrane protein complex. The CP43 subunit is one of several proteins that form the reaction center core complex. These proteins are essential for optimal catalytic activity. In this work, an oxidative post-translational modification of a tryptophan side chain, Trp-365 (Fig. 1A), to *N*-formylkynurenine (NFK) was identified in CP43. NFK can be formed from the reaction of singlet oxygen (¹O₂) with tryptophan, which results in a doubly oxidized ring cleavage product (Fig. 1B). Other modifications of this particular residue have been identified previously (1). The +32 mass shift observed by MS/MS analysis was consistent with oxidation of Trp-365 to form NFK. The presence of the NFK modification was confirmed by its unique optical absorption (Fig. 1C) and UV resonance Raman signal. In the 2.9 Å cyanobacterial structure (2), Trp-365 is located ~18 Å from the Mn₄Ca cluster (Fig. 1A). Three site-directed mutants (W365A, W365L, and W365C) at this position exhibit an increased rate of photoinhibition compared to a wildtype control. These data suggest that NFK functions in photoprotection of the Mn₄Ca cluster and the D1 protein during high light stress.

1. Anderson, L. B., Maderia, M., Ouellette, A. J. A., Putnam-Evans, C., Higgins, L., Krick, T., MacCoss, M. J., Lim, H., Yates, J. R., III, and Barry, B. A. (2002) *Proc. Nat. Acad. Sci. U.S.A.* **99**, 14676-14681.
2. Gustav, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A., and Saenger, W. (2009) *Nat. Struct. Biol.* **16**, 334-342.

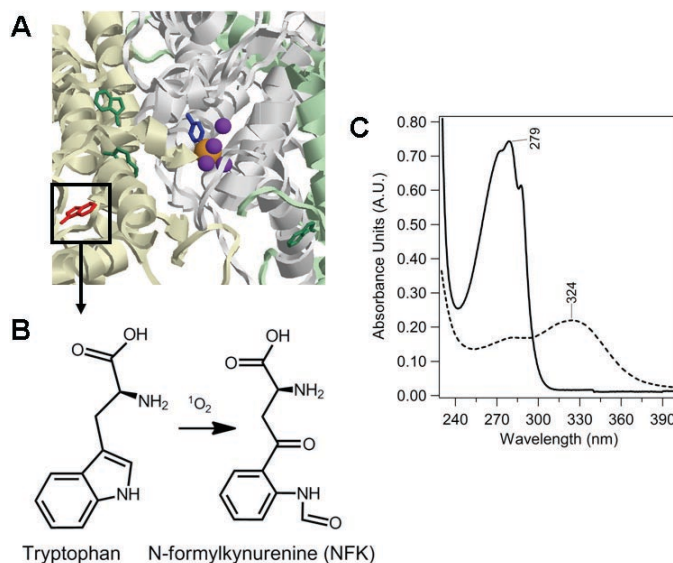


Figure 1. (A) The location of Trp-365 in cyanobacterial PSII (2) is highlighted in the box. The Mn₄Ca cluster is located in the center of the figure. The Y_z sidechain is shown above the Mn₄Ca cluster, and the other Trp sidechains within 20 Å of the active site are shown (Trp-359 and Trp-291 in CP43 and Trp-328 in D2). (B) Structures of tryptophan and NFK. (C) UV absorption spectra of tryptophan (solid line) and NFK-containing PSII peptides (dashed line).

Session V: Sunday Morning, October 31

Energy Transfer II

ONIOM METHOD FOR THE EXPLORATION OF DOUBLE DIFFERENCE SPECTRA OF UBIQUINONE IN THE Q_A BINDING SITE OF RB. SPHAEROIDES

Hari P. Lamichhane and Gary Hastings

Department of Physics and Astronomy, Georgia State University, Atlanta, Georgia

FTIR difference spectra associated with Q_A reduction in purple bacterial reaction centers are poorly understood. Certainly, no quantitative description of the vibrational properties of the Q_A ubiquinone is available. Here we have used QM/MM computational methods to calculate the vibrational properties of the ubiquinone bound in the Q_A site in purple bacterial reaction centers from *Rb. sphaeroides*. QM/MM calculations were undertaken using the ONIOM method in Gaussian03 software.

For calculations, the model considered is ubiquinone-3 (Q₃) embedded in a 10 Å sphere of the surrounding protein. ONIOM calculations were undertaken with neutral Q₃ treated using density functional theory at the B3LYP 6-31+G(d) level, and the protein environment treated at the molecular mechanics level using AMBER. Harmonic normal mode vibrational frequency calculations of unlabeled and specifically ¹³C and ¹⁸O labeled ubiquinone were undertaken. From these calculations isotope induced FTIR double difference spectra were constructed. Such spectra are directly compared to corresponding experimental spectra. From such a comparison we find quite astonishing agreement between calculated and experimental FTIR double difference spectra. However, we find that the calculated normal mode description of the bands in the spectra differ greatly from that proposed qualitatively based on the experimental spectra.

Experimentally, for ubiquinone in CCl₄ two bands observed at 1650 and 1611 cm⁻¹. These bands are due to the C₄=O and C=C groups, respectively. For the Q_A ubiquinone, bands are observed at 1628 and 1601 cm⁻¹, and are assigned to C=C and C₄=O stretching vibrations, respectively. These proposed assignments are based on band-shifts observed upon site specific isotope labeling of the Q_A ubiquinone, and are very different from that found for ubiquinone in solution. It was claimed that ~50 cm⁻¹ downshift of C₄=O band in the Q_A binding site might be due to the presence of doubly oxidized non-heme iron atom and hydrogen bonding to His 219 and Ser 222. However, it was unclear why the highest frequency C₁=O stretching mode is not affected by hydrogen bonding to amide nitrogen of Ala 260. Also, there was no strong explanation as to why the C=C stretching mode up-shifted by ~17 cm⁻¹. Here we use our computational results to propose a coherent description of isotope induced band-shifts observed in experimental spectra.

TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY OF THE D1-D2-CYT B559 REACTION CENTER COMPLEX: EXPERIMENTS AND MODELING

Kristin L. M. Lewis, Jeffrey A. Myers, Franklin Fuller, Patrick F. Tekavec, Charles F. Yocum, Jennifer P. Ogilvie

Department of Physics and Biophysics, University of Michigan, Ann Arbor, MI 48109

The photosystem II complex (PSII) reaction center is the heart of oxygenic photosynthesis, taking absorbed light from neighboring antenna complexes and creating a charge separation capable of splitting water. Despite the wealth of spectroscopic studies of the PSII reaction center, the basic energy transfer and charge separation dynamics remain poorly understood. With six chlorophyll a pigments and two pheophytins, the reaction center has a complex electronic structure; similar pigment absorptions and electronic coupling combine to create a broad linear absorption spectrum, making spectral assignments particularly difficult. In traditional nonlinear spectroscopy experiments, one must choose between temporal and spectral resolution, complicating the interpretation of these experiments. In addition, the degree of static disorder and electronic coupling in the system is difficult to discern. Two-dimensional electronic spectroscopy (2DES) provides a direct view of electronic coupling and femtosecond energy and charge dynamics without sacrificing spectral selectivity. We present low-temperature 2DES data of the D1-D2-Cyt b559 reaction center complex from the femtosecond to the hundred picosecond timescale. We discuss two-dimensional decay associated spectra (2D DAS), a decomposition technique that characterizes the spectral signatures of energy and charge separation. Within this framework, we discuss the sub-picosecond energy and charge transfer dynamics of the PSII reaction center. A number of competing excitonic models have been proposed in the literature. We discuss these models in the context of the data, and show preliminary simulations of the 2D spectra based on these models.

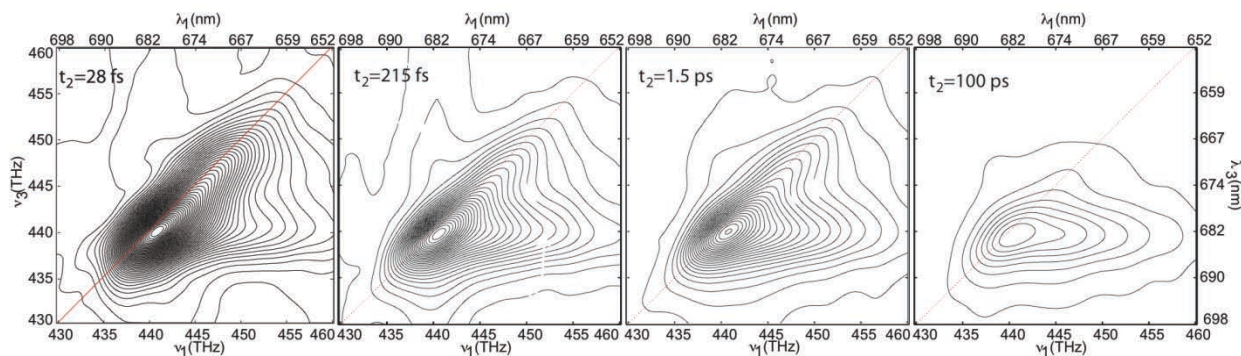


Figure 1: Experimental data showing the time evolution of 2D spectra of PSII reaction centers. At $t_2=28$ fs the 2D spectrum is elongated along the diagonal indicating large inhomogeneous broadening. Cross-peaks above and below the diagonal indicate excitonic coupling. As energy and charge transfer proceed, the system equilibrates to lower energy states and the spectra become elongated parallel to the excitation axis.

GRAPE SPECTROSCOPY: A NEW APPROACH TO REAL-TIME FEMTOSECOND 2D SPECTROSCOPY OF PHOTOSYNTHETIC LIGHT HARVESTING COMPLEXES

Elad Harel, Andrew Fidler, Phillip D. Long and Gregory S. Engel
Department of Chemistry, and The James Franck Institute
The University of Chicago, Chicago, Illinois

Here we present a new method to acquire an entire two-dimensional photon echo spectrum in a single laser shot with only conventional optics using analogies from magnetic resonance imaging and spectroscopy. This method, which we call GRAdient-Assisted Photon Echo Spectroscopy (GRAPES), can record electronic correlation maps in real time with femtosecond precision. We have applied this method to acquire the 2D spectrum of the light-harvesting complex LH2 for the first time using super continuum generation by eliminating the phase stability problems normally associated with multidimensional optical spectroscopy. I will discuss the implications of such broadband correlation spectroscopy on understanding the photophysics of energy transfer in photosynthetic systems.

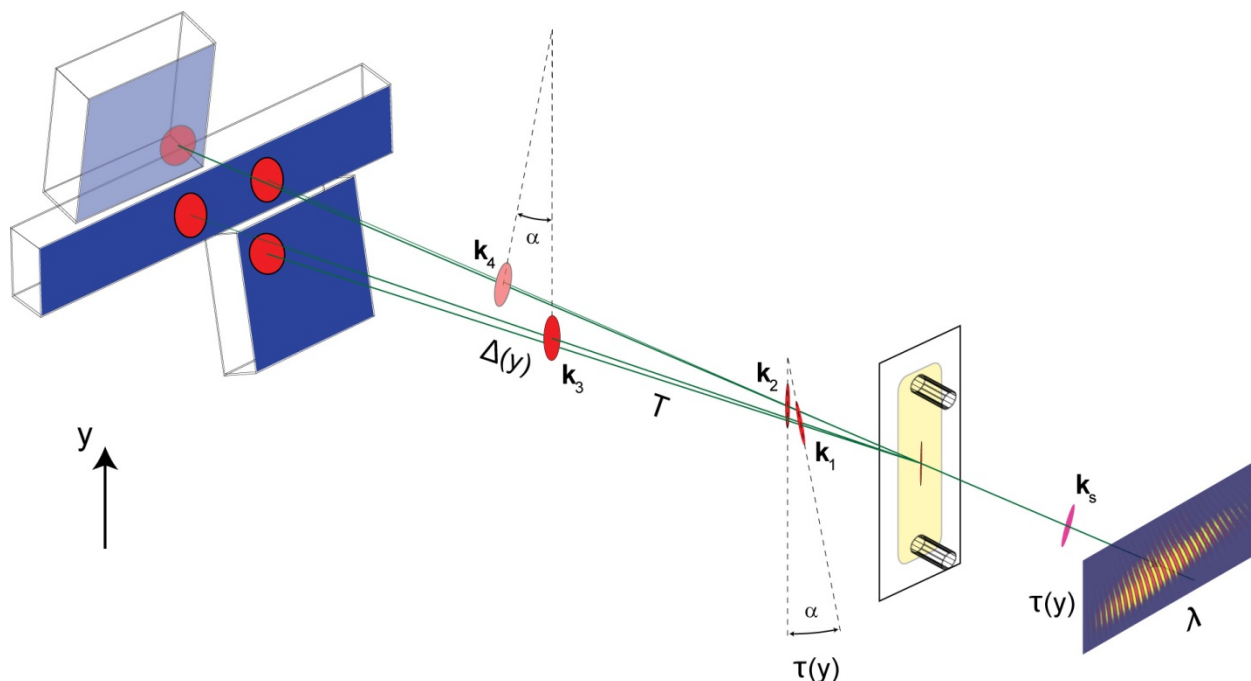


Figure 1: By controlling the tilt of the wavefronts, entire 2D electronic spectra can be acquired in a single laser pulse permitting broadband studies of photosynthetic complexes even with unstable light sources.

