Program and Abstracts

40th Midwest/Southeast Regional Photosynthesis Conference

Turkey Run State Park, Marshall, Indiana

October 24-26, 2014

Organizers

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Table of Contents

Welcome note 2
Sponsors 3
Schedule at a Glance 6
Detailed Schedule 7
Invited Lectures 11
Oral Presentation Abstracts 14
Poster Presentation Abstracts 29
Participants 67
40th Annual Midwest/Southeast Regional Photosynthesis Conference

A note from the Chair

It is my great pleasure to welcome you to the 40th annual Midwest/southeast regional photosynthesis conference.

If you have participated in previous meetings, you will recognize that the overall structure has not changed much. This year, there are four sessions representing a very loose categorization of research areas. This year we are fortunate to have three featured speakers, one from Canada, one from China, and one from the west coast.

As in previous years, to encourage student participation, we will be giving awards for best student speaker and best student poster. These awards have been made possible by a successful fund raising campaign. The donors that have provided financial support are listed by logo starting on page 3.

If you have any feedback about our conference for the current or future chairs, please, do not hesitate to contact me and make your suggestions.

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Sponsors

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# Conference Schedule at a Glance

**Friday, Oct. 24, 2014**
- 4:00 - 6:00 pm: Arrival and Registration
- 6:00 pm: Dinner
- 7:30 - 8:20 pm: Keynote Lecture I: Laszlo Kalman
- 8:20 - 9:10 pm: Keynote Lecture II: Jingdong Zhao
- 9:10 pm: Mixer and Poster Viewing

**Saturday, Oct. 25, 2014**
- 7:00 am: Breakfast
- 9:00 am: Session I: Contributed Papers
- 10:00 am: Coffee Break
- 10:25 am: Session II: Contributed Papers
- 11:45 pm: Lunch
- 1:00 - 4:00 pm: Leisure Time, Poster Viewing
- 4:00 - 6:00 pm: Poster Session, Refreshments
- 6:00 pm: Dinner
- 7:00 - 7:50 pm: Keynote Lecture III: Rick Debus
- 7:50 - 9:05 pm: Session III: Contributed Papers
- 9:05 pm: Mixer and Poster Viewing

**Sunday, Oct. 26, 2014**
- 7:00 am: Breakfast and Preliminary Check-Out
- 9:00 - 10:20 am: Session IV: Contributed Papers
- 10:20 - 10:45 am: Coffee Break and Checkout
- 10:45 am: Award Ceremony
- 11:00 - 11:20 am: Best Poster Talks
- 11:20 am: Closing Remarks and Departure
Detailed Conference Program

Friday Evening

4.00-6.00 PM       ARRIVAL AND REGISTRATION

6.00-7.25          DINNER

7:25-7.30          WELCOME AND OPENING REMARKS

7:30-8.20          Invited Lecture - Laszlo Kalman
                   NATIVE BACTERIAL REACTION CENTERS WITH UNIQUE
                   PHOTOCHEMICAL ABILITIES
                   Chair: Gary Hastings

8:20-9.10          Invited Lecture - Jingdong Zhao
                   PHYCOBILISOMES: THEIR STRUCTURES AND ASSOCIATION WITH
                   THYLAKOID MEMBRANES IN THE CYANOBACTERIA
                   Chair: Qingfang He

9.10 PM -          MIXER AND POSTER VIEWING

Saturday Morning

7:00-9.00 AM       BREAKFAST

9.00               SESSION I

Chair: Himadri Pakrasi

                  TANDEM MASTER REGULATORS INVERSELY CONTROL RESPONSES TO BLUE-GREEN LIGHT IN MARINE SYNECHOCOCCUS.
9.20-9.40
2. Lisa Wiltbank and David M. Kehoe
   REGULATION OF LIGHT-HARVESTING PROTEINS BY A TEAL-GREEN
   RESPONSIVE CYANOBACTERIOCHROME

9.40-10.00
   STRUCTURE-FUNCTION OF THE CYTOCHROME b6f COMPLEX: DOMAIN-
   SWAPPING IN THE HETERO-OLIGOMERIC MEMBRANE PROTEIN COMPLEX

10.00-10.25 COFFEE BREAK

10.25 SESSION II

Chair: Sergei Savikhin

10.25-10.45
4. Hiroki Makita and Gary Hastings
   TIME-RESOLVED VISIBLE AND INFRARED DIFFERENCE SPECTROSCOPY FOR
   THE STUDY OF PHOTOSYSTEM I WITH DIFFERENT QUINONES
   INCORPORATED INTO THE A1 BINDING SITE

10.45-11.05
5. Daniel Hartzler, Shigeharu Kihara, Jens Niklas, Gregory Orf, Donald Bryant,
   Robert Blankenship, Oleg Poluektov, Yulia Pushkar, and Sergei Savikhin
   UNCONVENTIONAL PHOTOPROTECTION MECHANISMS IN
   PHOTOSYNTHETIC SYSTEMS

11.05-11.25
6. Peter Dahlberg, Graham J. Norris, Cheng Wang, Subha Viswanathan, and Gregory
   Engel.
   OBSERVATION OF COHERENCE IN VIVO AND REAL TIME MODULATION OF
   ENERGY TRANSFER USING GRADIENT ASSISTED PHOTON ECHO
   SPECTROSCOPY

11.25-11.45
7. Moira Flanagan, Phil Long, Peter Dahlberg, Justin Caram, Andrew Fidler, Brian
   Rolczynski, Sara Chamberlin, and Gregory Engel
   SINGLE AMINO ACID SUBSTITUTION ALTERS QUANTUM COHERENCE IN
   THE BACTERIAL REACTION CENTER
11.45 PM LUNCH
1.00-4.00 PM EXHIBITS AND FREE TIME
4.00-6.00 PM POSTER SESSION (With Refreshments)
6.00-7.00 PM DINNER

Saturday Evening

7.00-7.50 PM Invited Lecture - Rick Debus
   FTIR STUDIES OF PHOTOSYNTHETIC OXYGEN EVOLUTION
   Chair: Rob Burnap

7.50 PM SESSION III

Chair: Charlie Yocum

7.50-8.10
   CONTINUOUS, STABLE PRODUCTION OF ISOPRENE BY SOLAR ENERGY AND CO2 CAPTURE IN FAST-GROWING SYNECHOCOCCUS CYANOBACTERIA

8.10-8.30 PM
9. Lian He, Stephen G. Wu, and Yinjie Tang
   SIMULATING CYANOBACTERIAL PHENOTYPES IN PHOTOBIOREACTORS BY COUPLING FLUX BALANCE ANALYSIS, KINETICS AND HYDRODYNAMICS

8.30-8.50
10. Le You, Lian He, Bert Berla, Himadri B. Pakrasi, Yinjie J. Tang
   \(^{13}\text{C-METABOLIC FLUX ANALYSIS (}^{13}\text{C-MFA) OF CYANOBACTERIAL PHOTO-FERMENTATION}

8.50 PM MIXER AND POSTER VIEWING
Sunday Morning
7.00-9.00 BREAKFAST AND PRELIMINARY CHECK-OUT

9.00 SESSION IV

Chair: Toivo Kallas

9.00-9.20 AM
DETERMINATION OF THE BINDING SITE OF THE PSB28 PROTEIN TO CYANOBACTERIAL PHOTOSYSTEM II USING CHEMICAL CROSS-LINKING AND MASS SPECTROMETRY

9.20-9.40
12. Andrew H. Ng, Po-Cheng Lin, Bert Berla, and Himadri B. Pakrasi
CHARACTERIZATION OF SEVERAL NOVEL NEUTRAL SITES IN SYNECHOCYSTIS SP. PCC 6803 AND THE STUDY OF STRONG PROMOTERS AT THESE SITES FOR INCREASED PROTEIN EXPRESSION

9.40-10.00
13. Bert M. Berla, Rajib Saha and Himadri B. Pakrasi
CYANOBACTERIAL ALKANES ENABLE LOW-TEMPERATURE GROWTH AND MODULATE THE RATIO OF CYCLIC TO LINEAR ELECTRON FLOW IN PHOTOSYNTHESIS

10.00-10.20
DEVELOPMENT OF AN ENGINEERED CYANOBACTERIAL BIO-REFINERY FOR D-LACTATE PRODUCTION

10.20-10.45 AM COFFEE BREAK

10.45-11.00 AM BEST POSTER AND TALK AWARDS

11.00-11.10 BEST UNDERGRADUATE POSTER TALK

11.10-11.20 BEST GRADUATE POSTER TALK

11.20 CLOSING REMARKS

DEPARTURE
Invited Lecture 1

NATIVE BACTERIAL REACTION CENTERS WITH UNIQUE PHOTOCHEMICAL ABILITIES
Sasmit S. Deshmukh, Matei-Alexandru Ivanescu, Charles Protheroe, Sarah Lag, Kai Tang, Laszlo Kalman
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Bacterial reaction centers (BRCs) from anoxygenic bacteria have extensively been used in the past as structural and (some extent) functional models for the more complex Photosystem II (PS II) of oxygenic photosynthesis. Reactions involved in the catalytic water splitting, however, could only be mimicked if multiple genetic modifications were made in the BRCs. For the best results so far the replacements of up to six residues were necessary. Here we describe our recent efforts, where environmental factors alone were able to trigger changes in BRCs that caused the oxidation-reduction potential of the primary electron donor (P) to differ in a range of ~180 mV in native Rhodobacter (Rba.) sphaeroides. These changes enabled RCs to acquire unique photochemical abilities at physiologically relevant temperatures without the necessity of genetic modification. Two examples will be presented: i.) delaying the charge recombination reaction up to hours and ii.) utilization of manganese ions as rapid secondary electron donors to P⁺. The former is preventing a naturally occurring electron transfer reaction to proceed and the latter is enabling a redox reaction that normally could not take place in native BRCs, only in multiple mutants and in PSII.

i.) Controlled light-induced structural changes in the vicinity of the bacteriochlorophylls resulted in decreased redox potential of P by ~80 mV and by ~150 mV in Rba. sphaeroides and Rba. capsulatus, respectively. Replacement of the surrounding detergent micelles with lipid bilayers combined with lipid binding to the carotenoid binding site and light-induced structural changes provided further stabilization and extended the lifetime of the charge-separated state by ~5 orders of magnitude. This new property allows the BRC to store electrical potential for hours.

ii.) Utilizing manganese ions as secondary electron donors to P⁺ in BRCs require opposite directional shifts of the oxidation potentials of P and the manganese ions. Systematic selection of ligands and environmental conditions led to a change in the coordination of manganese ions that resulted in complexes with Mn²⁺/Mn³⁺ oxidation potentials lowered to the range of 330 – 650 mV depending on pH. Association of these manganese clusters with BRCs not only altered the light-induced structural changes but also elevated the redox potential of P/P⁺ by up to ~100 mV, also in a pH-dependent manner. The combined effects of manganese coordination, binding, and light-induced structural changes enabled the native BRC to oxidize manganese ions with time constants of ~80 ms, in a reaction that is faster than the charge recombination.

The details of the molecular changes induced by the environmental modifications and the reaction mechanisms are presented in the talk.

