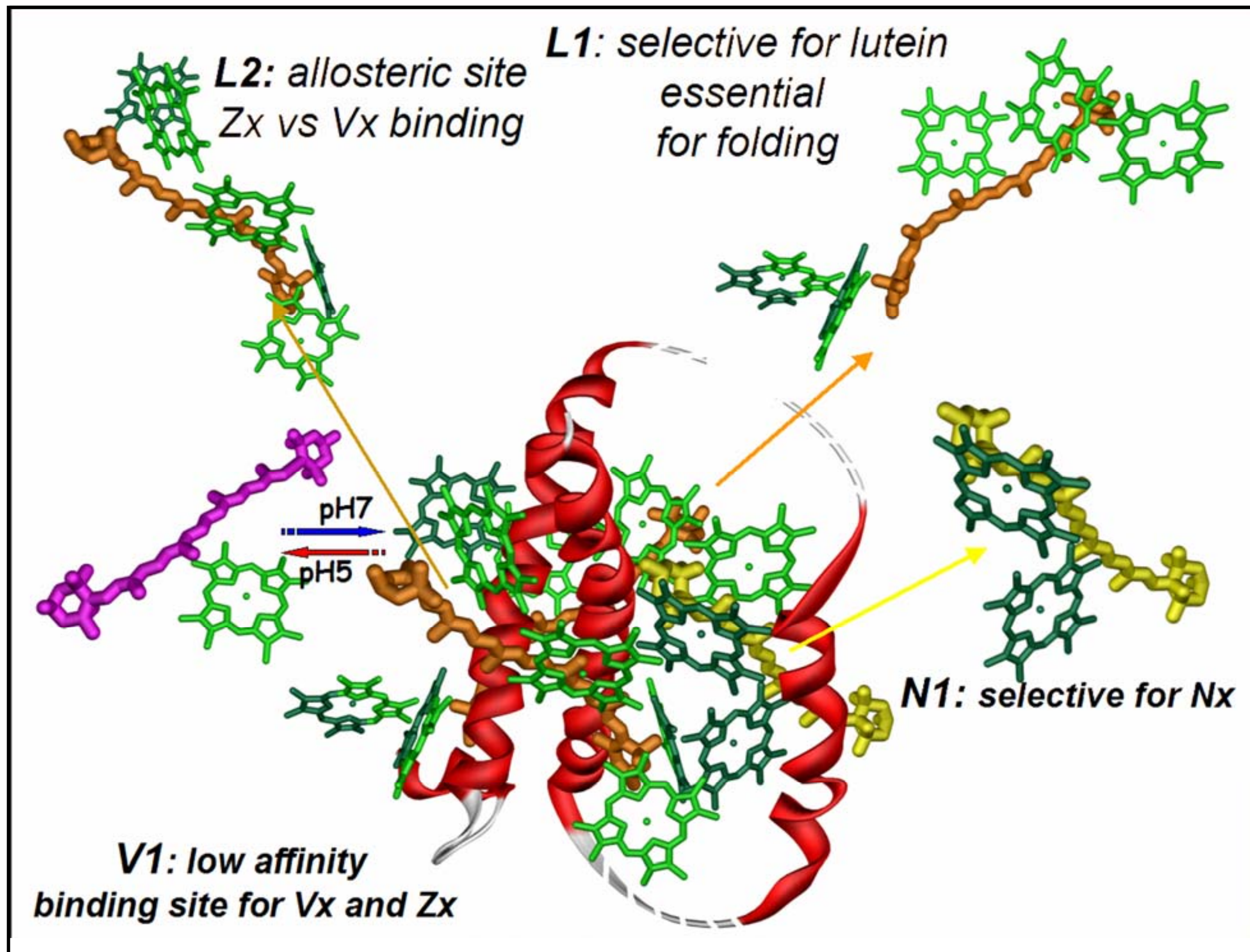
# Twenty Third Annual Eastern Regional Photosynthesis Conference