Session VI: Sunday Morning, October 31

Electron and Proton Transfer

PROTEIN STRUCTURAL DYNAMICS LINKED WITH REDOX CHANGES AND METAL ASSEMBLY IN RIBONUCLEOTIDE REDUCTASE

Adam R. Offenbacher, Jun Chen, Bridgette A. Barry, Georgia Institute of Technology, School of Chemistry and Biochemistry, 315 Ferst Dr., Atlanta, Georgia 30332

The b2 subunit of class Ia ribonucleotide reductases (RNRs) contains an antiferromagnetically coupled m-oxo bridged diiron cluster and a tyrosyl radical (Y122•).¹ Reduction of ribonucleotides is initiated by long-distance proton-coupled electron transfer reactions, which generate a reduced Y122 and a transiently oxidized, active site cysteine radical.^{2,3}

Assembly of the diiron cluster has been proposed previously to be rate limited by a protein conformational change,⁴ and allosteric effects have been reported to regulate metal binding.⁵ In our work, an ultraviolet resonance Raman (UVRR) difference technique was used to describe the structural changes, induced by the assembly of the iron cluster and by the reduction of the tyrosyl radical. Spectral contributions from aromatic amino acids were observed through UV resonance enhancement at 229 nm. We show that assembly of the iron cluster is accompanied by protonation of histidine. In addition, structural perturbations of tyrosine and tryptophan, which are attributed to changes in tyrosine conformation and in tryptophan hydrogen bonding and dihedral angle, were observed when the diferric cluster was removed. In summary, our work provides evidence that electrostatic and conformational perturbations of aromatic amino acids contribute to the structural perturbations, which are associated with metal cluster assembly in RNR.

- (1) Stubbe, J.; Riggs-Gelasco, P. *Trends Biochem. Sci.* **1998**, *23*, 438-443.
- (2) Uhlin, U.; Eklund, H. *Nature* **1994**, *370*, 533-539.
- (3) Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167-2202.
- (4) Tong, W. H.; Chen, S.; Lloyd, S. G.; Edmondson, D. E.; Huynh, B. H.; Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 2107-2108.
- (5) Pierce, B. S.; Hendrich, M. P. *J. Am. Chem. Soc.* **2005**, *127*, 3613-3623.

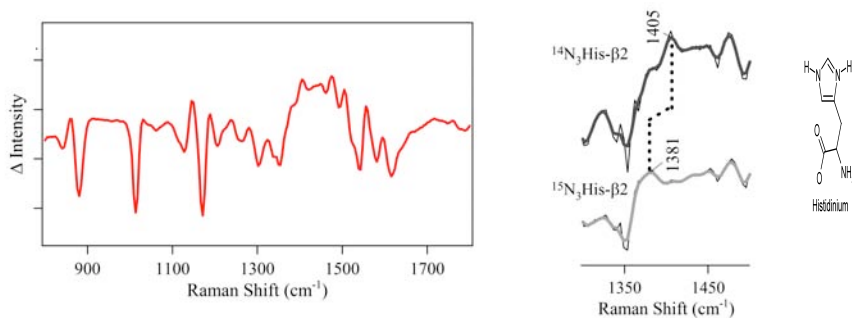


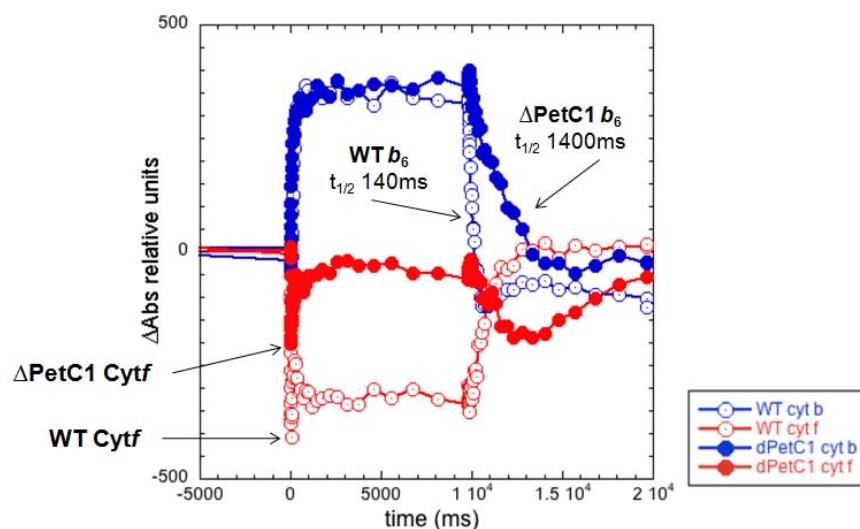
Figure: *Left:* UV resonance Raman difference spectrum of Apob2-minus-Metb2. *Center:* Comparison of ^{14}N His- and ^{15}N His-labeled Apob2-minus-Metb2 difference spectra. The dotted line illustrates the shift of the histidinium assignment. *Right:* Diagram of histidinium.

KINETICALLY COUPLED CYTOCHROME b_6 IN AN UNUSUAL CYTOCHROME- f DEFICIENT PETC2-RIESKE CYT bf COMPLEX OF *SYNECHOCYSTIS* SP. PCC 6803

Saurabh Ranade, Kenneth Toson, Matthew Nelson and Toivo Kallas

Department of Biology and Microbiology, University of Wisconsin, Oshkosh WI 54901

The cytochrome (Cyt) bf complex is essential for both photosynthetic and respiratory electron transport in cyanobacteria. In *Synechocystis* PCC 6803, PetC1 is the major Rieske iron-sulfur protein (ISP) subunit of the Cyt bf complex. PetC1 can be replaced to some extent by the PetC2 Rieske ISP. The function of PetC2 remains unclear. We investigated the catalytic efficiency of the PetC2 Rieske ISP, the nature of the PetC2-Cyt bf complex, and its impact on gene expression in a Δ PetC1 mutant of *Synechocystis* (Schneider et al., 2002). Expression of Rieske ISP (*petC1*, *petC2*, *petC3*) and Cyt f (*petA*) genes was investigated by reverse transcriptase quantitative PCR (RT-qPCR). Kinetic experiments were performed with a Joliot-type, LED spectrophotometer (BioLogic JTS-10). High-intensity actinic light was used to completely oxidize or reduce Cyt bf and photosystem I (PS I) centers to determine their relative quantities. Low-intensity actinic flashes were used to approach single turnover kinetics of the Cyt bf complex to determine the catalytic efficiency of the PetC2-Cyt bf complex in the Δ PetC1 mutant relative to the native PetC1-Cyt bf complex. RT-qPCR data revealed \sim 8-fold up-regulation of *petC2* and \sim 5-fold down-regulation of *petA* (Cyt f) gene expression in the Δ PetC1 mutant relative to wild type grown under optimal phototrophic conditions. Spectroscopic data suggested similar Cyt b_6 , plastocyanin and PS I contents but \sim 2.5 times lower Cyt f in the Δ PetC1 strain than in wild type. At low flash intensities, the half-time of Cyt f/c_6 re-reduction in the Δ PetC1 mutant approached that of the wild type suggesting that the PetC2 Rieske ISP may be as efficient as the major PetC1 ISP. These and other data suggest that the *Synechocystis* Δ PetC1 mutant carries an unusual PetC2-Cytochrome bf complex comprised primarily of core, cytochrome b_6 and PetC2-Rieske ISP subunits that may resemble a primordial 'Rieske-Cyt b ' complex.



Cytochrome f - b_6 redox kinetics in *Synechocystis* 6803 wild type and Δ PetC1. Redox changes were monitored during and after saturating actinic illumination. Note similar extents of b heme reduction but much lower Cyt f oxidation level in the mutant relative to wild type.

ALTERNATIVE COMPLEX III IN THE ELECTRON TRANSFER CHAIN OF THE PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

Xinliu Gao, Yueyong Xin, Jianzhong Wen, Patrick D. Bell, Robert E. Blankenship
Departments of Biology and Chemistry, Washington University in St. Louis, MO, 63130

The green photosynthetic bacterium *Chloroflexus aurantiacus* does not contain a cytochrome *bc* complex. Earlier work identified a potential substitute for the cytochrome *bc* complex, the menaquinol:auracyanin oxidoreductase, also named Alternative Complex III (ACIII). This complex has been purified, identified and characterized from *C. aurantiacus*. ACIII is an integral membrane protein complex with mass ~300 kDa. It contains 7 different subunits, including a 113 kDa FeS-containing polypeptide, a 25 kDa multi-heme *c*-containing subunit and a 23 kDa mono-heme *c*-containing subunit. The genes that code for the subunits are arranged in an operon in the genome. The copy number of each subunit and the number of hemes in the multiheme cytochrome subunit have been determined by LC-MS, potentiometric titration and intensity analysis of heme-stained SDS-PAGE. A preliminary structural model is proposed based on transmembrane and signal peptide analysis and chemical cross-linking combined with MALDI-TOF MS. The measurement of menaquinol:auracyanin oxidoreductase activity supports the view that ACIII functions as an electron carrier in the electron transfer chain of *C. aurantiacus*. The lack of sensitivity to the common inhibitors of the cytochrome *bc* complex suggests a different catalytic mechanism of the ACIII complex.

Poster Presentation Abstracts

(Listed alphabetically by first author)

P1

CLONING OF THE PHOTOSYNTHETIC ELECTRON TRANSPORT CARRIERS CYTOCHROME c_6 AND PLASTOCYANIN FROM ACARYOCHLORIS MARINA

Patrick Bell, Yisheng Connie Kang, and Robert E. Blankenship, Departments of Chemistry and Biology, Washington University in St. Louis, One Brookings Drive St. Louis MO 63130

Acaryochloris marina is a unicellular marine cyanobacterium that uses chlorophyll *d* as its primary photosynthetic pigment, which absorbs light at longer wavelengths compared to other chlorophylls. It is the only known oxygenic phototroph that uses this unique pigment, which has made it a species of interest for evolutionary analysis and possible bioenergy applications. This has led our lab to begin investigating the proteins and complexes involved in its photosynthetic apparatus, including its soluble electron carriers, cytochrome c_6 and plastocyanin. Cytochrome c_6 and plastocyanin are functionally interchangeable proteins involved in photosynthetic electron transport in cyanobacteria and algae. In many cyanobacteria and algae, plastocyanin is expressed under copper replete conditions, while cytochrome c_6 is expressed under copper depleted conditions. In *A. marina*'s genome, there are two genes coding for cytochrome c_6 and one gene coding for plastocyanin. In previous work in our lab, only one cytochrome c_6 from the genome was expressed from *A. marina* grown in iron-enriched Marine BG-11 media. This protein was purified by ammonium sulfate fractionation and ion exchange and gel filtration chromatography and subsequently characterized by mass spectroscopy, CD spectroscopy, isoelectrofocusing, and redox potentiometry¹. It was found to be a typical small (8.87 kDa), alpha helical, acidic (pI < 4), and high potential ($E_m = +327\text{mV}$ vs NHE) cytochrome c_6 in relation to cytochrome c_6 of other cyanobacteria.

Both cytochrome c_6 genes and plastocyanin in the *A. marina* genome have been successfully cloned into *E. coli* BL21-D3 by ligation independent *in vitro* homologous recombination. Efforts into the expression of the genes in *E. coli* will be presented.

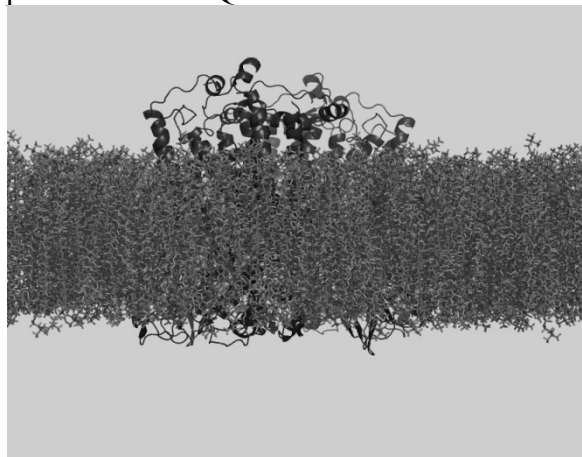
1. Bell P., Xin Y., and Blankenship R.E. (2009) Purification and Characterization of Cytochrome c_6 from *Acaryochloris marina*. *Photosynthesis Research*, **102**: 43-51.

P2

Q_o SITE OF *bc*₁ COMPLEX EPR; ANALYSIS OF SEMIQUINONES THROUGH RAPID MIX FREEZE QUENCHING.

RL Burton, C Wilson, CB Harrison, K Schulten, AR Crofts
University of Illinois, Urbana-Champaign Dept. of Biophysics; 419 Roger Adams Lab B-4, 600 S. Matthews Ave. Urbana, IL 61801

The mechanism behind the function of Q_o site of the *bc*₁ complex remains enigmatic despite decades of study. As one of the most critical regions of *bc*₁, the Q_o site is essential for its role in proton gradient generation, and it has also gained increasing attention for its roles as a powerful drug target against *P. falciparum* malaria parasite, and for its role as a superoxide producer in aging research. Our studies here focus on the structure-function relationship of the Q_o site; particularly on the glutamate of the conserved PEWY sequence and its role in both the second proton transfer of the Q cycle and in the switching of the bifurcated redox pathway. We are studying the Q_o site in the *bc*₁ complex of *Rhodobacter sphaeroides* through EPR on isolated semiquinone intermediates captured through rapid mix freeze-quenching on a millisecond time scale. Studies are being conducted on pure isolated *bc*₁ and on *R. sphaeroides* chromatophores activated through ubiquinol addition and a xenon flash, respectively. Wild type and mutant cytochrome b; most notably E295W, E295G, and E295Q are being employed. Rapid mix freeze-quench analysis of purified *bc*₁ complex has shown the presence of semiquinone intermediate in E295W with EPR. To bridge the gap between chromatophore and isolated *bc*₁ studies which show significantly different kinetics, *bc*₁ complex assembly into nanodiscs is also underway. EPR studies are being supplemented with QM/MM simulations on semiquinone movement.