PHYCobilisomes: Their Structures and Association with Thylakoid Membranes in the Cyanobacteria

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Phycobilisomes (PBSs) are light-harvesting antennae that transfer energy to photosynthetic reaction centers in cyanobacteria and red algae. PBSs are supermolecular complexes composed of phycobiliproteins (PBPs) bearing chromophores for energy absorption and transfer and linker proteins. Whereas the structures of some individual components have been determined by crystallography, the three-dimensional structures of the entire complex, critical to the understanding of energy transfer mechanism, remain largely unknown. Here, we report the structures of an intact PBS and its complex with photosystem II (PSII) from Anabaena sp. strain PCC 7120 using single-particle electron microscopy in combination with biochemical and molecular analyses. Based on the PBS structure, all PBP trimers and the conservative linker protein domains were unambiguously located and a global distribution of all chromophores was determined. We show that ApcE and ApcF are critical to the formation of an extrusion at the bottom of PBS, which plays an important role in PBS interaction with PSII and energy transfer to PSII reaction centers. We demonstrate that anionic lipids are important to the interaction of PBS with thylakoids. Our results provide insights into the molecular architecture of intact PBS in different assembly levels and a basis for understanding how the light absorbed by PBS to be transferred to PSII.
Invited Lecture 3

FTIR STUDIES OF PHOTOSYNTHETIC OXYGEN EVOLUTION

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The photosynthetic conversion of water to molecular oxygen is catalyzed by the Mn₄CaO₅ cluster in Photosystem II and provides nearly our entire supply of atmospheric oxygen. The Mn₄CaO₅ cluster accumulates oxidizing equivalents in response to light-driven photochemical events within Photosystem II and then oxidizes two molecules of water to oxygen. The Mn₄CaO₅ cluster converts water to oxygen much more efficiently than any synthetic catalyst because its protein environment carefully controls the cluster’s reactivity at each step in its catalytic cycle. This control is achieved by precise choreography of the proton and electron transfer reactions associated with water oxidation and by careful management of substrate (water) access and proton egress. The talk will describe FTIR studies undertaken to characterize the influence of the cluster’s metal ligands on its activity, to delineate the proton egress pathways that link the Mn₄CaO₅ cluster with the thylakoid lumen, and to characterize the influence of specific residues on the water molecules that serve as substrate or as participants in the networks of hydrogen bonds that make up the water access and proton egress pathways. This information will improve our understanding of water oxidation by the Mn₄CaO₅ catalyst in Photosystem II and will provide insight into the design of new generations of synthetic catalysts that convert sunlight into useful forms of storable energy.
Oral Presentation Abstracts

(In Order of Appearance)
Cyanobacteria of the genus *Synechococcus* are ubiquitous in oceans and are responsible for a significant portion of the primary productivity and oxygen production on Earth. These organisms contain phycobilisomes, which contain phycobiliproteins to which light harvesting molecules, known as chromophores, are attached. Some marine *Synechococcus* undergo blue-green chromatic acclimation, adjusting the composition of their phycobilisomes to optimize photon capture to drive photosynthesis in different light conditions. We study *Synechococcus* RS9916, which is a marine strain capable of blue-green chromatic acclimation that we are developing as a model system for the study of light harvesting and photosynthetic efficiency in the marine environment.

Using a reverse genetic approach, we have identified two genes encoding putative AraC-family transcriptional regulators whose presence in a number of *Synechococcus* strains is strongly correlated with the ability to undergo blue-green acclimation. These genes are contiguous in all of the genomes in which they occur and appear to form an operon. The interruption of either of these genes leads to a complete loss of this response, as judged by changes in phycobilisome composition. In addition, these mutants have lost proper RNA regulation of at least four blue-green regulated genes that appear to be important for proper acclimation.

Interestingly, the phenotypes of two mutants are completely opposite from each other. An interruption in the first of these two genes results in mutant cells that behave as if they are always in green light, regardless of the ambient light color. Interruption of the second gene results in mutant cells that act as if they are always in blue light. These results provide important genetic and biochemical information about the signal transduction pathway controlling the globally important process of blue-green chromatic acclimation in marine *Synechococcus*. 
Presentation 2

REGULATION OF LIGHT-HARVESTING PROTEINS BY A TEAL-GREEN RESPONSIVE CYANOBACTERIOCHROME

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

Cyanobacteriochromes are bilin-binding photoreceptors in cyanobacteria that have been shown to sense light colors across the visible spectrum of light. *Fremyella diplosiphon* has 27 cyanobacteriochromes: more than any other species of cyanobacteria with an available genome. Only two of these have been characterized. RcaE controls the process of Type III chromatic acclimation, wherein organisms optimize photosynthetic red to green ambient light by making changes to their light harvesting antennae. In *F. diplosiphon*, phycoerythrin (PE) levels are high in green light and low in red light. Conversely, phycocyanin (PC) levels are low in green light and high in red light. Thus, cells are tuned to efficiently absorb the wavelength of light available in the environment.

We have discovered a novel blue-green light responsive cyanobacteriochrome, DpxA, which controls levels of PE in cells with RcaE and controls both PE and PC levels in an *rcaE*-mutant. This indicates that two photoreceptors, sensing different colors of light, both regulate the same proteins and that one photoreceptor alters the way in which the other one regulates PE and PC. The *dpxA* gene is in an operon with a hybrid histidine kinase and a response regulator. The operon arrangement is conserved across many species of cyanobacteria, even some that do not contain PE, which suggests that the Dpx system could regulate different processes in other species. Overall, our research presents a network of photoreceptors in *Fremyella diplosiphon*, in this case controlling one of its important cellular processes. It also brings to light a novel role for blue-green light responsive photoreceptors that have thus far not been well-characterized on a functional level.
The mechanisms of electron transfer in a heterogeneous dielectric environment (1) partly ascribed to the many intra-protein lipids (2), trans-membrane proton transfer (3), and superoxide generation (4) can now be described in the context of a structure of the dimeric cytochrome b_{6}f complex with 2.5 Å resolution (2). The forces that stabilize the dimeric structure are not known, although it has been proposed to arise from domain swapping of the Rieske [2Fe-2S] protein, “ISP” [5-7]. Domain swapping has been implicated as a major mechanism of protein oligomerization, contributing to the stability of the biologically crucial multi-subunit complexes.

In the case of membrane protein assemblies, domain swapping is demonstrated by the hetero-oligomeric cytochrome b_{6}f and the bc_{1} complexes, the N-terminal trans-membrane α-helix of the ISP is attached to one monomer while the predominantly β-sheet extrinsic domain is inserted into the other in the functional dimer, and this domain swapping of the ISP subunit has previously been suggested to be crucial for structural stability of the dimeric complex [5-9]. Here, it is shown that ISP domain swapping serves a functional, rather than a structural, role in the cytochrome b_{6}f complex. The b_{6}f complex was purified from the cyanobacterium Fremyella diplosiphon SF33 with a sub-stoichiometric ISP content and contrary to the expectation, was found to be predominantly dimeric. Based on biochemical analysis, it is inferred that the ISP serves mainly a functional role. Stability of the complex is attributed to intra-membrane protein-protein and protein-lipid hydrophobic interactions based on in silico comparison with published crystal structures of b_{6}f. Thus, domain swapping not only contributes to protein oligomerization of proteins, but may also mediate function in higher order assemblies. [1 J. Phys. Chem B (2014); (2) Structure (2014); (3) PNAS, 2013; (4) Baniulis, 2013; (5) Kurisu, 2003; (6) Stroebel, 2003; (7) Cramer, 2006; (8) Yamashita, 2007; (9) Baniulis et al., 2009.

Domain swapping of ISP (Iron sulpher protein). The ISP subunit (highlighted in pink) provides a connection between the monomers of the dimeric cyt b_{6}f complex (PDB ID 2E74). The extrinsic domain is associated with one monomer while the transmembrane helix (TMH) interacts with the other monomer, to stabilize the dimer. The co-factors of one monomer are highlighted.

n-side, electrochemically negative side; p-side, electrochemically positive side
Presentation 4

TIME-RESOLVED VISIBLE AND INFRARED DIFFERENCE SPECTROSCOPY FOR THE STUDY OF PHOTOSYSTEM I WITH DIFFERENT QUINONES INCORPORATED INTO THE A₁ BINDING SITE

Hiroki Makita¹ and Gary Hastings¹
¹Department of Physics and Astronomy, Georgia State University, Atlanta, GA 30303

Room (298 K) and low (77 K) temperature time-resolved visible and infrared difference spectroscopy has been used to study photosystem I (PSI) particles with phylloquinone [2-methyl-3-phytyl-1,4-naphthoquinone (PhQ)], menadione [2-methyl-1,4-naphthoquinone (2MNQ)], plastoquinone-9 [2,3-dimethyl-5-prenyl-1,4-benzoquinone (PQ₉)], and dichlon [2,3-dichloro-1,4-naphthoquinone (Cl₂NQ)] incorporated into the A₁ binding site.

Measurements were undertaken using “standard” dilute samples and concentrated samples in short path-length (~5 µm) cuvettes. Concentrated PSI samples in short path-length cells form clear (non-scattering) films, enabling spectroscopic studies at low temperature without addition of cryoprotectants. This is important because different kinds of added cryoprotectants are known to influence electron transfer processes at low temperature. We observe no concentration induced alterations in the flash-induced absorption changes. Very concentrated samples in very short path-length cells are typically used in FTIR experiments, so flash-induced visible and infrared absorption changes can be made using the same sample under identical conditions.

At 298 K, for PSI with PhQ/2MNQ/PQ₉ incorporated, P₇₀₀⁺A₁⁻ recombination is characterized by a time constant of 80/14/3 ms, respectively. Forward electron transfer from A₁⁻ to Fₓ is characterized by a time constant of 0.309/3.1/211 µs, respectively. For PSI with Cl₂NQ incorporated, forward electron transfer is inhibited, and P₇₀₀⁺A₁⁻ recombination is observed with time constants of 3.8 µs and 139.5 µs.

Marcus electron transfer theory is used in combination with a quasi-equilibrium kinetic model to simulate the observed A₁⁻→Fₓ electron transfer process at 298 K. The model considers bidirectional electron transfer with the radical pair states being in thermodynamic equilibrium. From these simulations the midpoint potential of the different quinones in the A₁ binding site is estimated (see figure).

At 77 K, for PSI with PhQ/2MNQ/Cl₂NQ incorporated, P₇₀₀⁺A₁⁻ radical pair recombination is characterized by a time constant of 340/240/78 µs, respectively. A minor 2.4 µs decay phase is also observed for PSI with Cl₂NQ incorporated. Fractions of PSI reaction centers forming P₇₀₀⁺A₁⁻, P₇₀₀⁺Fₓ⁻, and P₇₀₀⁺FₓA/B states are quantified for PhQ/2MNQ/Cl₂NQ incorporated. The nature of heterogeneous electron transfer processes in PSI at low temperature is discussed in terms of the observed differences in P₇₀₀⁺A₁⁻ recombination lifetimes and fractions of each state for PSI with PhQ, 2MNQ, and Cl₂NQ incorporated.
UNCONVENTIONAL PHOTOPROTECTION MECHANISMS IN PHOTOSYNTHETIC SYSTEMS

Daniel A. Hartzler,1 Shigeharu Kihara,1 Jens Niklas,2 Gregory S. Orf,4 Donald A. Bryant,3 Robert E. Blankenship,4 Oleg G Poluektov,2 Yulia Pushkar,1 and Sergei Savikhin1

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Under full sunlight, unprotected (Bacterio)Chlorophyll ((B)Chl) molecules photodegrade in a matter of minutes. This is the result of the generation of highly reactive singlet oxygen (\(^{1}O_2\)) by energy transfer from the (B)Chl triplet state (\(^{3}(B)Chl\)) to the oxygen ground state, a process called \(^{1}O_2\) sensitization. Natural photosynthetic systems must protect themselves from the degrading effects of \(^{1}O_2\), typically done by positioning carotenoids within a few angstroms of each (B)Chl molecule to quench \(^{3}(B)Chl\) states. In this work, we present alternative, carotenoid independent, mechanisms which nature employs to prevent \(^{1}O_2\) sensitization by lowering the energy of \(^{3}(B)Chl\) below that of \(^{1}O_2\). First, we show it is possible that pigment-protein interactions can shift the triplet state energies of BChl \(a\) below \(^{1}O_2\), with a possible natural example being the Fenna-Matthews-Olson (FMO) complex. Evidence for this type of photoprotection includes the photostability of the complex (which contains eight BChl \(a\) molecules per monomer and no carotenoids) and recent measurements showing that the triplet state lifetime of FMO is independent of the oxygen concentration (Figure 1). Next we investigate the much stronger pigment-pigment interactions by measuring the triplet state coupling in (B)Chl dimers from their phosphorescence emission spectra (Figure 2). By quantifying the triplet state coupling of dimers with known structure, one can develop a calibrated model of triplet state coupling in other systems with strong pigment-pigment interactions, such as the BChl \(c\), BChl \(d\), and BChl \(e\) aggregates found in chlorosomal antenna. Results indicate that triplet exciton formation in these aggregates lower the triplet state energy significantly, to below that of \(^{1}O_2\), offering additional photoprotection beyond the carotenoid mechanism alone.

Figure 1: Triplet state dynamics of FMO at room temperature in aerobic (red) and anaerobic (blue) conditions.