Swope Center
Marine Biological Laboratory
Woods Hole, MA
April 21st-23rd, 2006

# *Twenty Third Annual Eastern Regional Photosynthesis Conference*

Swope Center
Marine Biological Laboratory
Woods Hole, MA
USA

April 21st – 23rd, 2006

## *This ERPC was sponsored by:*

**AgriSera**

**Brooklyn College of CUNY**

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**On the cover:** Structure of the LHCII showing the specific binding sites for Carotenoids.
**The figure was generously provided by Dr. Roberto Bassi from the University of Verona, Italy.**

# 23rd Annual Eastern Regional Photosynthesis Conference
## A note from the Chair

It is a great pleasure to welcome you to the 23rd annual meeting of the ERPC. Unfortunately, several of our colleagues could not make it to Woods Hole this year, but they send their greetings. If you participated in past meetings, you will recognize that the overall structure of the meeting remained the same. In several aspects we follow the modifications that were introduced last year. Although it was possible to create three sessions around special topics, various presentations were not grouped into sub-categories, because it was difficult to create fitting categories. In consequence this chairman looked at the abstracts and grouped them according to common themes. Also, continuing the tradition of previous meetings we have three featured speakers. In general, it was difficult to convince participants to give oral presentations, but we still managed to put a program together.
I wish to thank several people for their support that facilitated conference organization. Our previous chairman Tom Punnett did a wonderful job and provided me with plenty of valuable information. He already reserved the conference site at MBL for the next years (see below). Specifically, his tip to begin fundraising early resulted in the participation of multiple outside donors. This year we have several donors that provided generous financial and general conference support to allow us some of the extras we enjoy at this meeting. We are grateful for support to:

**AgriSera**
**Brooklyn College of CUNY**
**Bruker Biospin EPR**
**Li-Cor**
**Opti-Sciences**
**PP Systems**
**Qubit Systems**.

My thanks go to Ms. Karen Hwang who put our conference webpage together. The website for this year's ERPC will be kept in service and I hope that I will be able to provide this conference website to the next Chairman.

Please, find below the ERPC schedule for the next years:

| | | |
|---|---|---|
| ERPC-23 | 21st-23rd April, 2006 | Jürgen Polle, Chair |
| ERPC-24 | 20th-22nd April, 2007 | Gary Hastings, Chair |
| ERPC-25 | 18th-20th April, 2008 | Wade Johnson, Chair |
| ERPC-26 | 17th-19th April, 2009 | Michelle Mac, Chair |
| ERPC-27 | 23rd-25th April, 2010 | |

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

Jürgen Polle
Chair ERPC-23
jpolle@brooklyn.cuny.edu

## CONFERENCE SCHEDULE AT A GLANCE

*SESSIONS WILL BE HELD IN WHITMAN AUDITORIUM.*

**Friday:** April 21, 2006

| | |
|---|---|
| **4 - 6PM** | **ARRIVAL AND REGISTRATION** |
| **5:00PM** | **COCKTAILS** |
| **6:00PM** | **DINNER** |
| **7:30PM** | **WELCOME AND OPENING REMARKS** |
| **7:45PM** | ***SESSION A:*** Invited Lecture - Dr. Kenneth Sauer, CONTRIBUTED PAPERS |
| **9:00PM** | **MIXER AND POSTER VIEWING** |