*bc*₁ complex dimer in lipid bilayer

P3

EXAMINATION OF THE ROLE OF THE CYANOBACTERIAL PHYTOCHROME RCAP IN *FREMYELLA DIPLOSIPHON*

Adam N. Bussell and David M. Kehoe, Department of Biology, 212 S. Hawthorne Drive,
Indiana University, Bloomington, IN 47405

Complementary chromatic acclimation (CCA) has been characterized primarily in the freshwater filamentous cyanobacteria *Fremyella diplosiphon*. During CCA *F. diplosiphon* acclimates to environmental light color changes by altering its phycobilisomes (PBS) light-harvesting structures in response to changes in the ratio of red light (RL) and green light (GL). A two-component signal transduction system (termed Rca, for regulator for complementary chromatic adaptation) transcriptionally regulates the operons encoding phycocyanin and phycoerythrin and is composed of the photoreceptor RcaE and two response regulators, RcaF and RcaC. We have analyzed the recently sequenced genome of *F. diplosiphon* and identified a number of additional putative phytochrome-class photoreceptor genes. We have examined the expression of one of these putative cyanobacteriochromes, called RcaP, using quantitative polymerase chain reaction (QPCR). Our analysis of the expression of *rcaP* during growth of wild-type cells in RL and GL has revealed that its RNA accumulation is light dependent and that RNA accumulation during growth in GL is approximately eight-fold higher than during growth in RL. This increase in GL-dependent RNA accumulation is absent in both *rcaE* and *rcaC* mutant lines, demonstrating that the light color regulation of *rcaP* is controlled through the Rca system. RcaP contains three possible chromophore-binding GAF (cGMP phosphodiesterase/Adenylyl cyclase/FhlA) domains. We have demonstrated that two of these are capable of covalently attaching phycocyanobilin in *Escherichia coli*. These two domains show an unusual combination of photoreversibility properties.

P4

THE FIRST ELECTRON ACCEPTOR IN THE TYPE ONE REACTION CENTER FROM THE GREEN SULFUR BACTERIA *Chlorobium tepidum*.

Adrien Chauvet¹, Bharat Jagannathan², John H. Golbeck² and Sergei Savikhin¹

1. Department of physics, Purdue University, West Lafayette IN 47906, USA

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

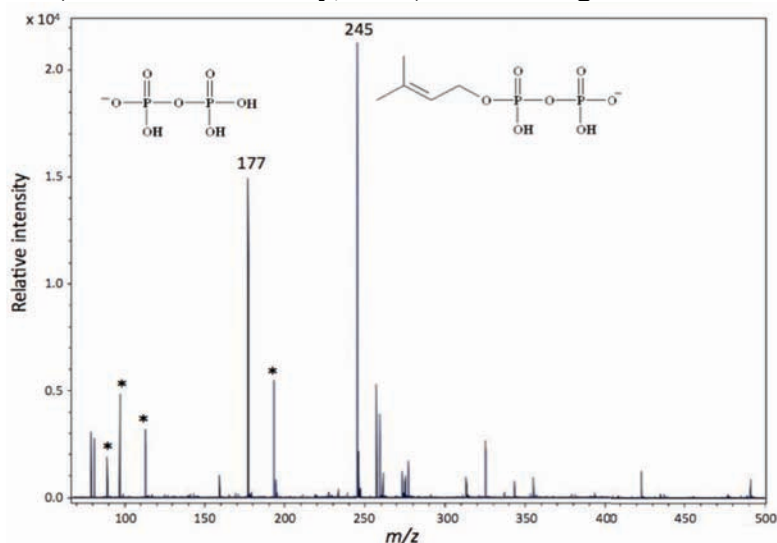
The green sulfur bacterium *Chlorobium tepidum* has one of the simplest type I reaction center (RC) complexes. While its structure is still unknown, biochemical and protein sequence analyses suggest that it is similar to photosystem I (PS I) in Heliobacteria, with two BChl *a* forming a special pair P840, four Chl *a* serving as pairs of accessory and primary electron acceptor (A0) pigments and 14 BChl *a* serving as an immediate RC antenna. This is a dramatic simplification compared to PS I RC, where 90 Chl *a* antenna pigments serve as antenna and 6 additional Chl *a* molecules function as electron transfer cofactors. The resulting spectral congestion has prevented direct visualization of ultrafast electron transfer processes within PS I RC and even the sequence of primary electron transfer processes in PS I is still under debate. The suggested presence of two types of pigments in RC from *Chlorobium tepidum* removes spectral congestion and opens a way to directly visualize electron transfer steps in type I RC using ultrafast spectroscopy, since the Chl *a* and BChl *a* pigments absorb at 670 nm and 800 nm, respectively. To confirm the proposed functional role of Chl *a* as electron transfer cofactor we performed extensive ultrafast pump-probe and steady state optical experiments on different preparations of RC complexes from *Chlorobium tepidum*, revealing energy/electron transfer rates between different groups of pigments. Surprisingly, we found that 60 % of the Chl *a* pigments do not transfer excitation energy to the BChl *a* antenna or to P840, which indicates that these pigments must be >20Å away from any other BChl *a* pigment. Moreover, upon direct excitation of the special pair, the Chl *a* signal does not exhibit the characteristics of an electron transfer observed clearly for Heliobacteria in similar conditions. The later results thus argues against the suggested presence of 4 Chl *a* in the reaction center core (RCC) complex. Additional analysis were done in order to refine the BChl *a*/ Chl *a* ratio and suggests a higher number of Chl *a* present per RC. Fluorescence analysis at room and low temperature reveals strong interaction between these active Chl *a* and the RCC, and we discuss the possible location and function of Chl *a* in the RC.

P5

PROTEOME RESPONSES OF *SYNECHOCOCCUS* sp. PCC 7002 TO HIGH-LIGHT INTENSITY AND ISOPRENE PRODUCTION VIA THE 2-C-METHYL-D-ERYTHRITOL 4-PHOSPHATE (MEP) PATHWAY

Abstract: The proteome of *Synechococcus* sp. PCC 7002 was analyzed by MALDI-TOF mass spectrometry to identify proteins involved in the response to high-light intensity and isoprene production via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. The analysis revealed a significant increase in the abundance of several proteins, including those involved in photosynthesis and carbon fixation. The presence of dimethylallyl diphosphate (DMAPP) was confirmed by MALDI-TOF mass spectrometry, indicating active isoprene production via the MEP pathway.

The ‘Proteome’ refers to all of the proteins of an organism or cell type at a defined instant. Comparative proteome analyses are useful for elucidating protein functions and interactions, and can be performed via mass spectrometry (MS) to measure mass-to-charge ratios (m/z) of peptide ions and fragmentation products. These data are compared against databases to identify proteins. MS signals are not quantitative because of variable ionization efficiencies. However, metabolic labeling of proteins with stable, heavy isotopes such as ^{15}N allows for relative quantification. Cyanobacteria are photosynthetic prokaryotes that require only light, atmospheric CO_2 , and inorganic nutrients for growth. They play key roles in global carbon and nitrogen cycling, oxygen production, and are of great interest for carbon-neutral biofuels. The cyanobacterium *Synechococcus* PCC 7002 is particularly interesting, because of its fast growth rate, amenability to genetic manipulation, and tolerance of salt, nutrient-deprivation, and extremely high light intensities. We are interested in mechanisms by which *Synechococcus* adapts to high-light intensity. This is a question of intrinsic biological interest and important for biofuels applications. We have introduced genes into *Synechococcus* for isoprene production via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (see abstract by Nelson et al.). Isoprenoids produced via this pathway are also important precursors for quinones, carotenoids, and chlorophylls. Thus we postulate that down-regulation of some of these competing pathways and incubations at high-light intensity might allow viable production of isoprene as a commercial feedstock. Toward that end, ^{15}N metabolic-labeling studies are in progress to investigate proteomic responses of *Synechococcus* 7002 to high-light exposures. Matrix-ion free MALDI-MS (Edwards & Kennedy, 2005) is also being used to identify metabolites of the MEP pathway.



MALDI-TOF mass spectrum of MEP pathway metabolite dimethylallyl diphosphate (DMAPP) using 9-aminoacridine as the matrix. Spectrum was obtained with a Bruker Reflex IV MALDI-TOF mass spectrometer in negative-ion mode. * indicates matrix peaks. Metabolites were identified using the online METLIN database. $[\text{M}-\text{H}]^-$ values for DMAPP = 245 m/z and pyrophosphate = 177 m/z .

P6

ALTERNATIVE COMPLEX III IN THE ELECTRON TRANSFER CHAIN OF THE PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

Xinliu Gao, Yueyong Xin, Jianzhong Wen, Patrick D. Bell, Robert E. Blankenship
Departments of Biology and Chemistry, Washington University in St. Louis, MO, 63130

The green photosynthetic bacterium *Chloroflexus aurantiacus* does not contain a cytochrome *bc* complex. Earlier work identified a potential substitute for the cytochrome *bc* complex, the menaquinol:auracyanin oxidoreductase, also named Alternative Complex III (ACIII). This complex has been purified, identified and characterized from *C. aurantiacus*. ACIII is an integral membrane protein complex with mass ~300 kDa. It contains 7 different subunits, including a 113 kDa FeS-containing polypeptide, a 25 kDa multi-heme *c*-containing subunit and a 23 kDa mono-heme *c*-containing subunit. The genes that code for the subunits are arranged in an operon in the genome. The copy number of each subunit and the number of hemes in the multiheme cytochrome subunit have been determined by LC-MS, potentiometric titration and intensity analysis of heme-stained SDS-PAGE. A preliminary structural model is proposed based on transmembrane and signal peptide analysis and chemical cross-linking combined with MALDI-TOF MS. The measurement of menaquinol:auracyanin oxidoreductase activity supports the view that ACIII functions as an electron carrier in the electron transfer chain of *C. aurantiacus*. The lack of sensitivity to the common inhibitors of the cytochrome *bc* complex suggests a different catalytic mechanism of the ACIII complex.

P7

RESOLVING THE LIPID SOLVATED PROTEIN BACKBONE OF PHOTOSYNTHETIC (MOSTLY) MEMBRANE PROTEINS USING DEEP-UV EXCITED RESONANCE RAMAN SPECTROSCOPY.

Christopher M. Halsey, Jian Xiong, Carol Roach, Renee D. JiJi and Jason W. Cooley
Department of Chemistry, University of Missouri, Columbia MO

Deep-UV excited resonance Raman (dUVRR) protein backbone vibrational modes have been shown to be sensitive to the solvation or hydration of the peptide backbone. Recently, this phenomena has been exploited to resolve discrete secondary structural domains associated with the inside and outside of large protein fibrils, which contain a solvent exposed and “water free” internal beta-sheet core. Yet, dUVRR, which has proven valuable for resolving ensemble secondary structure content and its changes in soluble proteins, has not been applied to lipid solubilized protein samples (membrane proteins) previously. Logically, the desolvation of the protein backbone should have a significantly different spectral signature than the aqueous solvated portion of a membrane protein, giving us a valuable tool for low-resolution structural analysis and potentially its changes in time. Here we present our initial findings in the analysis of membrane protein samples (many of which are photosynthetic samples) and begin to define the characteristic signatures of the lipid solvated protein backbone.

P8

HIGH PHOTO STABILITY OF STRONGLY COUPLED CHLOROPHYLL ANTENNAE AND AGGREGATES: WHAT MAKES THEM PHOTO STABLE?

Daniel A. Hartzler¹, Shigeharu Kihara¹, Hui Li², Yusuke Tsukatani², Jens Niklas³, Oleg Poluektov³, Donald A. Bryant², Sergei Savikhin¹

¹ Purdue University, West Lafayette, IN

² The Pennsylvania State University, University Park, PA

³ Chemical Sciences and Engineering Division, Argonne National Laboratory, Chicago, IL

Chlorophyll (Chl) molecules are known to produce highly toxic singlet oxygen under illumination as a result of energy transfer from their triplet excited states to oxygen. To prevent the formation of singlet oxygen, carotenoids (Car) are typically positioned close to Chl to ensure rapid triplet-triplet energy transfer from Chl to Car, which can then safely dissipate the energy of the excited states. Our recent studies revealed a new, unconventional, but very efficient photoprotection mechanism in strongly coupled natural and artificial light-harvesting complexes that does not rely on the presence of carotenoids. Experimental studies on carotenoid-free chlorosomes and artificial bacteriochlorophyll (BChl) aggregates show that these structures are at least three orders of magnitude more stable to photodamage than monomeric forms of BChl. It was proposed that this photoprotection in strongly coupled arrays of pigments is due to triplet exciton formation, which lowers the energy of the triplet exciton substantially below that of singlet oxygen.

In this report we present the results of our ongoing comprehensive study of the properties of triplet excited states of monomeric (B)Chls, (B)Chl aggregates and chlorosomes by means of EPR, time-resolved optical pump-probe spectroscopy and steady-state IR phosphorescence spectroscopy.