Figure 2: Phosphorescence emission spectra of a Chl \(a\) monomer (black) and dimer (blue) showing an approximately 35nm shift in the emission maximum.
Presentation 6

OBSERVATION OF COHERENCE IN VIVO AND REAL TIME MODULATION OF ENERGY TRANSFER USING GRADIENT ASSISTED PHOTON ECHO SPECTROSCOPY

Peter Dahlberg1,*, Graham J. Norris2, Cheng Wang2, Subha Viswanathan2, and Gregory S. Engel1,2

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Two-dimensional Electronic Spectroscopy (2DES) reveals detailed information regarding electronic structure, energy transfer, and energetic coupling in isolated photosynthetic complexes. The development of the Ultrafast Video-Acquisition Gradient-Assisted Photon Echo Spectrometer (UVA-GRAPES) has allowed 2DES experiments to be conducted in vivo for the first time. The fast acquisition offered by UVA-GRAPES (~ 5 minutes for 15000 2D spectra) allows for improved signal to noise through averaging, removal of significant scatter contributions in conjugate domains, as well as the observation of real time changes in energy transfer pathways. Here we present data that reveals coherences in vivo for the first time as well as changes in the energy transfer pathways in response to light and oxygen stimulation in the antenna from Rhodobacter sphaeroides.

2DES waiting time traces from live cells of sphaeroides grown in H2O and D2O media as well as isolated LH2 complexes. Oscillations at early waiting times indicate the first observation of coherence in vivo.
Presentation 7

SINGLE AMINO ACID SUBSTITUTION ALTERS QUANTUM COHERENCE IN THE BACTERIAL REACTION CENTER

Moira Flanagan\textsuperscript{1}, Phil Long\textsuperscript{1}, Peter Dahlberg\textsuperscript{1}, Justin Caram\textsuperscript{2}, Andrew Fidler\textsuperscript{2}, Brian Rolczynski\textsuperscript{2}, Sara Chamberlin\textsuperscript{2}, and Gregory Engel\textsuperscript{1,2}

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Since the observation of persistent coherences in a photosynthetic protein, there has been a flurry of spectroscopic experiments to measure coherences between pigments in a number of pigment protein complexes. The previously ignored quantum mechanical effect is thought to be involved in the efficient and fast energy transfer that takes place in the first steps of photosynthesis. In this study, we address the biological significance of quantum coherence in the reaction center complex from \textit{Rhodobacter sphaeroides}. Using broadband two-dimensional electronic spectroscopy, we show a novel coherence between the accessory (B) and special pair (P) bacteriochlorophyll absorption bands. If this coherence has a biological significance, the primary structure of the protein scaffold should couple to the bacteriochlorophylls. Here, we show that coherence signal is dependent on the protein at the amino acid level by studying a series of single and double amino acid mutations to the protein surrounding the special pair.

\textit{FIGURE- Coherence Signal Changes with Mutation.} A representative spectrum at waiting time $T=100$ fs is shown for the wild type reaction center. Waiting time traces taken at the BP crosspeak show an oscillation consistent with a BP coherence that is significantly damped with a single amino acid mutation. Fourier analysis reveals...
CONTINUOUS, STABLE PRODUCTION OF ISOPRENE BY SOLAR ENERGY AND CO₂ CAPTURE IN FAST-GROWING SYNECHOCCUS CYANOBACTERIA

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Cyanobacteria capture enormous amounts of solar energy and CO₂ and hold great potential for production of renewable bioproducts. One such product is isoprene (C₅H₈), a precursor for thousands of terpenoids including synthetic rubber, pharmaceuticals, fragrances, and bio-fuels. Isoprene can be made via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway whose products are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Cyanobacteria possess the MEP pathway but lack an isoprene synthase (IspS) enzyme for converting DMAPP to isoprene. We have introduced optimized IspS and IPP-DMAPP isomerase (IDI) genes from Populus sp. into Synechococcus sp. PCC 7002 cyanobacteria and produced isoprene at maximal rates some 80-fold higher than previously published for cyanobacteria. We have further inactivated genes for glycogen synthesis, a major competing carbon pathway, and introduced IspS-IDI genes into both chromosomal and plasmid sites to increase gene copy number. These strains have been tested in photobioreactors linked to a real-time, Fast Isoprene Sensor under both continuous light and light-dark cycles to assess isoprene production under natural conditions. Isoprene was produced continuously for 30+ days in a 5-liter bioreactor. Strains capable of glycogen synthesis also produced isoprene during darkness, presumably with energy and carbon from glycogen. A strain with IspS-IDI genes targeted to both the chromosome and a plasmid stably expressed these genes over time and produced ~1.5 times the isoprene of strains with genes in the plasmid only. These findings demonstrate important steps toward commercial isoprene production by solar energy and CO₂ capture in cyanobacteria. Patent application US-2014-0030785 has been filed with the aid of the WiSys Technology Foundation.

Stable isoprene production in Synechococcus 7002 (IspS-IDI) 5 L photobioreactor culture.
Presentation 9

Simulating cyanobacterial phenotypes in photobioreactors by coupling flux balance analysis, kinetics and hydrodynamics

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Cyanobacteria have emerged as promising platforms for producing biofuel and other economically valuable products. In photobioreactors, light intensity is attenuated as it passes through the culture. Cyanobacteria perform photoautotrophic growth in the “light zone”, while using energy-storage compounds (such as glycogen) to conduct heterotrophic metabolism in the “dark zone”. To describe dynamic and heterogeneous photobioreactor cultivations, we coupled genome-scale Synechocystis 6803 flux balance analysis (FBA) with photobioreactor hydrodynamics and growth kinetics. Via a static optimization approach, FBA models can resolve temporal metabolic flux distributions, contributing to the prediction of biomass growth and chemical production (D-lactate) under influence of N/P nutrients and CO₂ gas-liquid transport limitations. The integrated model suggests that: 1) biomass growth is sensitive to CO₂ mass transfer coefficients, light conditions, and bioreactor size/geometry; 2) biomass accumulation in the photobioreactor can lead to an expanding dark zone, and thus both light and CO₂ sufficiency need considering to maintain a high level of biomass; 3) biomass composition (e.g., glycogen content) and carbon fluxes can continuously change during cultivation period; 4) autotrophic cyanobacteria can have functional oxidative pentose phosphate pathway under uneven illuminations; and 5) for engineered D-lactate-producing strain, carbon flux from pyruvate pool towards biomass and lactate production needs to be carefully balanced to achieve the optimal productivity.

Keywords: cyanobacteria, gas-liquid transport, glycogen, static optimization, Synechocystis 6803
Presentation 10

**13**C-Metabolic Flux Analysis (**13**C-MFA) of Cyanobacterial Photo-fermentation
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Photo-biorefineries always face great challenges in the supply of adequate light and CO₂. Tubular or flat-plate photo-bioreactors with optimal light conditions are very costly to scale up. Meanwhile, continuous pumping enriched CO₂ into algal culture consumes large amounts of utility. To overcome these problems, photo-fermentation demonstrates great advantages. By supplying algal culture with organic carbon substrates (such as sugar), photo-biorefineries can significantly alleviate the light and CO₂ limitations. In contrast to heterotrophic fermentation, photomixotrophic growth maintains significant CO₂ fixation and harvests a large amount of light energy (ATP/NADPH) for cell maintenance and biosynthesis. Therefore, photomixotrophic conditions always give higher product yields from organic feedstock. In this study, we used the integrated analysis of **13**C-fluxomics and transcriptomics to investigate the cyanobacterial glucose metabolism under two photo-fermentation conditions.

First of all, we cultured cyanobacterium *Synechocystis* sp. PCC 6803 under light and carbon (glucose and bicarbonate) sufficient conditions. Via **13**C-isotopic tracing of free metabolite labeling and **13**C-MFA of pseudo-steady state metabolism, we delineate the central metabolism during photo-fermentation: 1) the relative flux of CO₂ fixation is six times higher than that of glucose uptake; 2) the oxidative pentose phosphate pathway is low (<2%), but this flux is enhanced when light became limited; 3) the in vivo flux through the TCA cycle reactions (α-ketoglutarate → succinate) is minimal, and the transformation of α-ketoglutarate to succinate is observed only when additional glutamate is supplied in the culture medium; 4) high flux through malic enzyme serves as a main route for pyruvate synthesis.

Second, we also explored the photoheterotrophic metabolism by blocking photosystems II using 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU), which inhibits O₂ evolution and linear electron transportation. An enhanced glucose uptake flux was observed compared to that under photomixotrophic conditions. A unique cyclic glucose catabolism pathway forms, including the oxidative pentose phosphate (OPP) pathway and the reversed glucose-6-phosphate isomerase (PGI) reaction. This metabolic cycle serves as an alternative NADPH source to photosystem I by oxidizing glucose. Unlike the phototrophic condition when CO₂ fixation is completely blocked by DCMU, CO₂ fixation through RuBisCO and anaplerotic pathway remains high activity to re-fix CO₂ for biomass synthesis under photoheterotrophic condition, though the complete Calvin cycle is not functional. **13**C-MFA also indicates that glucose metabolism did not have a net production of ATPs. Instead, the photosystem I activity is up-regulated and provide ATPs together with oxidative phosphorylation to support all the cell activities. The observed regulation of related pathways is further confirmed at transcriptional level using RNA-Seq.

Our **13**C-MFA results improve the understanding of the versatile metabolism in cyanobacteria and shed light on the photo-fermentation for photo-biorefineries.
Presentation 11

DETERMINATION OF THE BINDING SITE OF THE PSB28 PROTEIN TO CYANOBACTERIAL PHOTOSYSTEM II USING CHEMICAL CROSS-LINKING AND MASS SPECTROMETRY

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Photosystem II (PSII) is assembled in a complex series of steps. The PSII protein subunits, and their light-harvesting and redox cofactors, must be incorporated in a way that minimizes oxidative damage to the complex before it is fully functional. Psb28 is an accessory protein found mainly in the CP43-less PSII assembly intermediate RC47, and is believed to assist during assembly. Because Psb28 was not observed in any PSII crystal structure, its binding site has remained unknown. In this study, we have used chemical cross-linking combined with mass spectrometry to capture the transient interaction of Psb28 with PSII. Our results show that Psb28 interacts closely with the D1 protein on the cytosolic surface of PSII, directly above the non-heme iron and quinone binding sites. This binding site suggests that Psb28 may play a role in preventing acceptor-side photodamage during PSII assembly.

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CHARACTERIZATION OF SEVERAL NOVEL NEUTRAL SITES IN SYNECHOCYSTIS SP. PCC 6803 AND THE STUDY OF STRONG PROMOTERS AT THESE SITES FOR INCREASED PROTEIN EXPRESSION

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The engineering of biofuel production in bacteria has been one of the main goals of synthetic biology for the past decade, but unfortunately, progress in cyanobacteria has been limited thus far due to a lack of well-characterized tools for genetic engineering. Since the machinery for transcription and translation in cyanobacteria differs from *E. coli*, the synthetic biology toolbox that has been developed for *E. coli* over the past decade needs to be re-engineered for use in cyanobacteria. Essential components of a functioning toolbox for engineering an organism are well-characterized sites and promoters for heterologous protein production. One commonly used method for heterologous protein production in cyanobacteria such as *Synechocystis* sp. PCC 6803 involves the insertion of the gene of interest into a “neutral site” in the genome. Examples of neutral sites in *Synechocystis* include *psbA1* and slr0646. Although these sites may be considered neutral in that their deletion does not cause a phenotypic effect on the organism, they are not ideal since they interrupt coding regions of DNA. Additionally, although strong promoters have been previously identified for use in *Synechocystis*, no quantitative measure exists to compare promoter strength between commonly used promoters. In this work we discuss the development and characterization of several novel neutral sites and strong promoters for use in *Synechocystis*. Neutral sites were identified as the largest non-coding regions of DNA within the most recently available RNA-seq transcriptomic data set, in which many new small RNAs were found. The two largest sites, NSC1 and NSC2, in addition to a site on the endogenous *Synechocystis* plasmid pCC5.2 and a site on a replicative vector containing the RSF1010 replication origin, were investigated by integrating an expression cassette consisting of EYFP and a resistance marker via double homologous recombination to generate a library of individual mutants. We found that expression was identical across two distinct neutral sites on the chromosome, and that expression in the endogenous *Synechocystis* plasmid pCC5.2 allows for ten-fold higher expression than in the chromosome. Additionally three promoters: *P_{psbA2}, P_{Cpc560},* and *P_{trc},* were tested in each of the neutral sites. We found that *P_{Cpc560}* expresses about three times stronger than *P_{trc},* but that this effect is dependent on growth phase, and that *P_{psbA2}* expresses very weakly when compared to the other two promoters. These findings provide novel, characterized tools to support synthetic biology and metabolic engineering applications in cyanobacteria. These tools are currently being used for the engineering of *Synechocystis* to produce limonene, a monoterpenene with potential as a jet fuel.

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Presentation 13

CYANOBACTERIAL ALKANES ENABLE LOW-TEMPERATURE GROWTH AND MODULATE THE RATIO OF CYCLIC TO LINEAR ELECTRON FLOW IN PHOTOSYNTHESIS

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Cyanobacteria universally produce diesel-range hydrocarbons\textsuperscript{1}. While the pathways for their biosynthesis were only recently discovered\textsuperscript{2,3}, all strains studied so far include such alkanes or alkenes in their membranes at a concentration similar to chlorophyll. There exist two distinct and mutually exclusive alkane/alkene production pathways in cyanobacteria, and mutants of either pathway lacking these metabolites grow poorly at low temperatures. We have analyzed the mechanism of this growth defect in a mutant of \textit{Synechocystis} sp. PCC 6803 (6803-NOalk) by studying the redox kinetics of photosystem I using a Joliot-type spectrophotometer. At low temperatures where the mutant grows poorly, it exhibits enhanced cyclic electron transport relative to the wild-type. Based on this analysis, we believe that enhanced cyclic electron flow acts to maintain redox poise and ATP:NADPH balance despite restriction of growth. It is possible that the restriction of growth occurs due to reduced membrane fluidity and mobility of electron carriers such as plastoquinone in the absence of alkanes. We present results of \textit{in silico} metabolic modeling studies that indicate that enhanced cyclic electron flow and higher ATP:NADPH ratios could be more generally associated with reduced growth rates across a range of stressful or non-ideal growth conditions.

References


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DEVELOPMENT OF AN ENGINEERED CYANOBACTERIAL BIO-REFINERY FOR D-LACTATE PRODUCTION

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Recently, we engineered Synechocystis sp. PCC 6803 for D-lactic acid production using a mutated glycerol dehydrogenase, GlyDH*. D-lactic acid is an important precursor for the production of polylactic acid (used as biodegradable plastics), as well as in food and pharmaceutical industries. Codon optimization and cofactor balancing (NADH) through the heterologous expression of a soluble transhydrogenase from Pseudomonas aeruginosa were used to improve D-lactic acid productivity (by 2~3 folds). As Synechocystis 6803 is capable of mixotrophic growth, the strain was grown with different additional carbon sources such as glucose, pyruvate, and acetate. Amongst these, acetate was found to significantly enhance the productivity under photomixotrophic growth. To obtain cheap nutrients and acetate to promote cyanobacterial cultivation, we tested the use of effluents from the anaerobic digestion (AD) of municipal wastes as nutrient sources for cultivation. The effluents from AD processes contain nitrogen (N), phosphorus (P), and other nutrients, which can reduce algal medium costs. In addition, chemical or pH inhibition of methanogenesis during anaerobic digestion can accumulate acetate for mixotrophic cyanobacterial cultivations. Therefore, we investigated D-lactate production from the engineered cyanobacterial strain using sterilized AD. Results from our study indicated that a 1:4 volume-mixing ratio (acetate-rich AD effluent: BG-11 medium) could enhance D-lactate synthesis by ~40%. The engineered cyanobacteria accumulated over 1.2 g/L D-lactate in a 20-day culture period. In addition, the engineered cyanobacterium is highly sensitive to pH, and produces D-lactate only in alkaline conditions.

Metabolic engineering of Synechocystis 6803 for the synthesis of D-lactic acid
Poster Abstracts

(Alphabetically by First Author)
EXPRESSION AND FUNCTION OF CUPA PROTEIN FROM NDH1, COMPLEX OF SYNECHOCYSTIS PCC 6803

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Cyanobacteria have evolved different mechanisms to survive and adapt to adverse conditions. The CO$_2$ concentrating mechanisms (CCM) are responsible for increase on inorganic carbon (C$_i$) to be used in the Calvin Cycle. Synechocystis have five Ci uptake mechanisms: three HCO$_3^-$ transporters and two CO$_2$ uptake mechanisms. CO$_2$ uptake involves specialized NDH-1 complexes, NDH-1$_3$ (NdhF3/NdhD3/CupA/CupS) and NDH-1$_4$ (NdhF4/NdhD4/CupB). Subunit localizations with tagged proteins indicate that CupA and CupB proteins are present on thylakoid membrane (1,2). Mutants lacking these proteins presented impaired CO$_2$ uptake (3,4). It was suggested that CupA and CupB are possible carbonic anhydrases (3,5,6), however expression of soluble CupA and CupB and detection of CO$_2$ hydration activity were until now unsuccessful. These complexes appear to utilize redox energy of either ferredoxin or NADPH, but the mechanism of coupling this energy to CO$_2$ hydration and how the complexes fit into the overall patterns of cyclic electron transport (CET) is debated (3,4). We are studying the integration of the CCM with the PSET producing ATP and NADPH. Mutants affecting NDH complexes are being analyzed by chlorophyll fluorescence as an indicator of CO$_2$ uptake and P700 rereduction as a measure of CET. The results will be presented here. Also our group was able to heterologous express CupA, using a maltose binding protein fused with CupA from Synechocystis. Future studies with the protein may help to elucidate structure-function relationships implied by its putative CO$_2$-hydration activity.

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MUTATION DISTURBING WATER CLUSTER ALTER THE KINETICS OF THE FINAL STEPS OF WATER OXIDATION

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The hydrophobic Val185 in the D1 subunit of photosystem II (PSII) is located in the close vicinity of the oxo bridge atom, O5, of Mn$_4$CaO$_5$ cluster and faces the broad channel, allowing for the exchange for the substrates and products of the water oxidation reaction, as it passes the cluster [1-3]. Mutations on this residue may interfere with water molecules that intervene between the redox active tyrosine (Y$_Z$) and the putative proton gate residue, D1-Asp61 (Fig. 1). The asparagine substitution of D1-Val185 dramatically retards the rate of O$_2$ release during S$_3$→S$_0$ transition and results in an extension of the kinetic lag phase prior to O$_2$ release that is highly reminiscent of the effects of mutations produced at D1-Asp61 [4]. To understand how the alteration of D1-Val185 residue can have an influence on the kinetics of the final steps of water oxidation reaction, the dependence of O$_2$ release kinetics on pH/pD and temperature were studied separately by using time-resolved O$_2$ polarography and a comparison was made with wild-type and D1-Asp61 mutants. A strong H$^+$ product back-pressure on both the lag and slow phase of O$_2$ release kinetics is observed in wild-type, but no evidence for H$^+$ product back-pressure in D1-Val185Asn mutant. The Arrhenius plot of slow phase yield activation energy of 24 kJ/mol for D1-Val185Asn, as compared to 45 kJ/mol for wild-type. Correspondingly, the frequency factor of D1-Val185Asn is five orders of magnitude lower than the wild-type. The above results indicate new rate-determining steps (RDS) occur during the lag and dioxygen formation for D1-D1-Val185Asn. The new RDS may arise from the perturbation of structure rearrangement of H-bond network or water motions induced by mutation.


Fig. 1. Mutations were constructed in Synechocystis to D1 residues Val185 with the goal of disturbing the water molecules close to O5. A large retardation of O$_2$ release kinetics during S$_3$→S$_0$ was found in D1-V185N, similar to D1-D61N.

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SULFUR SPARING RESPONSE IN FREMYELLA DIPLOSIphon
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*Fremyella diplosiphon*, a freshwater cyanobacterium, is able to undergo a form of phenotypic plasticity called elemental sparing. *F. diplosiphon* remolds its light harvesting antennae, which are called phycobilisomes, under the conditions of low sulfate availability. These new phycobilisome proteins are made from a different set of genes, present in the *cpc3* operon, than those produced during sulfate replete conditions. This regulation occurs at the transcriptional level. Phycobilisomes make up approximately 60% of the total soluble protein in these cells, which makes the elemental sparing an important way to use sulfur economically when it is scarce in the environment. We have shown that this response provides a strong fitness advantage to cells in low sulfur conditions, compared to those that lack this response. We have found that some sulfur containing compounds are able to elicit a low sulfate response while others do not. For example, we have discovered that low levels of either sulfate or cysteine elicit the sulfur sparing response, while methionine does not. The long-term goal of my project is to determine the regulatory mechanism(s) that control this elemental sparing response.
UNLOCKING THE GATE OF THE BC1 COMPLEX; THE Qo SITE MECHANISM

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

bc1 complex basic Q cycle bifurcated reaction:
1. QH2 from reaction center enters Qo site, and Q enters Q1 site 2. First round of Qo site oxidation passes one H+/e- to high and low potential chains (Q1 semiquinone) 3. Second QH2 from reaction center enters Qo site 4. Second round of Qo site oxidation passes one H+/e- to high and low potential chains (Q1 quinol). Stigmatellin or Ascochlorin are inhibitors to the Qo site, and Antimycin-A is the inhibitor of the Q1 site.
INVESTIGATING CHANGES IN ENERGY TRANSFER PATHWAYS IN LH2 DUE TO A CAROTENOID SWITCH

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Carotenoids possess a dark S\(_1\)/intramolecular charge transfer (ICT) state that we believe plays an important role in photosynthetic energy transfer and photoprotection. The nature of the S\(_1\)/ICT state’s role in energy transfer is not well understood. In LH2 antenna complexes from *Rhodobacter sphaeroides* containing the well-studied carotenoid spheroidene, energy transfers from the spheroidene S\(_1\) state to bacteriochlorophyll (BChl) Q\(_y\) states with 82% efficiency. When LH2 is grown semi-aerobically, the complex incorporates the carotenoid spheroidenone in place of spheroidene. We hypothesize that this adaptation lowers the energy of the dark S\(_1\)/ICT state below the BChl Q\(_y\) energy to reverse the energy transfer from an excitation-donating pathway to an excitation-quenching pathway. Such a change would result in excess excitations being dissipated safely as heat and prevent the formation of damaging singlet oxygen. Our research efforts will explore the role of the carotenoid S\(_1\)/ICT dark state in energy transfer pathways. We propose to use two-dimensional electronic spectroscopy to study energy transfer within LH2 containing spheroidene, spheroidenone, and a carotenoidless mutant.

Linear absorption spectra and photographs of isolated LH2: carotenoidless mutant (dotted), spheroidene (dashed), and spheroidenone (solid). The carotenoidless mutant spectrum shows a dip in absorption from \(\sim 450 \text{ }–\text{ } 550\) nm where the carotenoids absorb. The spectrum of LH2 with spheroidene reveals three carotenoid S\(_2\) peaks corresponding to the three lowest vibrational levels of the S\(_2\) state. LH2 with spheroidenone shows a broader red-shifted carotenoid peak with a disappearance of the vibronic progression.
IRON CHELATOR 2,2’-DIPYRIDYL INDUCED SEVERE IRON LIMITATION IN SYNECHOCYSTIS SP. PCC 6803

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Iron is an essential cofactor in photosynthesis. The availability of iron strongly impacts the structure and function of photosynthetic apparatus and primary productivity of cyanobacteria. Traditionally, there are two methods for inducing iron limitation in cyanobacterial cells: repeated dilution and iron chelation. The iron chelator 2,2’-dipyridyl is widely applied for rapid and efficient depletion of iron. Cyanobacteria respond to iron limitation by expressing IsiA, which is controlled by the cis-encoded antisense RNA IsrR. The accumulated IsiA may form supercomplexes with photosystem I to increase the light capture ability of photosystem I. In this study, we investigated the growth and photosynthetic characters of cyanobacterium *Synechocystis* sp. PCC 6803 under iron-limiting conditions achieved by two iron-depriving methods. In comparison with cells grown in iron-free medium, 2,2’-dipyridyl-treated cells suffered more iron deficiency with retarded growth, lower intracellular iron content, more remarkable decrease in photosystem protein abundance and more severe impairment of photosynthetic activity. In addition, IsiA accumulated to a lower level in 2,2’-dipyridyl-treated cells than in dilution-treated cells. Further analysis using two-dimensional blue native/SDS-PAGE showed that 2,2’-dipyriyl treatment reduced the formation of IsiA-photosystem I supercomplexes. Northern blot revealed that the mRNA level of IsiA increased significantly in dilution-treated cells but not in 2,2’-dipyriyl-treated cells, whereas the accumulation of IsrR decreased in an inverse fashion. These data suggest that iron chelator 2,2’-dipyriyl elicits severe iron limitation in *Synechocystis* sp. PCC 6803 and suppresses the formation of IsiA-PSI supercomplexes by interfering with IsrR-regulated IsiA expression.
ENERGY TRANSFER IN LIGHT HARVESTING COMPLEX 2 ASSEMBLED USING NON-NATIVE CAROTENOIDS