P9

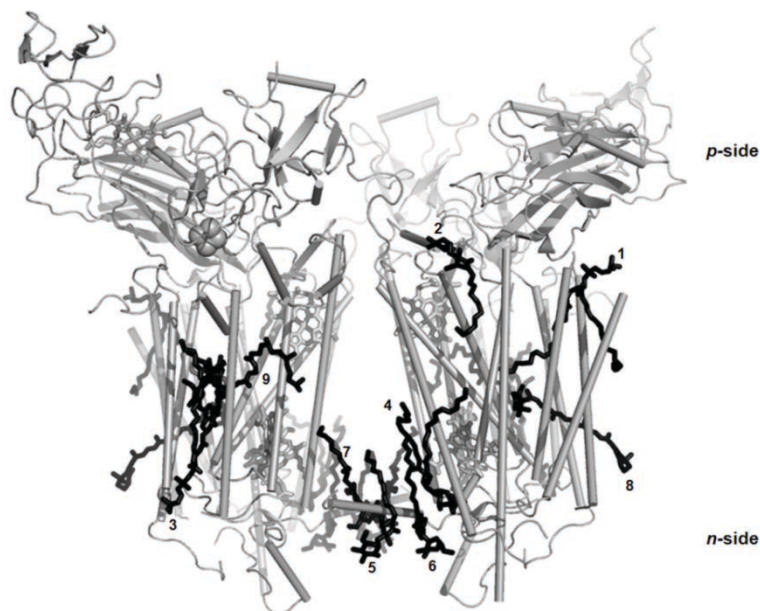
LIPID BINDING SITES IN CYTOCHROME *bc* COMPLEXES

S. Saif Hasan¹, Eiki Yamashita², William A. Cramer¹,

¹Hockmeyer Hall of Structural Biology, Dept. of Biological Sciences, Purdue University, West Lafayette, IN, USA, ²Institute of Protein Research, Osaka University, Osaka, Japan

Of the eight lipid/lipid binding sites, and two lipid-like (chlorophyll *a*, β -carotene) molecules in the hetero-oligomeric cyanobacterial^{2,6,8} or algal³ cytochrome *b₆f* complex, three lipid sites are recognized as homologous in the respiratory *bc₁* complex:⁷ (1) sulfoquinovosyl-diacylglycerol (SL, *b₆f*)-phosphatidic acid (PA, *bc₁*); (2) *n*-undecyl- β -D-maltopyranoside (UDM, *b₆f*)-cardiolipin (CL, *bc₁*); (3) UDM (*b₆f*)-PA (*bc₁*). Localization of anionic phosphatidylglycerol in the thylakoid stromal leaflet¹ and proximity of UDM (*b₆f*) to the anionic CL (*bc₁*) indicate the presence and conserved function of acidic lipid(s) at these UDM positions in the *b₆f* complex.⁴ Residues interacting with lipid head groups show conservation of hydrophobic/hydrophilic character^{4,5} and involve interactions via side chains and back-bone atoms. A fourth lipid site is shared on the *p*-side of the cyanobacterial (dioleoyl phosphatidyl choline, DOPC)^{2,6,8} and algal (natural galactolipid)³ *b₆f*. *p*-side (electropositive side of membrane) acyl tails have well resolved electron density in *b₆f*. Most *n*-side (electronegative side of membrane) acyl chains are disordered but have a hydrogen bond network between lipid/detergent head groups close to the inter-monomer cavity. This may represent a continuation of the bilayer and a pathway for quinone/quinol exchange. A defined function of peripheral lipids has been suggested by mutagenesis.⁹ Destabilization of peripheral CL- *bc₁* interaction leads to loss of *bc₁*-cytochrome *c* oxidase supercomplex stability.⁹ A β -carotene in *b₆f* lies proximal to this CL in *bc₁*. The β -carotene chain extends (~ 11Å) from the complex (disordered electron-density) and may have a role in stabilizing a *b₆f* - Photosystem I supercomplex.(Support:NIH GM38323).

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222S E21174042E7042w7622  
8 172c 7s297ri123e18 27s2  
218 072o2e18 77t772 772223t  
23277ei44272704277 2222  
cs2221e77ersi27742i4192s2  
7122 2277i 2mN7922WN2 3422  
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i322iec 2ce22y, 1, 3, 779 22g N2  
D 3422D 127042w7622  
0428 29is,77w22e1i29 22&N2  
292273 71e103r 7772TN2 432  
71923r 2e1 22e2192245 77e22  
27s121c 9229 2342718 072ot77
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References: (1) Douce and Joyard 1996; (2) Kurisu et al. 2003; (3) Stroebel et al. 2003; (4) Palsdottir and Hunte 2004; (5) Qin et al. 2007 (6) Yamashita et al. 2007; (7) Solmaz and Hunte 2008; (8) Baniulis et al. 2009; (9) Wenz et al. 2009

P10

REGULATION OF PHYCOBILISOME PROTEIN CONTENT IN RESPONSE TO LOW SULFATE CONDITIONS IN *FREMYELLA DIPLOSIPHON*

LaDonna M. Jones, Andrian Gutu, Charles R. Clark, and David M. Kehoe, Department of Biology, Indiana University, 1001 East Third Street, Bloomington IN 47405

Fremyella diplosiphon has a fascinating response to sulfate deprivation. At sulfate levels below 180 μ M, cells replace the sulfur rich phycobiliprotein isoforms PC1 and PC2 with PC3, which possess less sulfur containing amino acids. Our previous work has shown that the genes encoding PC3 are transcriptionally activated during this response, leading to the down regulation of the PC1- and PC2-encoding genes at the posttranscriptional level. Wild type cells grown in red light and sulfate-replete conditions have a PC absorption peak at 626 nm due to the PC1/PC2-containing phycobilisomes. Wild type cells grown in low sulfate conditions display a PC absorption peak at 621 nm, which is due to the PC3-containing phycobilisomes. We used this peak absorbance difference to screen for Tn5 mutants that fail to produce PC3 and/or fail to eliminate PC1/PC2 in the absence of sulfate. We have isolated and characterized a number of mutants whose PC absorption peak shift is not wild type in the absence of sulfate. We are in the process of analyzing a number of the most interesting mutants. One of these mutants is incapable of transcribing the operon encoding PC3. Six other mutants have minimal changes in PC1 and PC2 mRNA abundance levels in sulfate-replete conditions versus low sulfate conditions. Analyzing these mutants will expanding our understanding of the mechanisms through which cyanobacteria regulate their responses to low sulfate conditions.

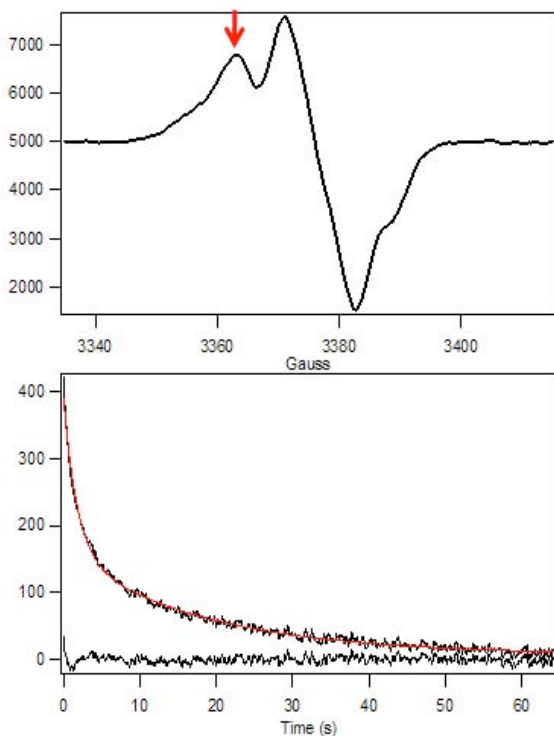
P11 PROTON COUPLED ELECTRON TRANSFER AND REDOX ACTIVE TYROSINES IN PHOTOSYSTEM II

James Keough, David Jenson, and Bridgette A. Barry

School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332

Photosystem II catalyzes the oxidation of water at a Mn_4Ca -containing oxygen-evolving center (OEC). Four flashes are required to produce oxygen from water. The OEC cycles among five S_n states, where n refers to the number of oxidizing equivalents stored. Photosystem II contains two redox active tyrosine residues, YD and YZ. These tyrosine residues are equidistant from the

Figure. (Top) EPR spectrum of the S₂YZ radical state at 190 K and pH 6.5. The arrow shows the magnetic field used for monitoring kinetics. (Bottom) EPR transient reflecting the decay of the YZ radical. The red line shows the fit, and the bottom trace is the residual.



chlorophyll donor, P680, but play different roles in catalysis. YZ is essential for oxygen evolution and mediates electron transfer between P680 and the OEC. YD is not essential for catalysis but may be involved in assembly of the OEC. YZ has an increased midpoint potential compared to YD. YZ radical is reduced by the OEC on the microsecond timescale, while YD decays on the order of minutes to hours. The placement of the OEC and neighboring amino acid side chains distinguish the two redox active tyrosines. Both tyrosines are active in proton coupled electron transfer (PCET) reactions, because when oxidized, the pK_a of the phenolic oxygen decreases. In these reactions, the nature of the proton transfer reaction may influence the rate of electron transfer. The proton and electron may be transferred simultaneously (CPET), the proton transfer may occur first (PTET), or the electron transfer may occur first (ETPT). Previously, we have shown that YD PCET mechanism changes as a function of pH. At low pH, a PTET mechanism was inferred. At high pH, the solvent isotope effect was consistent with a CPET mechanism (Jenson, D., Evans, A., and Barry, B. (2007) *J. Phys. Chem. B* 111, 12599-11264). We hypothesize that PCET mechanism distinguishes the two redox active tyrosines in PSII. To test this hypothesis, time resolved electron paramagnetic resonance was employed to study YZ.

Oxygen evolving PSII samples were prepared in the S₂ state, and a saturating flash was used to generate the YZ radical. Two kinetic phases of approximately equal amplitude were observed with rate constants of ~ 0.5 and 0.05 sec^{-1} . The decay kinetics of the radical was monitored as a function of pH in ¹H₂O buffers and as a function of p²H in ²H₂O buffers. The kinetics exhibited minimal pH dependence, and a significant isotope effect was observed at most pH values. These results may be consistent with a difference in PCET mechanism, when YZ is compared to YD. Supported by NIH GM43273.

P12

OXYGEN DEPENDENCE OF TRIPLET ENERGY TRANSFER FROM CHLOROPHYLL A TO B-CAROTENE INSIDE THE CYTOCHROME B₆F COMPLEX OF OXYGENIC PHOTOSYNTHESIS

Shigeharu Kihara¹, Stanislav Zakharov², William A. Cramer², Sergei Savikhin¹
¹Department of Physics and ²Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

The cytochrome *b₆f* (Cyt *b₆f*) complex in oxygenic photosynthesis mediates electron transfer between photosystem II (PSII) and photosystem I (PSI) and facilitates coupled proton translocation across the membrane. High resolution X-ray crystallographic structures of the Cyt *b₆f* complex show that a chlorophyll molecule (Chl) is an intrinsic component of the complex. The functional role of this Chl *a* remains unknown. However, a Chl molecule is known to produce highly toxic singlet oxygen (¹O₂) under light illumination as a result of energy transfer from its triplet excited state to oxygen. To prevent the formation of ¹O₂, carotenoids (Car) are typically positioned close (~4Å) to Chl to ensure rapid triplet-triplet energy transfer from Chl to Car, which can then safely dissipate its energy. However, the β-Car is positioned too far (~14Å) from the Chl *a* inside a Cyt *b₆f* complex to provide effective protection by the conventional Dexter-type energy transfer. From earlier work by our group, it was suggested that oxygen molecules in the intraprotein pathway of Cyt *b₆f* mediate the energy transfer from ³Chl to Car. To test this hypothesis, we tested the oxygen dependence of the energy transfer from Chl to Car using time-resolved pump-probe spectroscopy under aerobic and anaerobic conditions and also at low temperature when oxygen mobility is impeded. Since even trace amounts of oxygen could be sufficient for triplet energy transfer, we also performed a comprehensive modeling and testing of different deoxygenation techniques and developed a new protocol that allows to achieve very low oxygen levels in liquid samples with essentially no evaporation of the liquid.

P13

STRUCTURE-FUNCTION ANALYSIS OF AURACYANINS

Jeremy King¹ and Robert E. Blankenship.² ¹Graduate Program in Plant Biology ²Departments of Biology and Chemistry. Washington University in St. Louis, St. Louis, MO 63130

The auracyanins are blue copper proteins found in the filamentous anoxygenic phototroph, *Chloroflexus aurantiacus*. It is thought that these proteins mediate electron transfer in respiration and photosynthesis. *C. aurantiacus* encodes four distinct auracyanins, labeled A through D. Auracyanin C and auracyanin D were recently discovered and currently being characterized. Auracyanin A and auracyanin B share 38% amino acid identity and can be distinguished by their significantly different EPR and UV-Vis spectra. Both auracyanin A and auracyanin B show a major UV-vis peak at 600nm and a minor peak at 450nm. However, the ratio between the 450nm/600nm peaks is much larger in auracyanin A than auracyanin B. In addition, auracyanin A displays a rhombic EPR spectrum and auracyanin B displays an axial EPR spectrum. This is surprising as the two proteins show identical copper site dimensions and ligands in their crystal structures. In other blue copper proteins, the “copper loop”, a region containing the methionine, one of the histidines, and the cysteine ligands, has been implicated in modulating the spectral features. The primary sequence of the copper loops in the auracyanins is entirely different between the methionine and histidine ligands. Additionally, the auracyanin A loop is an amino acid shorter in this section. We are currently performing site-directed mutagenesis to determine the role of the copper loop in modulating the differences between the spectra of auracyanin A and auracyanin B.