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Carotenoids protect the photosynthetic apparatus against harmful radicals arising from the presence of both light and oxygen. They also act as accessory pigments for harvesting solar energy, and are required for stable assembly of many light-harvesting complexes. In the phototrophic bacterium Rhodobacter (Rba.) sphaeroides three sequential desaturations of the colourless carotenoid phytoene extend the number of conjugated carbon-carbon double bonds, N, from three to nine, giving the green carotenoid neurosporene; subsequent modifications culminate in the yellow/red carotenoids spheroidene/spheroidenone (N=10/11). In other bacteria phytoene desaturase (CrtI) catalyses another desaturation, which commits the pathway to the spirilloxanthin carotenoid pathway. Genomic crtI replacements were used to swap the native three-step Rba. sphaeroides CrtI for the four-step Pantoea agglomerans enzyme, which rerouted the pathway down the spirilloxanthin series, with 2, 2′ diketospirilloxanthin produced under semi-aerobic conditions. Premature termination of this new pathway by inactivating crtC or crtD produced strains with lycopene or rhodopin as the major carotenoid. All of the spirilloxanthin series carotenoids are accepted by the assembly pathways for LH2 and RC-LH1-PufX complexes. The efficiency of carotenoid-to-bacteriochlorophyll energy transfer for the N=15 carotenoid 2, 2′ diketospirilloxanthin in LH2 complexes is low, at 32%. High energy transfer efficiencies were obtained for neurosporene (N=9; 94%), spheroidene (N=10; 99%) and spheroidenone (N=11; 95%), whereas intermediate values were measured for lycopene (N=11; 65%), rhodopin (N=11; 62%) and spirilloxanthin (N=13; 41%). The variety and stability of these novel Rba. sphaeroides antenna complexes make them useful experimental models for investigating the energy transfer dynamics of carotenoids in bacterial photosynthesis.
Functional expression of the *Arachis hypogaea* L. Acyl-ACP thioesterases *AhFatA* and *AhFatB* enhance fatty acid production in *Synechocystis* sp. PCC6803

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Palmitoleic acid (C16:1) and stearic acid (C18:0) are precursors of polyunsaturated fatty acids, which are the focus of intensive global research due to their nutritional value, medicinal applications, and potential use as biofuel. Acyl-acyl carrier protein (ACP) thioesterases are intraplastidial enzymes that determine the types and amounts of fatty acids produced in plants and release these fatty acids into the cytosol to be incorporated into glycerolipids. Based on amino acid sequence identity and substrate specificity, these enzymes are classified into two families, FatA and FatB. In this study, we cloned *FatA* and *FatB* thioesterases from *Arachis hypogaea* L. seeds and functionally expressed these genes, both individually and in tandem, in a blue-green alga *Synechocystis* sp. PCC6803. The heterologous expression of these genes in *Synechocystis* altered the fatty acid composition of lipids, resulting in a 35%-74% increase in palmitoleic acid production and a 56%-72% increase in stearic acid production. Moreover, the transgenic *Synechocystis* cells also showed significant increases in levels of oleic acid (C18:1, OA), linoleic acid (C18:2, LA), and α-linolenic acid (C18:3n3, ALA). These results suggest that the fatty acid profile of algae can be significantly improved by the heterologous expression of exogenous genes. This study not only provides insight into fatty acid biosynthesis, but also lays the foundation for manipulating the fatty acid content of cyanobacteria.
Cyanobacteria Responses to Environmental Stress - Physiological and Biochemical Study of Excess of Cupper and Light Intensity on *Synechocystis* sp. PCC6803

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Copper is one of the commonly known trace metals required for the growth and survival of cyanobacteria, and acts as a cofactor in several enzymes including cytochrome c oxidase, laccase, superoxide dismutase, plastocyanin. The ability of copper to alternate between its cuprous Cu(I) and cupric Cu(II) oxidation states makes it an ideal biological cofactor which plays a major role in photosynthetic and respiratory electron transport chain. However, being a redox metal not only allow its participation in major redox reactions but also promote the production of reactive oxygen species (ROS) through the Fenton and Haber-Weis reactions, thereby increasing intracellular lipid peroxidation and protein oxidation. In this study we sought to examine the physiological and biochemical changes on *Synechocystis* 6803 cells under excess copper concentrations and elevated light intensity. Cells of wild type *Synechocystis*6803 and *slr*1573Δ strain, which encodes for the laccase gene, were subjected to different copper concentrations and light conditions for various periods of time. Here we report a cupper toxic dose of 5µM and a non inhibitory dose of 1.5 µM. The growth measurement and pigment analysis over a period of 5 days indicates that the toxicity of cupper appear at 3µM and appears aggravated under high light exposure. These results also indicate that the wild type strain appears to be more susceptible to the copper treatment under high light conditions than the mutant strain while under normal light *slr*1573Δ seems to be more sensitive to cupper. The 77k fluorescence measurement correlates theses results with a decrease in fluorescence intensity of PSI and PSII upon exposure to 3µM cupper.
Functional genomic Characterization of two *Chlamydomonas reinhardtii* mutants defective in photosynthesis.

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Photo-autotrophic growth under different light irradiances is modulated by a complex interplay that involves various physiological processes. *Chlamydomonas reinhardtii* is a model green micro-alga. It has a short haplontic life cycle, possesses a photosynthetic apparatus very similar to higher plants, can grow photo-autotrophically and heterotrophically (can metabolize exogenous acetate as a carbon source in the absence of light), has well developed chloroplast and nuclear transformation techniques and possesses a completely sequenced genome. Our laboratory has generated a mutant library of *Chlamydomonas* by random insertional mutagenesis. This library was screened under heterotrophic, mixotrophic and photo-autotrophic growth conditions, under different light intensities. The screening has resulted in the isolation of 20 mutants. Two of the isolated photosynthetic mutants are 10E1 and 10E35. 10E1 is light sensitive, chlorophyll deficient and non-photosynthetic under all light conditions. 10E1 can only grow heterotrophically and mixotrophically under dim light. 77K fluorescence emission spectra show that 10E1 has an overall high PSI fluorescence compared to 4A+ and an apparent abnormal PSII fluorescence signature, which is undocumented in any known *Chlamydomonas* mutants in the published literature. It also shows a blue shift of the PSI and PSII fluorescence peaks in the 77K emission spectra that indicate an uncoupling of the Chl antenna from the reaction center. Mutant 10E35 can grow in the dim and in the low light but fails to grow under high light in mixotrophic and photo-autotrophic growth conditions. 10E35 has two mutations affecting two genes namely, the well characterized, SAC3 which is a Snf1-like Serine/Threonine kinase (SNRK2.2) that functions as a sulfur stress response regulator on chromosome 12 and a novel functionally uncharacterized gene, g11686 on chromosome 11. g11686 has been functionally annotated as a nucleolar GTPase/ATPase p130 on Phytozome. We have compared the growth phenotype of 10E35 with that of a sac3 *Chlamydomonas* mutant. Unlike 10E35, the sac3 mutant is not high light sensitive in mixotrophic and photo-autotrophic sulfur replete-growth conditions. According to the published literature, sac3 mutant is only light sensitive under sulfur stress. The literature information and the growth analyses of 10E35 and the sac3 confirm that g11686, a novel functionally uncharacterized gene, is responsible for the 10E35 phenotype. We will be presenting our physiological and molecular research data on 10E1 and 10E35.
The reaction center (RC) complex of the green sulfur bacterium *Chlorobaculum tepidum* is composed of the Fenna-Matthews-Olson (FMO) antenna protein and the reaction center core (RCC) complex. The RCC complex has four subunits: PscA, PscB, PscC, and PscD. We studied the FMO/RCC complex by chemically cross-linking the purified sample followed by biochemical and spectroscopic analysis. Blue-native gels showed that there were two types of FMO/RCC complexes, which are consistent with complexes with 1 copy of FMO per RCC and 2 FMO per RCC. SDS-PAGE analysis of the samples after cross-linking showed that all the five subunits of the RC can be linked by three different cross-linkers: Bissulfosuccinimidyl suberate (BS3), Disuccinimidyl suberate (DSS) and 3,3'-Dithiobis-sulfosuccinimidyl propionate (DTSSP). The interaction sites of the cross-linked complex were also studied using LC-MS/MS. The results indicated that FMO, PscB, PscD and part of PscA are exposed on the cytoplasmic side of the membrane. PscD helps stabilize FMO to the reaction center and may facilitate the electron transfer from RC to ferredoxin (Fd). The soluble domain of the heme-containing cytochrome subunit PscC and part of the core subunit PscA are located on the periplasmic side of the membrane. There is a close relationship between the periplasmic portions of PscA and PscC, which is needed for efficient electron transfer between PscC and P840 (Fig. 1). The energy transfer of the RCC and FMO/RCC complexes were studied by fluorescence spectroscopy and pump-probe transient absorption (TA) spectroscopy. The energy transfer efficiency from FMO to RCC is calculated to be ~50%. Both samples showed a 20-30 ps charge separation lifetime component regardless of the excitation wavelength. For the FMO/RCC complex, the TA spectra could be interpreted by a combination of the excited FMO protein and RCC complex. A long-lived 826 nm band indicates energy trapping in the FMO lowest energy state.

Heat stress effects on soybean chloroplast ultrastructure were analyzed after one and seven days of stress and compared with control samples at the same time points. Transmission electron microscopy (TEM) analyses revealed heat stress effects on different aspects of chloroplast ultrastructure at different time points. Additional research is needed to characterize heat stress induced changes in chloroplast ultrastructure in relation to chloroplast function.

Soybean plants were grown in two growth chambers at 28 °C. After five weeks, the air temperature in one growth chamber was elevated to 45 °C for four hours each day while the other growth chamber was maintained at 28 °C. At the end of the first and seventh day of heat treatment leaf tissue was sampled from 28 °C and 45 °C treated plants. Samples were imaged by TEM and areas of grana were quantified using ImageJ. Bars represent standard error.
MAXIMIZING LIPID PRODUCTION IN CHLORELLA VULGARUS
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With the depletion of available fossil fuels and rising demand, an alternative source of energy will eventually be a necessity. A popular alternative is corn-based ethanol but the inherent problem with this solution is competition with food crops for agricultural space. In comparison, microalgae would only use 2.5% of existing U.S. cropping area to provide the nation with 50% of its energy needs. Although microalgal fuels are advantageous in terms of agricultural space it is still more expensive to produce biodiesel than traditional petroleum fuels. In an effort to make biodiesel more cost efficient, cultures of the algal species Chlorella vulgaris were grown in nitrogen-deplete medium in the presence of glycerol in an effort to maximize lipid production in the cells. To track the growth of the cells and the lipid accumulation, turbidity, dry weight, lipid extractions and chlorophyll a levels were measured daily. Cultures grown in nitrogen-deplete medium with glycerol were observed to have stunted cell growth compared to the control group which was grown in CV+ medium. The difference in growth at the end of the exponential phase between the cultures was approximately 32%. Another notable attribute among the nitrogen-deplete with glycerol cultures was that the concentration of chlorophyll a dropped from 5.5 µg/mL at the end of the exponential phase to 0.75 µg/mL by the end of stationary growth. The control cells on the other hand remained approximately at 5µg/mL. The results also showed that the nitrogen deplete with glycerol cultures had an average lipid concentration of approximately 24% per cell, while the control group only had 11% per cell.

Mechanism for cells increasing in lipid weight in the presence of glycerol.
IN VIVO FLUORESCENCE IN THE CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803
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The analysis of chlorophyll fluorescence is a well established method of assaying the physiological state of photosynthetic organisms. This is accomplished through analyzing the fluorescence transients obtained during the application and termination of an actinic light source. This transient, the Kautsky effect, has well defined early phases. Later phases that appear before a steady-state terminal fluorescence (F_T) appears are not as well defined ([1] and Figure 1). In addition, with the ability to simultaneously measure NADPH fluorescence, additional insights to cellular physiology can be obtained.
This work demonstrates that the chlorophyll fluorescence decline from F_M to F_T is largely dependent on inorganic carbon availability. The fluorescence decline from F_M to F_T coincides with a fluorescence decline in NADPH fluorescence from N_M to N_T. The N_M to N_T decline is also absent when a cell culture is treated with glycolaldehyde, a Calvin cycle inhibitor. Taken together, this provides evidence that Calvin cycle activity is the primary cause for the F_M to F_T decline.
This work also aims to quantify NADPH consumption through these physiological mechanisms through the application of techniques pioneered by other authors. Quantization of fluorescence transients into absolute concentrations of NADPH has previously been elusive. Techniques pioneered by Kauny and Sétif [2] to convert fluorescence into NADPH concentrations have been performed and evaluated.

Figure 1: Representative traces of fluorescence transients. Left: Chlorophyll fluorescence during and after exposure to an actinic light. Right: NADPH fluorescence during and after exposure to an actinic light. Parameters for each trace are labeled, and the subscripted labels are defined as follows: 0, dark-adapted minimum fluorescence; P, peak fluorescence; S, temporary stationary fluorescence; M, maximum fluorescence; T, terminal fluorescence; N, NDH-related fluorescence; R, post-illumination reduction event (pentose phosphate pathway).

EXPLORING THE TEMPERATURE DEPENDENCE OF THE OXYGEN-EVOLVING COMPLEX OF PHOTOSYSTEM II

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Deciphering the complexities of the oxygen-evolving complex (OEC) is imperative to the advancement of renewable and cleaner energies. In order to examine the OEC, photosystem II (PSII) enriched particles from spinach leaves were procured using the Bertholt, Babcock, and Yocum method\(^1\). These particles contain the OEC, a metal cluster in PSII that is composed of one calcium and four manganese atoms. The function of the OEC is to oxidize water using electron holes generated within PSII. The OEC is oxidized stepwise through a sequential extraction of electrons via a photooxidized chlorophyll \(a\) pair (P680)\(^2\). Once the OEC is sufficiently oxidized, electrons are extracted from water with molecular oxygen and protons begin generated as products. This water oxidation chemistry is temperature sensitive. The specific focus of this research is to determine the temperature at which the OEC is most catalytically efficient. Temperature titrations of PSII have so far indicated that in \textit{Spinacia oleracea}, Buxus sp., \textit{Chlorella vulgaris}, and \textit{Chlamydomas reinhardtii}, the temperature maximum is strongly correlated with the type of preparation. Intact organisms and chloroplasts show a maximum close to 38°C, while some subthylakoidal preparations show a maximum close to 28°C. In the algae and subcellular preparations, addition of 2,6-dichloro-1,4-benzoquinone results in a maximum of close to 28°C. Additional experimentation is being done in order to uncover the nature of this observed temperature dependence discrepancy.


Iron Homeostasis in *Synechocystis* PCC 6803: Role of Bacterioferritin Genes (*sll1159*, *slr0242* and *sll0221*)

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Iron is crucial ingredient as prosthetic component of proteins that plays role in all living organisms. Despite being an abundant element, the bioavailability of iron is very limited. The photosynthetic apparatus of cyanobacteria contains high amount of iron-containing proteins and is highly vulnerable to iron limitation. Iron deficiency lead to an increase production of ROS which affect greatly PSI and PSII causing oxidative stress. In this study we are examining the function of iron homeostasis in cyanobacterium *Synechocystis* sp. PCC 6803. We deleted *sll1159*, *slr0242* and *sll0221* (bacterioferritin comigratory protein encode genes) genes from *Synechocystis* sp. PCC 6803 and achieved completely segregation of single, double and triple mutants.

To induce iron stress cells were cultured with BG-11 without ferric ammonium citrate in the presence of 10 µM 2,2’-dipyridyl, an iron chelator for various period of time. The early results indicate that single mutants grow similarly with type strain in normal BG-11. However, in iron free medium all mutant grow poorly compared to wild type except Δ *sll1159+ sll0221*, which grows more than wild type.

![Graph showing growth curve under iron limited condition](image)

Single, triple mutants and wild type growth curve under the iron limited condition contained 10 µM 2,2’-dipyridyl
Cyanobacteria are oxygenic photosynthetic prokaryotes that use CO\textsubscript{2} as the sole carbon source for autotrophic growth. However, oxygen produced during the process of photosynthesis irreversibly inactivates nitrogenase, the enzyme responsible for nitrogen fixation. There are various mechanisms present in diazotrophic cyanobacteria to resolve these two conflicting processes, oxygen-evolving photosynthesis and oxygen-sensitive nitrogen fixation. Cyanothece sp. ATCC 51142 is a unicellular cyanobacterium that temporally separates these two processes by performing photosynthesis and accumulating glycogen during daytime, and consuming oxygen by high rate of respiration to supply a suitable intracellular environment for nitrogen fixation during nighttime. The model cyanobacterium Synechocystis sp. PCC 6803, a close relative of Cyanothece, is a non-diazotrophic strain ideal for synthetic biology applications, because of its mature genetic modification system. In order to learn the principles of engineering a non-diazotrophic cyanobacterium to fix nitrogen, genes for nitrogen fixation were introduced from Cyanothece into Synechocystis.

In Cyanothece, the nif genes are found together in an intact-cluster of 35 genes, 28kb in length. This entire gene region was integrated into a plasmid and successfully transformed into Synechocystis, where it stably replicated in cells. All of the nif genes in the cluster were expressed in Synechocystis, using the native promoters from Cyanothece, especially under nitrogen deprivation conditions. Another plasmid was constructed that contained only the core region of the nif-cluster (20kb in length). Nitrogenase activity, assayed by acetylene reduction to ethylene, could be detected in Synechocystis strains containing both of these plasmids. The highest activity at present 3\% of that assay for Cyanothece, showed that a functional nitrogenase enzyme could be made in Synechocystis. Work is in progress to refactor the nif-genes using exogenous promoters to find the minimal genes required for activity, which will be important for tuning genes to respond environmental changes, like the light/dark cycle.

Genome-scale metabolic models allow for insights into the metabolism and optimal genetic perturbations to achieve desired results. And these models were previously developed for both Cyanothece and Synechocystis. The Synechocystis model has been updated to incorporate additional reactions using recent publications, which has led to a reduction in the number of blocked metabolites in the model. This model will be used to aid in the genetic regulation necessary for nitrogen fixation in Synechocystis.

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Mass Spectrometry Footprinting Reveals the Structural Rearrangements of Cyanobacterial Orange Carotenoid Protein upon Light Activation

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The orange carotenoid protein (OCP), a member of the family of blue light photoactive proteins, is required for efficient photoprotection in many cyanobacteria. Photoexcitation of the carotenoid in the OCP results in structural changes within the chromophore and the protein to give an active red form of OCP that is required for phycobilisome binding and consequent fluorescence quenching. We characterized the light-dependent structural changes by mass spectrometry-based carboxyl footprinting and found that an α helix in the N-terminal extension of OCP plays a key role in this photoactivation process. Although this helix is located on and associates with the outside of the β-sheet core in the C-terminal domain of OCP in the dark, photoinduced changes in the domain structure disrupt this interaction. We propose that this mechanism couples light-dependent carotenoid conformational changes to global protein conformational dynamics in favor of functional phycobilisome binding, and is an essential part of the OCP photocycle.


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INVESTIGATING PHOTOSYSTEM II CHARGE SEPARATION KINETICS WITH TRANSIENT-GRATING STARK SPECTROSCOPY

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The investigation of Photosystem II (PSII) charge separation kinetics is vital for understanding the role that protein structure plays in tuning the electronic coupling of pigments in the PSII reaction center. Progress in structural and spectroscopic studies of PSII yielded an understanding of the arrangement of pigments in the reaction center, and several mechanisms describing their interactions to form a charge separated state were proposed. However, comparing theoretical models to experimental data is complicated by the ultrafast time scales of the charge transfer (CT) processes involved and the fact that these CT states are typically non-radiative and do not have clear spectroscopic signatures. Linear Stark spectroscopy has proven to be a valuable method for uncovering CT states in photosynthetic reaction centers and other systems. This approach has been extended to pump-probe experiments in the presence of an external electric field to investigate the kinetics of charge transfer in biological and organic semiconductor systems. The magnitude of the electric-field-dependent kinetics is often small so a sensitive spectroscopic method is required. Heterodyne-detected transient-grating spectroscopy has been shown to have significantly better signal-to-noise than pump-probe spectroscopy due to the detection being background-free. We propose a new transient-grating Stark (TG Stark) spectroscopy method to enable sensitive, high time-resolution detection of charge-transfer kinetics. We demonstrate the method on the D₁D₂-cyt-b559 PSII reaction center complex.

Panel A: A background-free diffractive optic 2D TG electronic spectroscopy setup. The pump and probe pulses are produced by non-collinear parametric amplifiers (NOPAs). The incoming pulses are then time overlapped and focused on the diffractive optic (DO) and re-imaged on the sample. The signal is collected and detected with a spectrometer.

Panel B: Transient Stark signal obtained with our setup at 77K on D₁D₂. The TG signal was acquired in background-free geometry using spectral interferometry. The kinetics of the two peaks shown above were fit to a bi-exponential function (inset).
ASSEMBLY OF LIGHT-HARVESTING COMPLEX 2 IN LIPID NANODISCS

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A major challenge in understanding membrane protein complexes such as light-harvesting and reaction center protein complexes involves obtaining these in a functionally active, water-soluble and monodisperse form. Incorporating membrane protein complexes into nanodiscs, lipid patches surrounded by a boundary protein, makes it possible to observe them in native like, homogeneous membrane environments. By using this technique, intact complexes can be studied through native electrospray ionization mass spectrometry or mass spectrometry-based footprinting. By integrating ESI MS and nanodisc assembling approaches we can study protein conformations and protein-protein interactions. Membrane-embedded LH2 light-harvesting complexes from purple bacteria were used as a model system in this current project. The protein-lipid nanodisc assembly was isolated by using a gel filtration column and the size was confirmed by dynamic light scattering. Static fluorescence emission spectra of nanodisc samples excited at 375 nm shows a slight red shift compared to detergent sample. Incorporation of the LH2 complex into the nanodisc and analysis using ESI MS is underway.
LOCALIZATION OF PHYOCOBILIPROTEINS UNDER FAR RED LIGHT GROWTH CONDITIONS IN CHLOROPHYLL \textit{f} CONTAINING CYANOBACTERIUM \textit{Halomicronnema hongdechloris}

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The filamentous cyanobacterium \textit{Halomicronnema hongdechloris} was the first organism found that contains chlorophyll \textit{f}. This unique chlorophyll is expressed under red light growth conditions and appears to help the organism adapt to the light condition. In this work, we explore the effect of Far Red (FR) light growth conditions on the phycobiliproteins in \textit{H. hongdechloris} using biochemistry, spectroscopy, confocal microscopy, electron microscopy and hyperspectral imaging. Our preliminary results indicate a polar localization of phycobiliprotein next to the septum of the cells with an accompanying 10nm red shift in fluorescence emission in the FR grown cells compared to the White Light (WL) grown cells. Likewise, biochemical isolation of the photosynthetic proteins suggests a difference in constitution of the photosynthetic apparatus under FR growth conditions. We are using TEM of freeze substituted cells to see if there are corresponding changes in cell ultrastructure. Together, these data suggest significant rearrangement the photosynthetic apparatus in favor of eco-physiological adaptation growth.

Confocal microscopy image of \textit{H. hongdechloris} with bright spots showing fluorescence emission from the 650 nm region and grey areas showing emission over 700 nm.
STRUCTURAL ANALYSIS OF DIHEME CYTOCHROME C BY HYDROGEN-DEUTERIUM EXCHANGE MASS SPECTROMETRY AND HOMOLOGY MODELING

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A lack of X-ray or NMR structures of proteins inhibits their further study and characterization, motivating the development of new ways of analyzing structural information without crystal structures. The combination of hydrogen-deuterium exchange mass spectrometry (HDX-MS) data in conjunction with homology modeling (HM) can provide improved structure and mechanistic predictions. Here a unique diheme cytochrome c (DHCC) protein from *Heliobacterium mosdesticaldum* is studied with both homology modeling and HDX to bring some definition of the structure of the protein and its role. Specifically, HDX data were used to guide the homology modeling to yield a more functionally relevant structural model of DHCC.