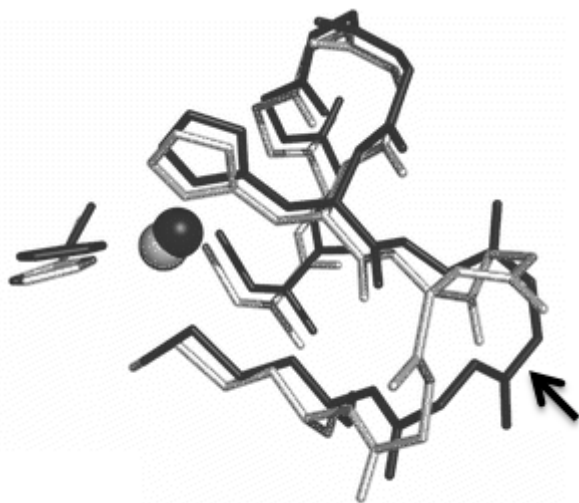


Figure: The Copper Loops of Auracyanin A (dark grey) and Auracyanin B (light grey). The copper loops show identical dimensions. The region between the methionine and histidine ligands is highlighted with the arrow. Auracyanin A is displaced upward for clarity.

P14

ELUCIDATING THE PROTEIN'S ROLE IN COHERENT ENERGY TRANSFER IN THE PHOTOSYNTHETIC REACTION CENTER

Phillip D. Long, Elad Harel, and Gregory S. Engel

Biophysical Sciences Program, Department of Chemistry, and The James Franck Institute
The University of Chicago, Chicago, Illinois

Photosynthetic bacteria utilize highly sophisticated mechanisms to channel excitation energy to reaction center complexes within the cell membrane. Although often associated with the subsequent electron transfer which drives synthesis of ATP, the reaction center itself serves as an effective model complex to study energy transfer prior to the primary charge separation event. The chromophores embedded within the *Rhodobacter sphaeroides* reaction center exhibit three distinct excitons, or delocalized excited states, named P (primarily special pair bacteriochlorophylls), B (primarily accessory bacteriochlorophylls), and H (primarily bacteriopheophytins). While past work has suggested the role of quantum transport mechanisms within the reaction center complex, definitive room-temperature data involving all three excitons remains elusive. Furthermore, the role of the protein matrix in such quantum transport must be clarified. Here we present preliminary data from a new method to capture the energy transfer dynamics of all three excitons simultaneously using broadband two-dimensional electronic spectroscopy with femtosecond resolution. Upon characterizing the behavior of wild-type reaction centers, we demonstrate methods for pinpointing the level of evolutionary fine-tuning in the protein matrix by manipulating the complex locally, regionally, and globally.

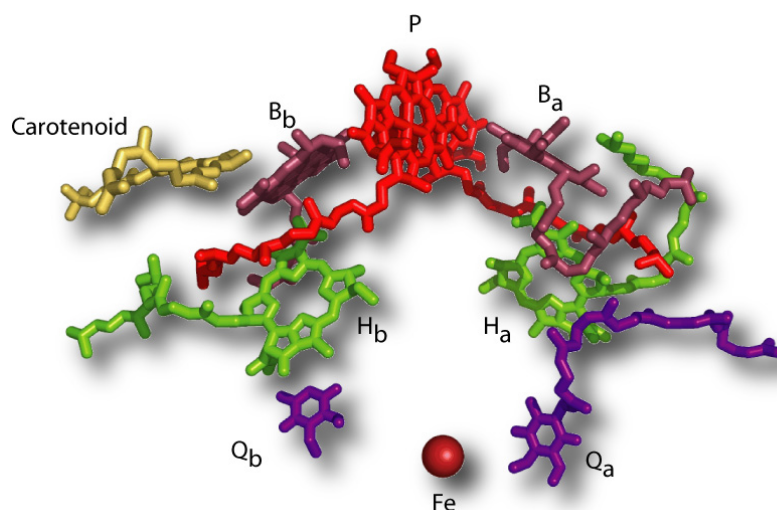


Figure 1: Chromophores within the reaction center. The reaction center enables both energy transfer toward the special pair (P) as well as electron transfer away from the special pair toward the quinone (Q_a). Observing the coupling to the special pair and wavefunction collapse at the special pair remains a spectroscopic challenge demanding new techniques.

P15

INACTIVATION OF *rpaA*, A RESPONSE REGULATORY GENE IN *SYNECHOCYSTIS* SP. PCC 6803 LEADS TO A LIGHT-SENSITIVE PHENOTYPE.

Waqar Majeed, Yong Xue and Qingfang He

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

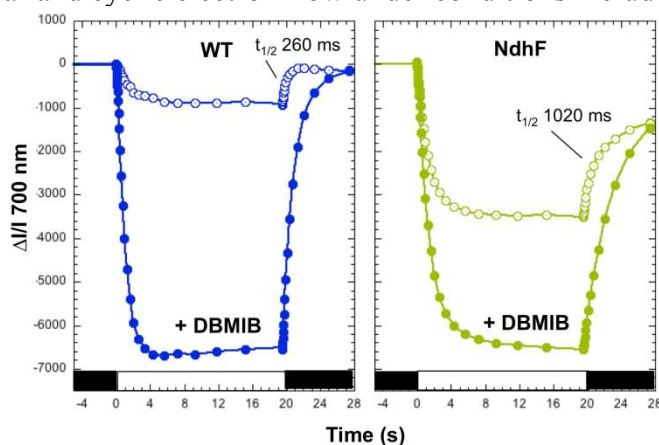
The response regulatory gene *rpaA* (*slr0115*) was discovered through genetic screening aimed to find genes that affect the expression of *hliA*. The gene was studied by targeted mutagenesis in *Synechocystis* sp. PCC 6803. Homoplasmic mutant, designated *rpaA*⁻, was obtained. The mutant was found to grow slower under photoautotrophic conditions than the wild type, and it is high light sensitive. The mutant cultures were somewhat bleached when they were exposed to HL for 3 or more days whereas wild type remained green. Pigment analysis and 77K fluorescence results revealed *rpaA*⁻ accumulate more carotenoid per chlorophyll basis and have an impact on PSI assembly which caused decreased PS1/PS2 ratio in whole cell and thylakoid membrane. However, total carotenoid content per optical density at 730nm was reduced in the mutant cells grown in high light as compared to wild type. Furthermore, the 77K fluorescence analysis of PSI trimer, PSII and PSI monomer revealed decreased PSI fluorescence in the mutant. Western blot analysis of PSII and PSI monomer for PSI associated protein, revealed lesser *psaD* accumulation, suggesting the heavy impact on PSI assembly under high light conditions. RT-PCR analysis of the mutant revealed that the *psaA*, *crtB*, *crtR* genes were up regulated following exposure to high light. It also revealed that the RpaA is a regulator of *psaA*. These results suggest that the regulatory gene *rpaA* is important for cell fitness and for modulating the expression of photosynthetic genes under high light conditions.

P16

ROLE OF THE NADH DEHYDROGENASE (NDH-1) COMPLEX IN CYCLIC ELECTRON FLOW AND PLASTOQUINONE POOL REDOX POISE IN THE MARINE CYANOBACTERIUM *SYNECHOCOCCUS* SP. PCC 7002

Anuradha Marathe¹, Jessica Dorshner¹, Alina Ott², David E. Rivera Aponte³, Brandon Thomas¹, Matthew Nelson¹ and Toivo Kallas¹. ¹Department of Microbiology, University of Wisconsin, Oshkosh; ²Department of Biology, University of Wisconsin, Stevens Point; ³Department of Biology, University of Puerto Rico, Bayamon.

Photosynthesis involves light energy capture by photosystems (PS) II and I and electron transfer between them via the plastoquinone (PQ) pool and cytochrome (Cyt) *bf* complex. A PS I cyclic electron flow also occurs that returns electrons to PS I via the PQ pool and/or Cyt *bf* complex. Recently a supercomplex has been isolated from plant chloroplasts that contains PS I – Cyt *bf* complex – ferredoxin NADPH oxidoreductase (FNR) – ferredoxin (Fd) and catalyzes cyclic electron transfer in vitro (Iwai et al., 2010 *Nature*). In cyanobacteria, the NADH dehydrogenase (NDH-I) complex oxidizes NAD(P)H and delivers electrons to the PQ pool. In darkness, NDH-1 and succinate dehydrogenase play important and seemingly different roles in cyanobacteria in maintaining PQ pool redox poise. Cyclic electron flow mediated by the NDH-1 complex appears to be the major cyclic electron route in cyanobacteria (Yu et al., 1993 *Plant Physiol.*). The relative contributions and importance of linear (which generates both ATP and NAD(P)H) and cyclic (which generates ATP only) electron flow under different environmental conditions remain poorly understood. We have re-examined cyclic flow in wild type and an NdhF (NDH-I) mutant of *Synechococcus* PCC 7002 by means of a Joliot-type kinetics spectrophotometer (BioLogic JTS-10) that is well suited for these measurements. We find that the NDH-I route accounts for ~75% of cyclic flow in cells harvested from optimal photosynthetic conditions. During dark aerobic or anaerobic incubations, Cyt *bf* turnover declines in the wild type but to a much greater extent in the NdhF mutant demonstrating an important role of the NDH-1 complex in PQ redox poise under these conditions. Experiments are in progress to determine the relative contributions of linear and cyclic electron flow under conditions including high-light exposure.



Cyclic electron flow in *Synechococcus* wild type and NdhF mutant. Oxidation-reduction of PS I P700 during far red, PS I specific illumination in the presence and absence of the Cyt *bf* inhibitor DBMIB.

P17

DECAY ASSOCIATED ANALYSIS OF MULTIDIMENSIONAL SPECTROSCOPY ON PHOTOSYSTEM II REACTION CENTERS

Jeffrey A. Myers, Kristin L. M. Lewis, Franklin Fuller, Jennifer P. Ogilvie
Department of Physics and Biophysics, University of Michigan, Ann Arbor, MI 48109

The initial photophysical steps of oxygen evolution in Photosystem II (PSII) can be studied in the D1-D2 cyt.b559 PSII reaction center preparation, which retains only six Chlorophylls and 2 pheophytins. We present low temperature two-dimensional electronic spectroscopy (2DES) data of the Q_y band of the D1-D2 cyt.b559 reaction center aimed at understanding the events of energy transfer and primary charge separation in this system. In a system such as the PSII RC, where both energy and charge transfer processes lead to spectral changes over a broad range of time scales, appropriate models must correctly predict these spectral changes and their associated kinetics. To date, validating models via 2DES has consisted primarily of qualitatively comparing lineshapes and temporal behavior of simulated 2D spectra to experimentally observed spectra. In the spirit of transient absorption spectroscopy analysis, we extract kinetics directly from the spectra and compare these to the model parameters. In transient absorption spectroscopy, global-fitting of the characteristic exponential signal decay gives “decay associated spectra”, which allow kinetic models to be assigned to spectral features. In our analysis of 2D spectra we produce analogous 2D decay associated spectra (2D DAS) to reveal the excitation dependence of the kinetic processes in the PSII RC. We demonstrate that this method provides an incisive tool for examining multimetric models of the photophysics of the PSII reaction center.

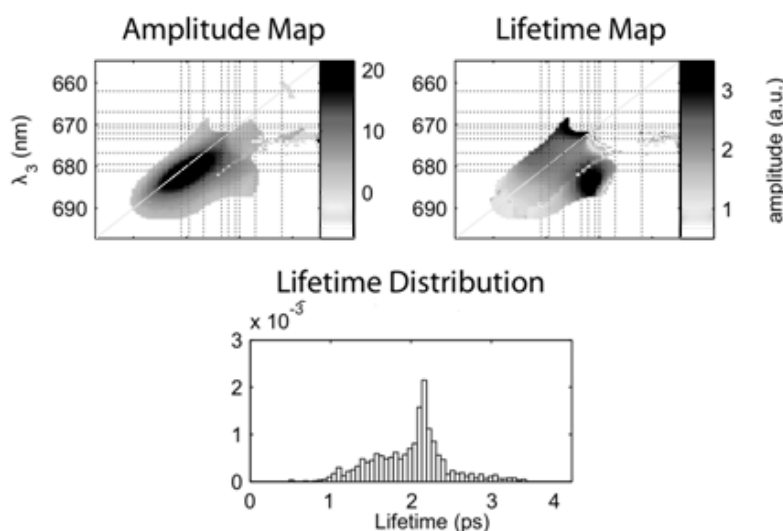


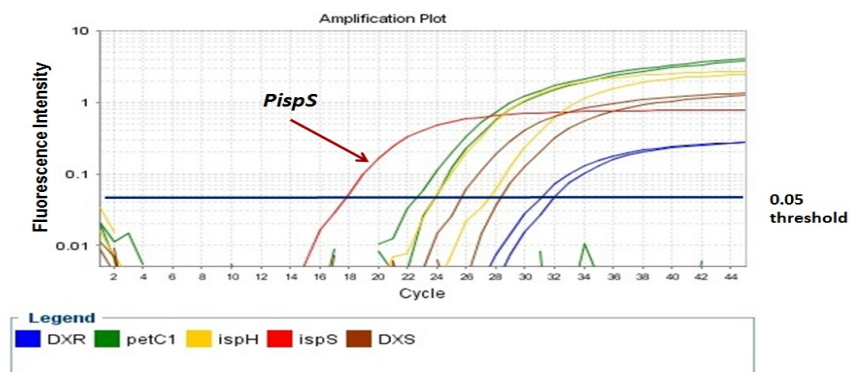
Figure 1: 2D decay associated spectrum of a selected distribution of time scales near 2 ps are shown as frequency-frequency correlated amplitudes and rates. This time scale cluster clearly indicates excitation dependence (x-axis) of the fitted decay rates, which supports recent models that posit two separate charge separation pathways.