Homology Model of DHCC with highlighted sections representing the different rates of HDX observed. Top Left, low-exchange, helix region; Top Right, very-low exchange helix bundle; Bottom Left, high exchange, loop region; Bottom Right, medium exchange, helix/loop region. Dashed lines represent the oxidized form of the protein and solid lines are the reduced sample. *Biochemistry, 2014*, 53 (35), pp 5619–5630
POINT MUTATIONS IN THE WATER OXIDATION SITE OF PHOTOSYSTEM II OF SYNECHOCYSTIS SP PCC 6803 CAUSE CHANGES IN THE RATE OF WATER OXIDATION.
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In Photosystem II (PSII), a Mn$_4$CaO$_5$ cluster is the active site for water oxidation, and it is surrounded by water molecules for substrate in a cavity between the D1 and CP43 subunits of PSII. The water molecules undergo poorly understood hydrogen bonding networks within the cavity that may help modulate the function of the Mn$_4$CaO$_5$ cluster. The water also interacts with the first and second sphere amino acid ligands, V185 and D61, which are also thought to modulate the Mn$_4$CaO$_5$ cluster. Mutations to these ligands were made, changing the Val185 to Ile and Leu, Ser169 to Leu and Cys, and Asp61 to Lys and Val. Observations were then made over each mutant’s ability to assemble functional PSII proteins, as evidenced by fluorescent measurement of the chlorophyll, as well as observing the oxygen evolution to measure the oxygen production by each mutant compared to the wild type. Many of the mutants proved to have affected accumulation of PSII, and often far too much for our investigation. However, some of the mutants proved to have relatively unaffected accumulation of PSII, and instead had affected oxygen evolution rates, which, when normalized to the wild type, begin to paint a clearer picture of the interactions water undergoes inside of PSII near the Mn$_4$CaO$_5$ cluster.

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STUDYING THE STRUCTURAL AND ELECTRONIC CONFIGURATIONS DURING PHOTOCATALYTIC ACTIVATION OF OXYGEN AT A DIIRON (II) COMPLEX.

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Several mono and carboxylate-bridged diiron nuclear enzymes are critical in activating dioxygen in various biological processes such as DNA synthesis, hydrocarbon metabolism and cell proliferation¹. Iron-containing enzymes such as cytochrome P450, peroxidases, catalases and methane monooxygenase (MMO) have been shown to activate dioxygen by using two electrons and protons to produce iron (IV) oxo intermediates². The remarkable efficiency of these enzymes is often attributed to the formation of these iron(IV) cations which serve as active oxidants in enzymatic reactions and are able to attack the C-H bonds of a wide range of hydrocarbon substrates³. It is widely postulated that ferryl-oxo species are key intermediates in the mechanism of cytochrome P450 and MMO. However due to the complexity of the protein environments in biological iron enzymatic systems¹, monitoring the structural changes occurring during dioxygen activation is a complex undertaking. This project serves to study the light driven activation of a well characterized artificial analogue of an diiron MMO enzyme, (µ-peroxo) (µ-carboxylato) diiron(III) complex, [Fe₂(µ-O₂)(N-EtHPTB)(µ-PhCO₂)]²⁺ in a chromophore/diiron complex assembled unit. Such types of assemblies provide new avenues for study of catalytic reaction mechanisms. They are promising examples of artificial molecular systems leading to dioxygen activation as visible light is efficiently absorbed at the chromophore, and light energy is in turn converted into a chemical potential via an electron relay through charge accumulation processes at the iron metal center. Formation of high valent iron peroxy species have been shown by UV-Vis, EPR and Resonance Raman spectroscopy as well as X-ray spectroscopic analysis. XANES and EXAFS revealed formation of an iron peroxy and oxo species with different coordination numbers.

EXPLORING ANTENNA SYSTEMS IN CYANOBACTERIA

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Photosynthetic antenna systems, such as those found in cyanobacteria, function in the process of harvesting sunlight for conversion into cellular fuel. In the cyanobacterium Synechocystis sp. PCC 6803, light harvesting is accomplished by a combination of membrane intrinsic pigment-proteins, such as CP43, CP47 and IsiA, and large extrinsic phycobilisome complexes. We are using a combination of approaches to understand the principles of light harvesting and the specificity of antenna association with photosystems. In addition, we are engineering Synechocystis strains to have modified light harvesting systems with new functionalities.

We have examined the consequences of phycobilisome modification in a series of mutants with varying degrees of antenna truncation, and have found that these strains evolve oxygen at high rates but are defective in S-state transitions. Global proteomic analyses of phycobilisome mutants showed widespread changes to processes distantly related to photosynthesis. Isolated IsiA-PSI complexes are being examined using chemical cross-linking to reveal the molecular-level associations of IsiA with PSI. Using a synthetic biology approach, we are re-engineering natural antenna complexes by generating artificial light harvesting proteins called maquettes and then tuning the localization and pigment binding of these proteins in vivo in cyanobacteria. Synthetic biology approaches and omics-aided strategies are also being applied to the dynamic processes of phycobilisome assembly and disassembly. Together, these studies are unraveling the different aspects of light harvesting and the various strategies that cyanobacteria employ to optimize energy capture.

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REDOX-SENSITIVE CYSTEINE RESIDUES GATE EXCITATION ENERGY TRANSFER IN THE FENNA–MATTHEWS–OLSEN ANTENNA COMPLEX

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The Fenna–Matthews–Olson (FMO) complex is a light-harvesting, homotrimeric bacteriochlorophyll a (BChl a) containing antenna protein found in green sulfur bacteria and the phototrophic Acidobacterium Chloracidobacterium thermophilum. Two decades ago, it was discovered that the protein contains a redox-dependent energy quenching pathway to protect itself from transferring energy in the presence of atmospheric oxygen. In the intervening years, little progress had been made on determining the mechanism of this pathway. Using high-resolution tandem mass spectrometry coupled with de novo peptide sequencing, as well as cysteine alkylation, we have discovered that redox-active cysteine residues are necessary for the energy transfer quenching in purified FMO protein from the model organism Chlorobaculum tepidum. Absorption, circular dichroism, and fluorescence spectroscopies confirm that considerable photophysical changes occur in the complex upon cysteine modification. Direct protein electrochemistry indicates a midpoint potential of -267 mV vs. SHE for a 2 H⁺/2 e⁻ proton-coupled electron transfer process at physiological pH. This electrochemical response disappears upon cysteine modification. The cysteines of interest lie less than 5 Å from, and likely coordinate to, the C-3¹ oxygen atoms of BChl a #2 and #3. These results demonstrate that the charge state of the cysteine residues play an essential role in controlling the exciton delocalization among the protein’s pigments. To our knowledge, this cysteine-assisted excitation quenching has not been seen in any other known photosynthetic system.
We propose using Two-Dimensional Electronic Spectroscopy (2DES) to study the coherent dynamics of monomeric Chlorophyll a, the most abundant pigment molecule found in the photosynthetic molecular complex Photosystem II (PSII). Recent 2DES studies of the PSII Reaction Center [1] have observed long-lived coherent oscillations during the energy and charge transfer steps that precede the formation of a charge-separated state. This and previous observations of coherent dynamics in photosynthetic systems [2-5] indicate the presence and potential importance of long-lived coherent states for the functionality of these systems, though the origin of these coherences is still not clear. This study seeks to help determine the physical origin of observed coherent oscillations, whether they are vibrational, electronic, or mixed vibrational-electronic, and to aid in the development of a computational model for the spectrally-congested PSII reaction center complex. Chlorophyll a is a good model system due to its relative simplicity, the extent to which it has been studied previously, and its prominence and participation in charge and energy transfer processes in the PSII reaction center. In our 2DES studies of Chlorophyll a we observe prominent coherent dynamics which persist at both 77 K and Room Temperature. We use simulations of simple dimer models [6] to determine the origin of the observed coherences.

References:
CONFORMATIONALLY GATED ELECTRON TRANSFER IN NATIVE BACTERIAL
REACTION CENTERS FROM A MANGANESE CLUSTER

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Oxygenic photosynthesis uses a manganese cluster to catalyze the oxidation of water to molecular oxygen. Oxygenic photosynthetic organisms descended evolutionarily from their more ancient anoxygenic relatives. Until recently, the utilization of manganese as a secondary electron donor in anoxygenic photosynthesis has only been shown in mutant bacterial reaction centers (BRCs) from *Rhodobacter sphaeroides*1,2. In the search for this evolutionary transition we recently succeeded in enabling even native bacterial reaction centers (BRCs) to utilize manganese ions as secondary electron donors through coordination of several manganese ions into a complex, and changes in the BRC’s environment (see also plenary lecture by Kalman). However, the details of the binding of this complex to the BRC and the corresponding redox reactions have yet to be fully understood. In our current work we attempt to localize the manganese binding site and characterize the second-order electron transfer from manganese to the bacteriochlorophyll dimer. This is accomplished by altering the local dielectric constant by incorporating various hydrophobic molecules into the BRC, by screening of electrostatic interactions, and by removal/reconstitution of the secondary quinone. The common feature of all of these probes is that they change the occupancy of the cavity near the bacteriochlorophyll monomer B. The diffusion and binding of the manganese to the proposed binding site was also found to be facilitated by light-induced conformational changes, resembling the photoactivation process of the oxygen evolving complex of Photosystem II.


Bacterial reaction center in detergent micelle. Diffusion of the manganese complex to the proposed binding site in the cavity near the inactive bacteriochlorophyll monomer.
COMBINED VISIBLE AND FTIR SPECTROSCOPIC CHARACTERIZATION OF BC₁ COMPLEX COMPONENTS

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Difference mode Attenuated Total Reflection Fourier Transform Infrared (ATR FTIR); thin film FTIR; and visible spectroscopy together provide valuable information regarding the nature of binding of quinones and the redox state of the bc₁ complex. Detailed kinetic studies of the binding and subsequent reactions require starting with the identification of relevant peaks and the best possible characterization of the peaks. Relevant peaks for Q₁ site appearance of the semiquinone have been tied to bH titrations using visible absorption spectra of the midpoint potential where a so-called b₁₅₀ species appears as an increase in potential in the titration. This should represent the formation of the semiquinone and the higher potential for reduction of bH being caused by interaction of the semiquinone with bH. These combined spectroscopies taken across various redox spans reflect this and characterize a target for viewing the kinetics of the mechanism involved in the reduction of quinone at the Q₁ site of the bc₁ complex of Rhodobacter sphaeroides.
AFFINITY IS A DOUBLE EDGED SWORD FOR SOLUBLE ELECTRON CARRIERS
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Full sunlight provides about ten times more energy than photosynthetic organisms can successfully use for carbon fixation under ambient CO2 concentrations. The great majority of the solar energy absorbed throughout the day therefore never enters the photosynthetic electron transport chain, but is instead dissipated as heat to avoid oxidative damage to the photosystems. In considering strategies to increase photosynthetic yields, the disproportionate abundance of solar energy with respect to the availability of substrates for its utilization is widely recognized as the greatest source of inefficiency, and the most promising area for improvement. While most approaches focus in one way or another on increasing the effectiveness of carbon fixation, this project is instead interested in the feasibility of constructing alternative electron transport pathways from PSI to electron sinks outside the cell. Engineering such a pathway is expected to be feasible based on the fact that they already exist in the non-photosynthetic electrogenic bacteria Geobacter and Shewanella. Using annotated sequences of soluble cytochromes from Shewanella we have created a library of electron carrier proteins purified from expression in E. coli. Using in-vitro spectroscopic techniques these cytochromes were characterized for their ability to act as efficient electron acceptors from PSI. We report that many characteristics such as midpoint potential, protein size, and number of charge carriers are relatively unimportant, whereas rapid release of the protein after charge transfer is absolutely crucial.