P18

METABOLIC ENGINEERING OF ISOPRENE PRODUCTION IN THE HIGH-LIGHT, HALO-TOLERANT CYANOBACTERIUM, *SYNECHOCOCCUS* SP. PCC 7002

Matthew E. Nelson¹, George L. Weir IV¹, Jessica Dorschner¹, Amy E. Wiberley², Eric L. Singaas² & Toivo Kallas¹, ¹Department Biology-Microbiology, Univ. Wisconsin Oshkosh & ²Wisconsin Institute for Sustainable Technology, Univ. Wisconsin Stevens Point

Microalgae capture enormous amounts of solar energy and convert atmospheric carbon into polymers that hold great potential as feedstock chemicals and carbon-neutral biofuels. One such feedstock is isoprene (C₅H₈; 2-methyl 1,3-butadiene), a monomer of natural rubber, currently made industrially from petroleum. Isoprene is volatile and ideally suited for capture from the head-space of algal bioreactors. Our goal is to develop a process for isoprene production in *Synechococcus* PCC 7002, a rapidly growing, high-light and halotolerant cyanobacterium. Isoprene production has been demonstrated in *Synechocystis* sp. PCC 6803 (Lindberg *et al.*, 2009 *Metabolic Engineering*). In plants, isoprene is produced from dimethylallyl diphosphate (DMAPP) by the enzyme isoprene synthase (IspS). DMAPP is made via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which is conserved among plants and many bacteria. Cyanobacteria lack the crucial *ispS* gene. We used a recombination strategy to target forms of the *ispS* gene from poplar to the high copy plasmid pAQ1 of *Synechococcus* (Xu *et al.*, 2009 *Photosynth Res Protocols*). The *ispS* genes are controlled by the strong c-phycocyanin β -subunit promoter (*cpcB*) of *Synechocystis* 6803. Introduction of the *ispS* genes into *Synechococcus* has been confirmed by PCR. Reverse transcriptase quantitative PCR (RT-qPCR) data show *ispS* gene expression in *Synechococcus* at high levels, ~32-fold higher than *petC1* for the major Rieske iron-sulfur protein. Gas chromatography, mass spectrometry (GC-MS) is in progress to evaluate isoprene production. Mass spectrometry is also being used to assess enzymes and metabolites of the MEP pathway. We anticipate that metabolic engineering and culture strategies will enable viable isoprene production in the robust, *Synechococcus* PCC 7002 strain.



RT-qPCR amplification plot of selected MEP pathway genes. *petC1* was used as a control for MEP pathway genes. Note that *PispS* reaches an arbitrary fluorescence threshold almost 5 cycles sooner than *petC1* indicating approximately 32-fold higher expression relative to the highly expressed *petC1*.

P19

GENERATING MOLECULAR HYDROGEN WITH COBALOXIME LINKED TO PHOTOSYSTEMS – A MULTI FREQUENCY EPR STUDY OF COBALOXIME'S ELECTRONIC STRUCTURE

Jens Niklas, Rakhim R. Rakhimov, Karen L. Mulfort, Kristy L. Mardis[#], David M. Tiede, Oleg G. Poluektov

Division of Chemical Sciences and Engineering, Argonne National Laboratory, Argonne, IL 60439

[#]Department of Chemistry and Physics, Chicago State University, Chicago, IL 60619

Molecular hydrogen is generally considered as an ideal energy source, being essentially pollution-free. The electrons needed for this process can be provided by Photosystems linked to hydrogen catalysts. Cobaloximes (Cobalt complexes with diglyoxime ligands) rank among the most promising transition metal complexes for the reduction of protons to molecular hydrogen. Their catalytic properties are strongly dependent on the surrounding solvent, and in particular on the direct ligand(s) to the central metal ion. The knowledge of the electronic properties is essential for an understanding of the catalytic properties of the complex. Electron Paramagnetic Resonance (EPR) is an excellent tool to achieve this goal.

In this work, the cobaloxime $\text{Co}(\text{dmgBF}_2)_2$ has been studied in a variety of different solvents by multi-frequency EPR spectroscopy at X-band (9 GHz), Q-band (34 GHz), and D-band (130 GHz) microwave frequencies. The multi-frequency approach allows us to determine g-factor anisotropy and hyperfine splitting due to the central metal (^{59}Co) and coordinating solvents/ligands (L) that have ^{14}N , ^{15}N magnetic nuclei. Two basic types of Co(II) coordination can be distinguished, five-coordinated $\text{LCo}(\text{dmgBF}_2)_2$ and six-coordinated $\text{L}_2\text{Co}(\text{dmgBF}_2)_2$, the former having substantially greater g-factor anisotropy. The influence of the nature of the ligand, nitrogen or oxygen is also investigated. The results obtained experimentally are correlated with DFT calculations on $\text{Co}(\text{dmgBF}_2)_2$ in different surroundings.

P20

BISUBSTRATE ACTIVATION OF OXYGEN EVOLUTION ACTIVITY IN PHOTOSYSTEM II BY CALCIUM AND CHLORIDE IONS

Brandon M. Ore, Rachel A. Reed, and Alice Haddy

Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402

Photosystem II (PSII), which produces molecular oxygen from water in higher plants and cyanobacteria, requires calcium and chloride ions as inorganic cofactors. Calcium is known to be complexed as a part of the catalytic Mn_4Ca cluster at which O_2 is produced, while one or two chloride ions are believed to bind approximately 7 Å away from the Mn_4Ca cluster. In this study, the interdependence of calcium and chloride in activating oxygen evolution was examined in PSII-enriched membranes from which the PsbP (23 kDa) and PsbQ (17 kDa) extrinsic subunits had been removed by NaCl washing. For study of calcium dependence of activity at varying chloride concentrations, NaCl-washed PSII preparations were further depleted of calcium by exposure to light and EDTA. It was found that the maximum calcium-dependent activity increased as the chloride concentration increased and vice versa. The interdependence of the O_2 evolution activity on calcium and chloride concentrations was analyzed using a bisubstrate enzyme kinetics model, treating the two ions as substrates. The data were observed to be consistent with a ternary complex mechanism, as expected, although a distinction between random and ordered sequential models could not yet be made. For the study of calcium dependence at varying chloride concentrations, the Michealis constants (K_M) were found to be 0.26 mM for Ca^{2+} and 2.9 mM for Cl^- . The data were complicated by some apparent substrate inhibition by calcium at low chloride concentrations. Results that were similarly indicative of bisubstrate enzyme kinetics were found for the study of chloride dependence of activity at varying calcium concentrations. These results emphasize the importance of considering the effects of the second ion when either calcium or chloride activation of O_2 evolution is under study. (This work was supported by the National Science Foundation and the UNCG Office of Research).

P21

CONSEQUENCES OF MODIFIED ANTENNA SYSTEMS IN CYANOBACTERIA

Lawrence Page, Michelle Liberton, and Himadri B. Pakrasi
Department of Biology, Washington University, St. Louis, MO 63130

Light harvesting in cyanobacteria is accomplished by a combination of large peripheral phycobilisome antenna complexes and integral antenna closely associated with the reaction center. In order to examine the consequences of modified antenna systems on growth and cellular function, we used a *Synechocystis* PAL mutant in which genes necessary for phycobilisome assembly and function have been deleted (1). It has been suggested that in photosynthetic organisms such as green algae, down regulation of light harvesting capability results in increased cell growth and photosynthetic efficiency (2). In order to determine if this is the case in cyanobacteria, we used an optimized photobioreactor system (3) for precise regulation of growth parameters and collected data over a wide range of culture conditions, including different CO₂ and light regimes, for both wild-type (WT) and PAL cultures. Figure 1 compares results obtained from ambient and 2% CO₂ growth conditions. Our data show that lack of phycobilisome antenna do not provide a substantial advantage to *Synechocystis* cells under any of the conditions tested. These studies aid our understanding of how light harvesting is accomplished in cyanobacteria and the impact of antenna function on photosynthetic efficiency.

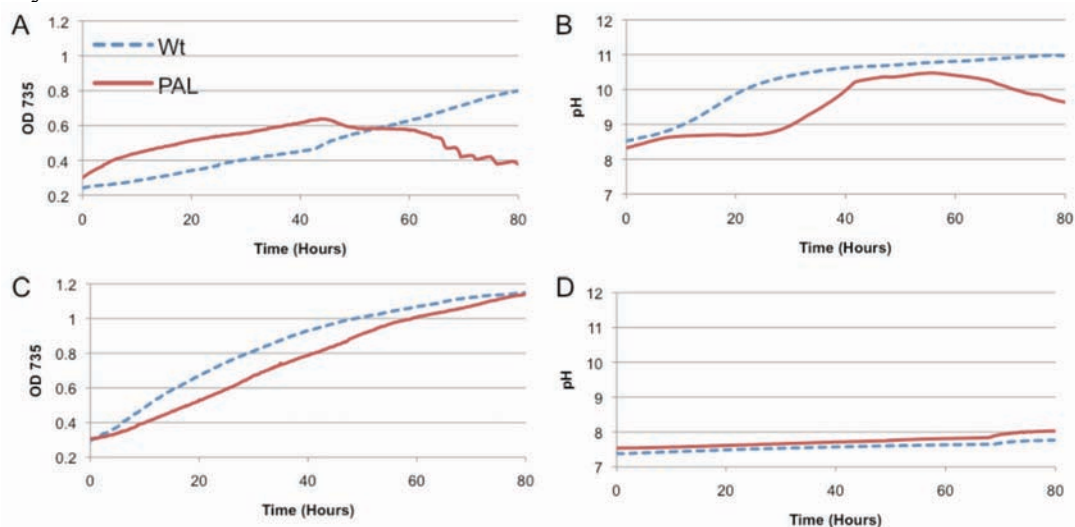


Figure 1. Cell growth and pH of WT and PAL cultures in ambient and high CO₂ conditions. With air bubbling, growth inhibition of PAL compared to WT (A) coincided with a rapid rise in pH (B). Growth in 2% CO₂ (C) stabilized pH (D) and allowed PAL to reach growth levels similar to WT.

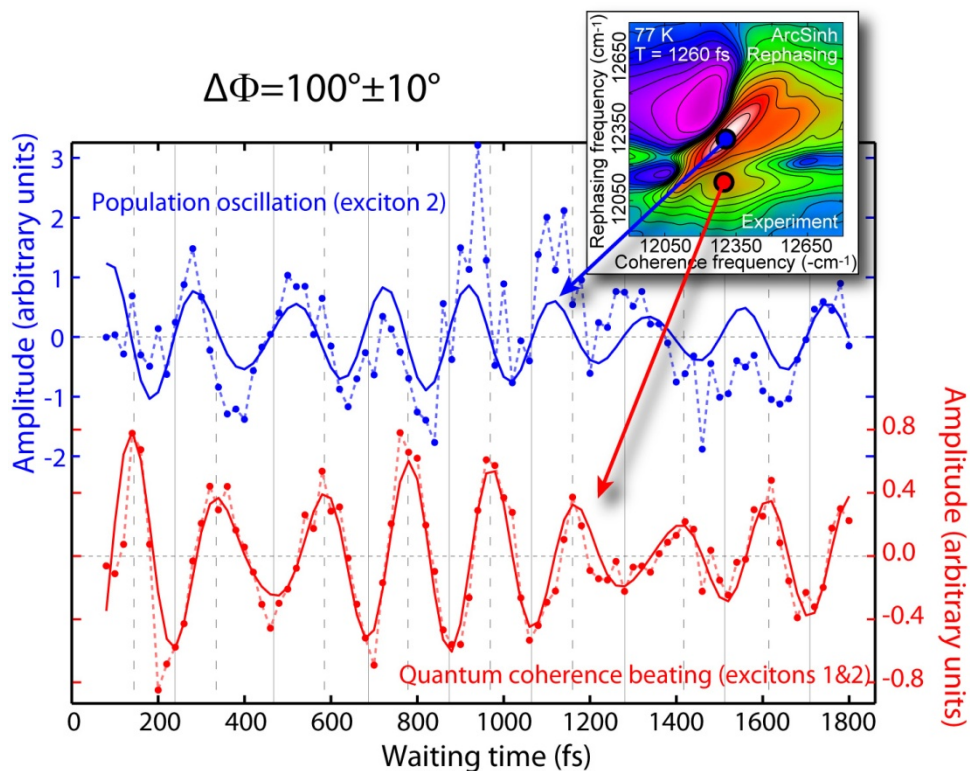
1. Ajlani and Vernotte (1998) *Plant Mol Biol* 37: 577-80.
2. Mussnug et al. (2007) *Plant Biotechnol J* 5: 802-14.
3. Nedbal et al. (2008) *Biotechnol Bioeng* 100: 902-10.
4. This work was supported by the U.S. Department of Energy, Grant DE-SC0001035, through the Energy Frontier Research Centers (EFRCs): Photosynthetic Antenna Research Center (PARC) at Washington University in St. Louis.

P22

QUANTUM TRANSPORT IN PHOTOSYNTHETIC LIGHT HARVESTING COMPLEXES

Gitt Panitchyankoon, Dugan Hayes, Kelly Fransted. and Gregory S. Engel
Department of Chemistry and The James Franck Institute, The University of Chicago,
929 E. 57th Street, Chicago, IL

Quantum effects in biology have been widely postulated to enhance energy transfer in photosynthetic complexes. Currently, no experimental evidence exists to support the claim. Performing two-dimensional electronic spectroscopy on the Fenna-Matthews-Olson (FMO) complex, we probe the existence of quantum transport in the photosynthetic energy transfer pathway. Our experimental data demonstrates a coupling between population and coherence, which enables mixing among the two. This coupling leads to a significant increase in coherence lifetime and to oscillations in the probability of observing an excited state (population). The result provides direct evidence of quantum transport in FMO. The observed dynamics can arise only when the excited states mix with the protein bath dynamically. Because the excited states of FMO are non-degenerate in energy, oscillating populations necessarily implies an oscillation of excitonic energy. The system directly trades energy with the protein bath. This time-dependent mixing of "system" and "bath" demands a rethinking of the nature of electronic excitations and their dynamics in complex environments.