Single-flash spectra of different cytochromes with purified spinach thylakoids reveal very different binding behaviors responsible for their overall reduction kinetics.
STRUCTURE-FUNCTION STUDIES OF TRANS-MEMBRANE SIGNALING IN THE CYTOCHROME $b_6f$ COMPLEX

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The cytochrome $b_6f$ complex of oxygenic photosynthesis catalyzes proton-coupled redox reactions of the lipid soluble substrate plastoquinone (PQ)/plastoquinol (PQH$_2$), to generate a trans-membrane proton electrochemical gradient through vectorial transfer of protons from the electrochemically negative (n, stromal) side of the thylakoid membrane, to the electrochemically positive (p, lumen) side. Free energy stored in the trans-membrane gradient is utilized for ATP synthesis. The p-side PQH$_2$ deprotonation-oxidation (Q$_o$) site within the cytochrome $b_6f$ complex has previously been suggested to function as a “redox-sensor”, to control the pathway of photosynthetic state transitions (¹, ²). PQH$_2$ binding within the trans-membrane Q$_o$-site has been implicated in activation of a 76 kDa Stt-7 kinase, whose catalytic site is located on the n-side, within a 68 kDa extrinsic domain. PQH$_2$-dependent activation of the Stt-7 kinase leads to phosphorylation and redistribution of LHCII accessory pigment-protein molecules between photosystems II and I (³, ⁴). Interaction between the cytochrome complex Q$_o$-site and the Stt-7 kinase domain requires trans-membrane communication over a distance of >40 Å through a presently unknown signaling mechanism (hypothetical model shown in Figure).

To gain insights into the mechanism of signaling between the Q$_o$-site and the Stt-7 kinase domain, structure-function studies of the cytochrome Q$_o$-site were performed (⁵, ⁶). An unusual feature of the Q$_o$-site is the presence of a single chlorophyll-a molecule (Chl). It was observed that the presence of the Chl molecule significantly occludes the Q$_o$-site. The Chl molecule interacts with PQ/PQH$_2$ within the Q$_o$-site, leading to enhanced retention of the PQ molecule through modified intra-protein PQ/PQH$_2$ dynamics (⁵). A consequence of PQ-retention within the Q$_o$-site is the formation of semi-quinone, which leads to enhanced superoxide production by the cytochrome complex (⁶). The prolonged life-time of PQH$_2$/PQ may be significant for the generation of the signal that transmits information from the intra-complex Q$_o$-site to the surface-associated Stt-7 kinase.

Further insights will be obtained from a co-crystal structure of the cytochrome $b_6f$ complex and the Stt-7 kinase, by identification of, (i) the binding site of the kinase on the cytochrome surface, and (ii) residues involved in signal transduction.

CHARACTERIZATION OF TWO CHLAMYDOMONAS REINHARDTII MUTANTS WHICH ARE DEFECTIVE IN CHLOROPHYLL BIOSYNTHESIS AND PHOTOSYNTHESIS.

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The green micro-alga Chlamydomonas reinhardtii possesses photosynthetic apparatus analogous to higher plants and a completely sequenced genome, as well as photo-autotrophic and heterotrophic (exogenous acetate acts as carbon source) growth ability. These attributes make it a versatile model for oxygenic photosynthesis studies. This project aims to identify molecular components pertinent to chlorophyll (Chl) biosynthesis and photosynthesis in Chlamydomonas.

Chl and heme are essential tetrapyrroles. Synthesized via a common branched pathway, they function in energy metabolism in photosynthetic organisms. One enzyme of this pathway is the multi-trimeric Magnesium chelatase (MgChel), which consists of three subunits: CHLD, CHLI, and CHLH. By inserting Mg\(^{2+}\) into Protoporphyrin IX, MgChel, yields the first biosynthetic intermediate specific to the Chl branch. Most plants have two isozymes of CHLI (CHLI1 and CHLI2). Although the functional role of CHLI1 is well characterized, that of CHLI2 is not. GUN4 (genomes uncoupled 4) is a regulator of MgChel.

This project aims to identify molecular components that play a role in Chl biosynthesis in Chlamydomonas employing forward genetics. Two random DNA insertional chlorophyll deficient mutants defective were generated by our lab, namely 5A7 and 6F14. Mutant 5A7 lacks detectable Chl. It lacks the CHLI1 gene but possesses an intact CHLI2. 5A7 also lacks eight additional uncharacterized genes. 5A7 is the first known CHLI1 mutant to be identified in green algae. Mutant 6F14 is GUN4 deficient, green in the dark, but photo-bleaches under higher light intensities. 5A7 and 6F14 have been complimented with a functional CHLI1 and GUN4 gene, respectively. We present our recently published research results on the characterization of two DNA insertional mutants defective in Chl biosynthesis: 6F14 and 5A7.
TIME-RESOLVED NANOSECOND CIRCULAR DICHRONIC SPECTROSCOPY FOR PHOTOSYNTHESIS RESEARCH

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Fenna-Matthews-Olson (FMO) complex is a photosynthetic light harvesting protein that channels electronic excitations from chlorosomal antenna to reaction center in green sulfur bacteria. Each subunit of this trimeric complex contains 8 bacteriochlorophyll \textit{a} (BChl \textit{a}) molecules enclosed into a betta-sheet of a proteins. Close spacing leads to strong interaction between the BChl \textit{a} molecules and causes excitonic delocalization of electronic excited states over several pigments. Excitonic nature of the excited states gives a rise to coherences and quantum beats between different states and it has been suggested that such quantum interference can enhance the efficiency of energy transfer along the complex (Fleming, Shulten). This phenomenon, however, relies on a particular realization of the excitonic excited state structure within FMO, defined by the energies of individual noninteracting pigments (diagonal elements of a Hamiltonian matrix) and interactions between the pigments (off-diagonal elements). Since the diagonal energies are not directly observable, conclusions about their values are made using exciton modeling of available experimental data, resulting in 10 different Hamiltonian matrices. Our recent studies on triplet excited state dynamics suggested that current models are insufficient for describing the experimental data. Formation of localized triplet states on individual pigments excludes these pigments from excitonic Hamiltonian and allows to decouple the properties of this pigment from the rest of the pool. The latest date obtained in this way suggests, for example, that the lowest singlet exciton state is shared between 3 BChls, not 2 as proposed by the best current models. It is clear that further refinement of exciton models is needed to properly understand energy transfer processes in this complex.

In this work we develop and apply time-resolved circular dichroism (CD) nanosecond spectroscopy to study exciton structure of the FMO (via triplet state dynamics) and refine the exciton model parameters. Unlike unpolarized optical spectra, excitonic CD is extremely sensitive to mutual orientation of interacting pigments, which will allow to greatly enhance the sensitivity of the model to model parameters. With modifications, this new technique will allow us to measure changes in CD spectrum of $\Delta A_{R-L} \sim 10^{-6}$, which should be sufficient to measure a change in CD absorption of the FMO protein.
INVESTIGATION OF SECOND PROTON RELEASE FROM Q₀ SITE IN BC₁ COMPLEX

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One function of the cytochrome bc₁ complex is to generate a proton gradient for use in ATP synthesis. Each turnover of the quinol oxidation site results in the release of two protons on the p-side of the membrane. The mechanism for release of the first proton is well established, but just how the second proton escapes from the active site has not been determined. Our model is that several key residues stabilize a chain of water molecules, leading from the active site past the b₃ heme, allowing the proton to reach the bulk solvent via a Grotthus-type mechanism. We are investigating proton release both indirectly, through monitoring the redox kinetics of the b₃-hemes of the low potential chain, and directly, using the pH-sensitive dye neutral red. Neutral red is able to cross the chromatophore membrane, and after the photosynthetic chain is activated by a flash of light we can observe the color change of the dye as protons are released into the interior. We are studying the effects of mutations at residues R94, Y147, and N279 in Rb. sphaeroides.
DETERMINING THE INTERACTIONS BETWEEN PHYCOBILISOMES AND REACTION CENTERS IN A EUKARYOTIC RED ALGAE C. MEROLAE

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Abstract:
\textit{Cyanidioschyzon merolae}, an extremophilic red alga adapted to the environment in acid hot springs (pH 2.0-3.0), uses one type of photosynthetic antenna called the phycobilisome (PBS) to harvest light energy. This energy is then transferred to the reaction centers (RCs, PSI and PSII) to drive the splitting of water and the photosynthetic electron transport chain, which provides cellular energy for carbon fixation. We are interested in the structural basis of efficient excitation energy transfer from PBS to two photosystems, particularly, whether or not a megacomplex exists in \textit{C. merolae} as suggested by our work in cyanobacteria. Chemical cross-linking was accomplished by using isolated thylakoid membranes. The resulting cross-linked protein complexes were then separated on a step sucrose density gradient through ultracentrifugation. Fluorescence and absorbance data suggest that the PBS is cross-linked to both PSI and PSII in our extracts, and that these components are energetically coupled. Using liquid chromatography-mass spectrometry (LC-MS/MS), we have confirmed that the bands collected contain PBS and RCs, and current experiments are being done to determine the nature of their interactions in these cross-linked extracts. Future work will involve optimization of the cross-linking procedure, peptide cross-linking analysis by LC-MS/MS, and time resolved fluorescence spectroscopy on extracted complexes to elucidate the mechanisms of energy transfer within the complex.
SYNECHOCOCCUS ELONGATUS UTEX 2973, A FAST GROWING CYANOBACTERIUM FOR BIOTECHNOLOGICAL APPLICATIONS

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Photosynthetic microbes are of emerging interest as production organisms in biotechnology because they can grow autotrophically using sunlight and CO₂. A rapidly growing cyanobacterial strain that can be genetically manipulated would serve as an ideal candidate for broad research purposes, including studies that focus on understanding cyanobacterial systems and those that utilize cyanobacteria to produce valuable products. Unfortunately, none of the currently used model cyanobacterial strains can grow under autotrophic conditions as fast as yeast or other microbes used in industrial applications. Here we describe *Synechococcus* UTEX 2973, a unicellular cyanobacterium capable of rapid autotrophic growth, comparable to heterotrophic industrial hosts. *Synechococcus* UTEX 2973 can be readily transformed for facile generation of desired knockout and knock-in mutations. Genome sequencing coupled with global proteomics analyses revealed that *Synechococcus* UTEX 2973 is a close relative of the widely studied cyanobacterium *Synechococcus elongatus* PCC 7942, an organism that grows more than two times slower. A small number of nucleotide changes are the only significant differences between the genomes of these two cyanobacterial strains. Thus, our study has unraveled genetic determinants necessary for rapid growth of cyanobacterial strains of significant industrial potential. *Synechococcus* UTEX 2973 could serve as an ideal model cyanobacterial system for a wide range of applications.

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PHOTOPHYSICAL PROPERTIES OF PERYLENE-PORPHYRIN PANCHOMATIC ABSORBERS

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Natural photosynthetic pigments (chlorophylls and bacteriochlorophylls) absorb strongly in the blue and red or near-infrared regions of the solar spectrum, but weakly elsewhere. For next generation solar conversion systems, synthetic chromophores must not only absorb over a broad spectral range, but also possess a relatively long excited state lifetime and synthetically tailorable structure. A high fluorescence quantum yield also would find utility. The favorable photophysical properties should be maintained in nonpolar and polar media. Such a molecule would be an excellent light-harvesting tool. To combine panchromaticity with any or all of the aforementioned traits is a difficult task that has no set template. Here we describe the photophysical properties of a set of porphyrins strongly electronically coupled to one to four perylenes. Absorption and fluorescence spectra, fluorescence and triplet quantum yields, singlet and triplet excited-state lifetimes, and the associated rate constants are presented. The results illustrate the favorable characteristics of the perylene-porphyrin panchromatic absorbers as solar light-harvesting units.

Absorption spectra of perylene-porphyrins in toluene.
A THYLAKOID LUMENAL REDOXIN INVOLVED IN THE REGULATION OF PHOTOSYSTEM STOICHIOMETRY IN CYANOBACTERIA

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In oxygenic photosynthetic organisms, the adjustment of photosynthetic complex stoichiometry is directly related to cellular redox homeostasis. The thylakoid lumen provides an environment for numerous redox reactions; however, how the lumenal proteins are involved in the regulation of photosystem stoichiometry remains largely unknown. There are eight predicted thioredoxin-like proteins in the thylakoid lumen of the cyanobacterium Synechocystis sp. PCC 6803. We have named them as thylakoid lumenal redoxin (TLR)1 to 8. The deletion of the gene encoding TLR8 resulted in a significant increase in the PSII to PSI ratio and an over three-fold increase in the phycocyanin to chlorophyll ratio. Mutant cells grown under low light had lower photosynthetic activity on a per cell basis than wild type, but slightly higher when grown under high light. P700 redox kinetics indicated that PSI activity was significantly reduced in the mutant cells. These results indicate that the thylakoid lumenal protein TLR8 is a strong determinant of photosystem stoichiometry in cyanobacteria.

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