P23

A SINGLE MUTATION PROVIDES INSIGHTS INTO THE EVOLUTION OF PsbO, THE PHOTOSYSTEM II MANGANESE STABILIZING PROTEIN

Hana Popelkova¹, Aaron Wyman², and Charles Yocum¹

¹Departments of MCD Biology and Chemistry, University of Michigan, Ann Arbor, MI 48109, ²Biology Department, Aurora University, Aurora, IL 60506

High rates of O₂ evolution require PsbO, a 26.5 kDa extrinsic protein that is tightly bound to photosystem II (PSII). This family of proteins shows a high degree of sequence identity in all species that have been examined so far. An N-terminal sequence truncation accounts for the lack of a second copy of PsbO in cyanobacteria, but overall these proteins contain about the same number of amino acids. Deletion of 18 N-terminal amino acids drastically weakens the binding of spinach PsbO to PSII, and insertion or deletion of a single amino acid into the sequence of the cyanobacterial protein has a similar effect. A possible explanation for this behavior may be due to the necessity to maintain interactions between the C- and N-termini of these proteins as they fold and bind to PSII. The C-termini of PsbO's exhibit a high degree of sequence identity. The conserved cyanobacterial phenylalanine (F) residue is replaced by tryptophan (W) in eukaryotes. A W241 → F mutation in spinach PsbO (Figure 1) exhibits weakened binding and ineffective restoration of O₂ evolution, as well as increased thermostability, which is unusual given the conservative nature of the substitution. We have now shown that truncation of the N-terminus of this spinach mutant by six residues to create Δ L6MW241F recovers binding and activity and restores structural flexibility in solution. We propose that in cyanobacteria a truncated N-terminus allows the presence of F at the C-terminus, because it prevents an excessively strong interaction between the N- and C-termini that is likely the cause for a rigid structure in the spinach W241F PsbO mutant. Therefore, replacement of F with W is necessary in the eukaryotic PsbO protein with the extended N-terminus to maintain the solution flexibility required for competent assembly of PsbO into PSII.

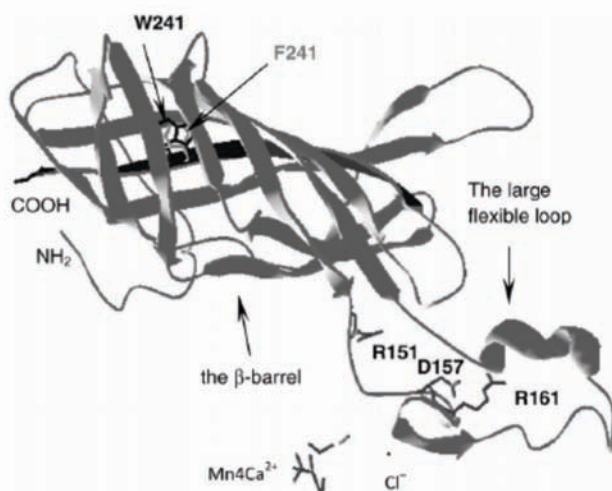


Figure 1. Overlap of the 3D-homology models of WT PsbO and the W241F PsbO mutant from spinach in the PSII-bound form. The crystal structure of *T. elongatus* PsbO [PDB entry 3bz2] was used as a template (not shown). The N- and C-termini are labeled along with the location of F or W near the C-termini of the proteins.

P24

STUDY OF COMPARATIVE GROWTHS OF CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803 UNDER PHOTOTROPHIC, AUTOTROPHIC AND MIXOTROPHIC CONDITIONS ON GLUCOSE AND XYLOSE

Saurabh J Ranade and Qingfang He

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

Xylose is a major building block of hemicellulose which consists of 30% of the plant matter. In this study, ability of cyanobacterium, *Synechocystis* sp PCC 6803 to utilize xylose in comparison with glucose and photoautotrophy was studied with the objective of further exploiting it for conversion of plant based matter into bioenergy. To compare the growth patterns of the organism under given conditions; in first set of experiment, flasks containing BG-11 as control and BG-11 plus glucose (ranging from 5mM to 150mM) were maintained in triplicates under LAHG conditions at 30⁰C as well as under optimal light (50 μ M m⁻²s⁻¹) with ample aeration. In second set of experiment, flasks containing BG-11 as control and BG-11 BG-11 plus xylose (ranging from 5mM to 150mM) were maintained in triplicates under LAHG conditions at 30⁰C as well as under optimal light (50 μ M m⁻²s⁻¹) with ample aeration. Optical densities of the cultures were tracked every 15-24 hours. When optical density of a culture exceeded 0.6, pigment contents were measured followed by the measurement of oxygen evolution. For both sets of the experiment, apart from the growth curves, graphs were plotted to indicate ability of *Synechocystis* sp PCC 6803 to utilize or withstand the sugar concentrations. Ability of the strain to utilize remaining building blocks of hemicellulose and the prospective of cloning cellulose and/or hemicellulose degrading genes will be tested in near future.

P25

DEVELOPMENTAL DEFECTS IN ARABIDOPSIS MUTANTS OF THE PSBP DOMAIN 5 (PPD5) PROTEIN

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

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

Plants contain an extensive family of PsbP-related proteins termed PsbP-like (PPL) and PsbP domain (PPD) proteins, which are localized to the thylakoid lumen. The founding member of this family, PsbP, is an established component of the Photosystem II (PSII) enzyme, and the PPL proteins have also been functionally linked to photosynthetic processes. However, the functions of the PPD proteins remain poorly understood. To elucidate the function of the PPD5 protein in Arabidopsis (At5g11450), we have characterized T-DNA insertion lines and RNAi knockdown lines of this gene. While these *ppd5* mutants do not have notable defects in photosynthetic electron transfer, they show striking plant development and morphology defects. The *ppd5* mutants flower earlier relative to wild-type plants and display defects related to axillary bud formation. These include the formation of additional rosettes originating from axils at the base of the plant as well as aerial rosettes formed at the axils of the first few nodes of the shoot. Possible mechanisms by which the PPD5 protein might function as a negative regulator of axillary bud development are discussed.

P26

TYPE IV CHROMATIC ACCLIMATION IN *SYNECHOCOCCUS* sp. RS9916

Animesh Shukla¹, Andrian Gutu¹, Avijit Biswas², Wendy M Schluchter², Frederic Partensky³ and David M. Kehoe¹

¹*Department of Biology, Indiana University, 1001 E. Third Street, Bloomington, IN 47405, U.S.A*

²*Department of Biological Sciences, University of New Orleans, LA 70148, U.S.A.*

³*Station Biologique CNRS, Roscoff, France*

Synechococcus sp. RS9916 belongs to a group of marine cyanobacteria that plays a major role in global oxygen and carbon cycles. In changing light color conditions, this organism optimizes its light harvesting efficiency by undergoing a process called Type IV chromatic acclimation (CA4). Unlike other known acclimation processes, CA4 involves varying the accumulation of the light harvesting chromophores, phycoerythrobilin (PEB) and phycourobilin (PUB) within the phycobilisome rods. Green light (GL) absorbing PEB accumulates during growth in GL, while the blue light (BL) absorbing PUB accumulates during growth in BL.

We are working to better understand the molecular physiology and regulation of CA4 in *Synechococcus* sp. RS9916. Toward this end, we have compared the transcriptomes of cells grown in GL versus BL in order to reveal the differential expression of genes during CA4. We have identified about 80 genes that are significantly up-regulated in BL growth and about 25 genes that are significantly up-regulated in GL growth. These data suggest that CA4 must be regulated, at least in part, at the transcriptional or post transcriptional level.

We have also studied the temporal expression pattern of some of the genes that show significant up-regulation during the changing light conditions. The RNA accumulation patterns of genes that are up-regulated early in the acclimation process are being used to generate an action spectrum for the CA4 response.

We also seek to uncover the mechanism behind BL absorbing PUB biosynthesis. We have identified potential PEB-PUB lyase-isomerase that is approximately 6 fold up-regulated in BL. In addition, we have recently developed a method to transform *Synechococcus* RS9916. We currently can introduce autonomously replicating plasmid, and we are working to achieve allelic replacement via homologous recombination and transposon mutagenesis to identify genes encoding components that regulate CA4 response.

P27

LIGHT AND NEUTRON SCATTERING OF PHOTOSYNTHETIC ANTENNAS

Joseph K.H. Tang¹, Liying Zhu², Volker S. Urban³, Jianzhong Wen¹, Aaron M. Collins¹, Pratim Biswas², Robert E. Blankenship^{1*}

¹Department of Biology and Department of Chemistry, ²Department of Energy, Environmental and Chemical Engineering, Washington University, St. Louis, MO 63130 USA, and ³Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

Green photosynthetic bacteria harvest light and perform photosynthesis in low light environments, and contain specialized antenna complexes to adapt to this condition. Here, we first present small-angle neutron scattering (SANS) studies to elucidate structural information about the photosynthetic apparatus, including the peripheral antenna chlorosomes, the integral membrane light-harvesting B808-866 complex, and the reaction center (RC) in the thermophilic green phototrophic bacterium *Chloroflexus aurantiacus*. Using contrast variation, SANS suggests that (1) the B808-866 complex is wrapped around the RC; (2) the overall size and conformation for the B808-866 complex of *Cfx. aurantiacus* is roughly comparable to the LH1 complex of the purple bacteria; (3) the RC of *Cfx. aurantiacus* is smaller than the RC of the purple bacteria; and (4) chlorosomes are a lipid body with rod-like shape (Tang et al, (2010) *Biophysical Journal*, in press). Moreover, our recent studies indicate that chlorosomes remain intact up to 75 °C, and that salt induces the formation of chlorosomal aggregates. No internal structural changes are suggested for the aggregates. The formation of salt-induced aggregation is more efficient with divalent metal ions than with monovalent metal ions. With heat treatment at 98 °C for 2 min, chlorosomes are undamaged and the baseplate complex is dissociated. Chlorosomes without the baseplate remain rod-like shape and are 30-40% smaller than with the baseplate attached. Also, chlorosomes are stable within pH 5.5 to 11.0. Together, our report demonstrates that chlorosomes are highly heat-resistant, stable in wide pH range, and excellent candidates for developing biomimetic solar cell nanodevices.

P28

USE OF A GENETIC SCREEN TO UNCOVER THE COMPONENTS OF THE CGI REGULATORY SYSTEM OF COMPLEMENTARY CHROMATIC ACCLIMATION IN *FREMYELLA DIPLOSIPHON*

Lisa B. Wiltbank, Andrian Gutu, David M. Kehoe
Department of Biology, Indiana University, Bloomington, IN 47405

Phycobilisomes in cyanobacteria aid in the efficient use of light from the environment for photosynthesis. By controlling expression of the phycobiliproteins which make up the phycobilisomes, cyanobacteria gain the ability to optimally utilize a variety of wavelengths of light for photosynthesis. A well-known example of this is that of complementary chromatic acclimation (CCA), which occurs in many cyanobacteria, including the aquatic species *Fremyella diplosiphon*. During CCA, cells express the genes for production of the phycobiliprotein phycocyanin (PC) in red light growth conditions and express the genes for production of phycoerythrin (PE) in green light growth conditions. In *F. diplosiphon*, switching between production of PC and PE in red versus green light is regulated by the well-characterized Rca signal transduction pathway. In cells where the Rca system is disrupted, genes involved in the production of PC are expressed at the same low level in red and green light. This suggests that the regulation of PC is solely under the control of the Rca system. However, even in Rca-disrupted cells, the expression of PE-related genes is three-fold lower in red light than in green light. Thus, another system is postulated to contribute PE inhibition in red light.

We have developed and are conducting a mutant screen to find components of this second system: the Cgi system. Our molecular studies of the Cgi system demonstrated that it represses PE production in red light-grown cells. Therefore, after transposon mutagenesis of Rca-deficient *F. diplosiphon*, we screened for cells that displayed a brown phenotype in red light, indicative of increased production of PE. Through this screen, we have identified four genes, which we consider to be strong candidates for components of the Cgi system. Future research is needed to determine if these putative components function in the Cgi system and how they operate together to regulate production of PE in red light.

P29

DE NOVO PRODUCTION OF RESVERATROL IN METABOLICALLY ENGINEERED ESCHERICHIA COLI

Yong Xue, Ronnie C Ridley and Qingfang He

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

Trans-Resveratrol, a polyphenol compound, is an important plant secondary metabolite produced under stress conditions. It is known to exert beneficial effects on human health for its anticancer, anti-toxic and anti-inflammatory functions. Resveratrol can be synthesized either from phenylalanine or from tyrosine by plants. Three enzymes are involved in the pathway that converts tyrosine to resveratrol, i.e. TAL (Tyrosine ammonia-lyase), 4CL (Coumaroyl-CoA ligase), and STS (Stilbene synthase). Cyanobacteria *Synechocystis* is photoautotroph which can be used as a 'photo-bioreactor' for biosynthesis of nutraceuticals after genetic modifications. In this study, codons of the genes encoding TAL (from *Saccharothrix espanaensis*), 4CL (from *Nicotiana tabacum*) and STS (from *Vitis vinifera*) were optimized to enhance their expression in *Synechocystis* sp. PCC 6803. Modified TAL, 4CL and STS genes were assembled together into an expression plasmid pACYCDuet-1, which was transformed into *E. coli* BL21 DE3 strain. Resveratrol was detected by HPLC and verified by LC/MS from culture medium of *E. coli* growing in 2xYT medium without adding any substrates. Formation of p-coumaric acid, the immediate precursor for resveratrol biosynthesis, was also found, and its amount decreased rapidly as biosynthesis of resveratrol proceeds. Construction of *Synechocystis* sp. PCC 6803 mutant expressing these three genes is in progress. This work demonstrates that microorganisms are highly suitable for resveratrol bioproduction.

P30

IDENTIFICATION AND PURIFICATION OF *HELIOBACTERIUM MODESTICALDUM* NADH:MENAQUINONE OXIDOREDUCTASE AND CYTOCHROME *bc* COMPLEX

Hai Yue and Robert E. Blankenship¹

Departments of Biology and Chemistry, Washington University in St. Louis, MO 63130

Heliobacteria (Hb) are unique anaerobic anoxygenic photoheterotrophs. In terms of carbon metabolism, Hb are obligately heterotrophic. Phylogenetically, Hb are the only phototrophic organisms that group within the bacterial phylum Firmicutes, most of which are Gram positive. Finally, Hb are unique among all phototrophs in that they produce endospores. Hb also contain the simplest known photosystem, with a homodimeric core reaction center (RC) complex and no peripheral antenna. Finally, sequence comparisons suggest that the heliobacterial cytochrome *bc* complex is a hybrid between the *bc*₁ and *b₆f* complexes. With all of these remarkable characteristics, however, our knowledge is limited in terms of the structure and function of almost every component of the Hb photosystem. Based on previous biochemical and genomic research, we think that the NADH:menaquinone oxidoreductase (NDH-1) complex may have an important function in the photosynthetic electron transfer chain. To characterize NDH-1 in Hb, we have utilized transcriptomics and proteomics methodologies, including Real Time PCR, blue-native and SDS-PAGE, mass spectrometry and enzymatic activity assays. Based on preliminary results, we think that NDH-1 is expressed at a low level, yet is detectable within the membrane in terms of its NADH dehydrogenase activity. After having identified its existence, we are now trying to develop and optimize a protocol to purify this huge complex and further analyze it with enzymatic and biochemical techniques. For heliobacterial cytochrome *bc* complex, no biochemical purification protocol, yielding an intact enzyme, is available so far. Next, we will use spectroscopic and electrochemical methodologies to further broaden our understanding of the heliobacteria. This research is supported by the Exobiology Program of NASA.

P31

THE *SLR0551* GENE IS REQUIRED FOR PHOTOAUTOTROPHIC GROWTH UNDER CHLORIDE-LIMITING GROWTH CONDITIONS IN THE CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803

Shulu Zhang, Laurie K. Frankel and Terry M. Bricker

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

Random transposon insertional mutations were created throughout the genome of a wild-type strain of *Synechocystis* sp. PCC 6803 using the hyperactive *in vitro* transposition system EZ-Tn5/KAN-2. Upon screening of about 5,000 kanamycin-resistant transformants, 15 transposon insertional mutants were identified as chloride-requiring mutants that failed to grow or grew very poorly photoautotrophically under chloride-limiting growth conditions. The mutant 1D2, 1 of the 15 identified mutants, grew photoautotrophically in the growth medium containing chloride but not in the low chloride growth medium (<20 μM Cl⁻). However, the mutant grew photoheterotrophically in the presence or absence of chloride in the growth medium at comparable rates to its wild-type control. Addition of chloride ions into the low chloride medium can restore the mutant's photoautotrophic growth ability. Through inverse PCR and DNA sequencing analysis, a single transposon insertion was found to occur between the nucleotides 1437 and 1438 of the *Slr0551* gene. This insertion causes a translational disruption of the Slr0551 protein, predicted to be a hypothetical protein of 640 amino acids. Genetic complementation with the full-length wild-type *Slr0551* gene confirmed that the transposon insertion into *Slr0551* resulted in the chloride-requiring growth phenotype of 1D2. Further studies were performed to examine the mutant's photosynthetic electron transport capacity. The results indicate that 1D2 cells have seriously reduced (ca. 25% of the control) photosynthetic electron transport activity. This suggests that the Slr0551 protein may be involved in photosynthetic electron transport, and the disruption of the protein in 1D2 may result in insufficient carbon fixation to support photoautotrophic growth under chloride-limiting growth conditions.

P32

TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY FOR THE STUDY OF A₁, THE SECONDARY ELECTRON ACCEPTOR IN PHOTOSYSTEM I

Nan Zhao and Gary Hastings

Department of Physics and Astronomy, Georgia State University, Atlanta, GA 30303

The A₁ binding site in photosystem I (PSI) particles from *Synechocystis* sp. 6803 is occupied by a phylloquinone (PhQ) molecule (2-methyl, 3-phytyl, 1, 4-naphthoquinone). In *menB* mutant strains, the biosynthetic pathway of PhQ is blocked and a plastoquinone-9 (PQ9) molecule occupies the A₁ binding site instead of PhQ, or the binding site is empty. By incubating *menB* mutant PSI particles in a large molar excess of PhQ (or PhQ analogues), we have shown that it is possible to incorporate fully functional PhQ into the A₁ binding site. To probe the molecular properties of the introduced PhQ (or analogue) in both the neutral and anion radical states, we have been using time-resolved FTIR difference spectroscopy. These difference spectra are somewhat convoluted, with protein bands and pigment bands overlapping. To distinguish bands associated with neutral and reduced PhQ from protein bands in time-resolved FTIR difference spectra we have adopted a variety of isotope labeling strategies. Here we describe the reconstitution of unlabeled (¹²C) PhQ into the A₁ binding site in fully ¹³C labeled *menB* mutant PS I particles. We show that by comparison of FTIR difference spectra obtained for PhQ reconstituted unlabeled and ¹³C labeled *menB* PSI particles we can distinguish bands of neutral and reduced PhQ from bands of the protein. In particular we propose that negative bands at 1661/1632 cm⁻¹ are due to the non H-bonded (C₁=O) and H-bonded (C₄=O) C=O modes of neutral PhQ. A negative band at 1593 cm⁻¹ is due to a C=C mode of neutral PhQ, and a band at 1494 cm⁻¹ is due to a C=O mode of the PhQ anion. We also propose that a positive band at 1413 cm⁻¹ is due to another C=O mode of PhQ⁻.

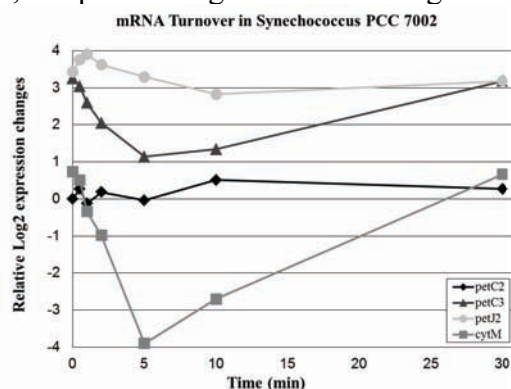
P33

REVERSE TRANSCRIPTASE QUANTITATIVE PCR ANALYSIS OF ELECTRON TRANSPORT GENES AND mRNA TURNOVER IN *SYNECHOCOCCUS* PCC 7002

Justin Zangl¹, George L. Weir IV¹, Alina Ott², David E. Rivera Aponte³, Jessica Dorschner¹, Anuradha Marathe¹, Matthew Nelson¹ and Toivo Kallas¹.

¹Department of Biology-Microbiology, University of Wisconsin, Oshkosh, ²Department of Biology, University of Wisconsin, Stevens Point

Cyanobacteria, like plants and algae, obtain their energy from photosynthesis. These organisms are important for life on earth because of the high volume of oxygen they produce and the atmospheric carbon dioxide (CO₂) they consume during photosynthesis. Photosynthesis involves harvesting of light energy to drive electron transport through a series of reactions in the thylakoid membrane protein complexes photosystems II and I, and the cytochrome (Cyt) *bf* complex. Our goal is to understand how regulation of electron transfer and metabolic pathways allows cyanobacteria to adapt to changing environments. To address the role of alternative Rieske iron-sulfur proteins (PetC1, PetC2, and PetC3) of the *bf* complex and cryptic cytochromes, we used reverse transcriptase, quantitative PCR (RT-qPCR) to track the expression of these genes (*petC1*, *-C2*, *-C3*, *petJ1*, *-J2*, and *cytM*) in cyanobacteria shifted from optimal, high CO₂ photosynthesis to dark aerobic and anaerobic conditions. The *psaC* gene of PS I and the *zwf* gene for glucose-6-phosphate dehydrogenase were used as controls. Expression levels of these genes was also measured to determine how quickly mRNA turnover can occur in this organism. Dark anaerobiosis resulted in a steep decline in Cyt *bf* turnover (measured as Cyt *f/c*₆ reduction) of ~5 fold over a 12 h period and much steeper declines in the expression levels of most of the genes tested with the exception of *petC2*. These data raise interesting questions as to whether the decline in electron flow results from, depletion of reductant pools, down-regulation of Cyt *bf* proteins, or from PetC2-Cyt *bf* complexes that may be less efficient. The expression levels of some genes declined as much as 16-fold within 5 minutes of the environmental shift, indicating that mRNA turnover occurs extremely rapidly. The RT-qPCR data also validated microarray data, which showed global, steep down-regulation of most genes during dark anaerobiosis.



mRNA turnover in *Synechococcus* PCC 7002. Expression levels of selected genes in cells shifted from optimal growth conditions (3% CO₂, 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 39°C) to a lower temperature (20°C), light intensity (15 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and ambient CO₂. The expression of *cytM* changed 16 fold within 5 minutes.

List of registered participants

First	Surname	University	Email
Pegg	Anderson	Univ. of Chicago	pegg@uchicago.edu
Warren	Beck	Michigan State Univ.	beckw@msu.edu
Patrick	Bell	Washington University, St Louis	pdbell@go.wustl.edu
Adam	Bussell	Indiana Univ.	bussella@indiana.edu
Jan	Cervený	UCLA/PSI	Jan.Cervený@psi.cz
William	Cramer	Purdue Univ.	waclab@purdue.edu
Jessica	Dorschner	Univ. of Wisconsin, Oshkosh	dorscj55@uwosh.edu
Tina	Dreaden	Georgia Tech	tdreaden@gatech.edu
Greg	Engel	Univ. of Chicago	gsengel@uchicago.edu
Franklin	Fuller	Univ. of Michigan	fullerf@umich.edu
Xinliu	Gao	Washington University, St Louis	xgao@go.wustl.edu
Alice	Haddy	UNC Greensboro	aehaddy@uncg.edu
Elad	Harel	Univ. of Chicago	elharel@uchicago.edu
Dan	Hartzler	Purdue Univ.	dhartzle@purdue.edu
Saif	Hasan	Purdue Univ.	sshasan@purdue.edu
Gary	Hastings	Georgia State	ghastings@gsu.edu
Dugan	Hayes	Univ. of Chicago	dugan@uchicago.edu
LaDonna	Jones	Indiana Univ.	jones404@indiana.edu
Toivo	Kallas	Univ. of Wisconsin, Oshkosh	kallas@uwosh.edu
Connie	Kang	Washington University, St Louis	srhconnie@gmail.com
David	Kehoe	Indiana Univ.	dkehoe@indiana.edu
Shigeharu	Kihara	Purdue Univ.	kihara@purdue.edu
Jeremy	King	Washington University, St Louis	jdking@wustl.edu
Hari	Lamichhane	Georgia State	hlamichhane1@student.gsu.edu
Kristin	Lewis	Univ. of Michigan	klmlewis@umich.edu
Michelle	Liberton	Washington University, St Louis	mlliberton@biology2.wustl.edu
Haijun	Liu	Washington University, St Louis	hliu6@wustl.edu
Phil	Long	Univ. of Chicago	plong1@uchicago.edu
Anuradha	Marathe	Univ. of Wisconsin, Oshkosh	marata75@uwosh.edu
Angus	McCuskey	Purdue Univ.	amccusko@purdue.edu
Matthew	Nelson	Univ. of Wisconsin, Oshkosh	nelsonm@uwosh.edu
April	Nesbit	Indiana Univ.	adnesbit@indiana.edu
Jens	Niklas	Argonne Nat'l Lab.	jniklas@anl.gov
Kris	Niyogi	UC Berkeley	niyogi@berkeley.edu
Adam	Offenbacher	Georgia Tech	gtg467u@mail.gatech.edu

Cynthia	Pagba	Sacramento	cynthia.pagba@gmail.com
Lawrence	Page	Washington University, St Louis	lepage@wustl.edu
Himadri	Pakrasi	Washington University, St Louis	pakrasi@wustl.edu
Gitt	Panitchayangkoon	Univ. of Chicago	gitt@uchicago.edu
Kevin	Redding	Arizona State	kevin.redding@asu.edu
Johnna	Roose	Louisiana State	jroose@lsu.edu
Animesh	Shukla	Indiana Univ.	ashukla@indiana.edu
Robin	Sibert	Georgia Tech	gtg429s@mail.gatech.edu
Jason	Stofleth	Purdue Univ.	jstofle@purdue.edu
Junlei	Sun	Pennsylvania State University	stoneman1984@gmail.com
Joseph	Tang	Washington University, St Louis	j.tang@wustl.edu
Brandon	Thomas	Univ. of Wisconsin, Oshkosh	thomab24@uwosh.edu
Ken	Toson	Univ. of Wisconsin, Oshkosh	KenToson@Gmail.com
George	Weir	Univ. of Wisconsin, Oshkosh	weirg32@uwosh.edu
Lisa	Wiltbank	Indiana Univ.	lisawilt@indiana.edu
Hai	Yue	Washington University, St Louis	hyue@go.wustl.edu
Justin	Zangl	Univ. of Wisconsin, Oshkosh	zanglj35@uwosh.edu
Pawel	Zatwarnicki	University of Wroclaw, Poland	p.zatwarnicki@gmail.com
Shulu	Zhang	Louisiana State	slzhang@lsu.edu
Nan	Zhao	Georgia State	nzhao2@student.gsu.edu