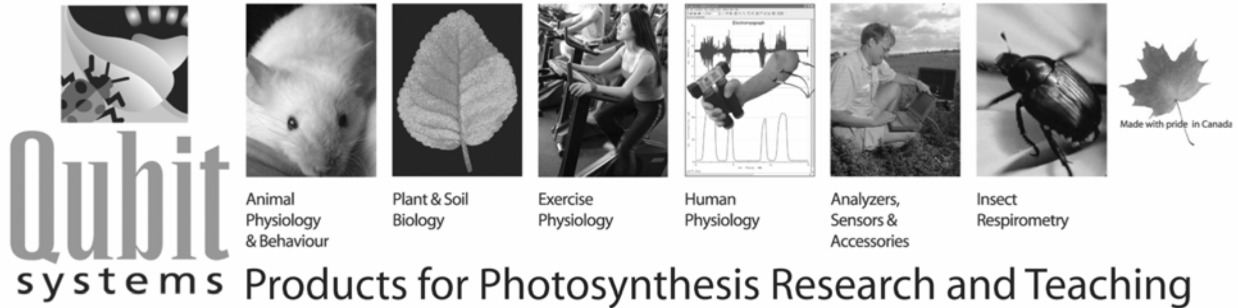
**Saturday:** April 22, 2006

| | |
|---|---|
| **7:30-8:30AM** | **BREAKFAST** |
| **8:45AM** | **SESSION A:** OXYGEN EVOLUTION (CONTINUED) |
| **9:00AM** | **SESSION B:** PHOTOSYSTEM I – CONTRIBUTED PAPERS |
| **10:15AM** | **COFFEE BREAK** |
| **10:45AM** | **SESSION B:** PHOTOSYSTEM I (CONTINUED) |
| **11:00AM** | **SESSION C**: CONTRIBUTED PAPERS |
| **12:15PM** | **LUNCH** |
| **1:30-4:00PM** | **EXHIBITS AND FREE TIME** |
| **4:00PM** | **POSTER SESSION** |
| **5:00PM** | **COCKTAILS, POSTER SESSION CONTINUES** |
| **6:00PM** | **DINNER** |
| **7:00PM** | **SESSION D: LIGHT HARVESTING,** Invited Lecture - Dr. Roberto Bassi |
| **8:00PM** | **MIXER AND POSTER VIEWING** |

**Sunday:** April 23, 2006

| | |
|---|---|
| **7:30-8:30AM** | **BREAKFAST AND PRELIMINARY CHECK-OUT** |
| **8:45AM** | **SESSION D: LIGHT HARVESTING (CONTINUED)** |
| **10:15AM** | **COFFEE BREAK** |
| **10:30AM** | **MEETING OF PI'S IN THE LECTURE HALL** |
| **10:45AM** | **SESSION E:** Invited Lecture - Dr. G.C. Dismukes |
| **11:45AM** | **CLOSING REMARKS** |
| **12:00PM** | **LUNCH** |
| **1:00PM** | **DEPARTURE** |

Leader in the design of research instrumentation
for measurement of leaf gas exchange, soil respiration,
chlorophyll fluorescence and vegetation reflectance

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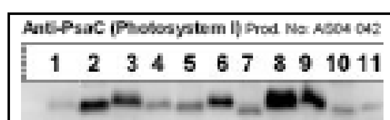
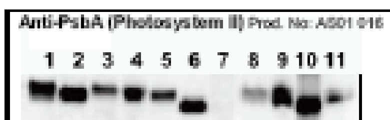
# Antibodies for Research in Photosynthesis

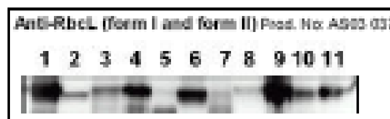
Primary antibodies for: Light Harvesting Complexes (LHC)
All proteins of Photosystem I
All proteins of Photosystem II
Rubisco, Elip and many more

## Global Antibodies & Protein Standards

Global Antibodies – for Multi-species target recognition




Lane description, Multi-species Western Blot:
(Loading based on equal Chlorophyll a - 0.25 µg/line)
1. Higher plants
2. Cyanobacteria
3. Glaucocystophyte
4. Ochrophyte - Raphidophyte
5. Ochrophyte - Diatom
6. Euglenoid
7. Prasinophyte
8. Green Algae -Chlorophyte
9. Red Algae
10. Dinoflagellate
11. Haptophyte

AgriSera Global Antibodies:
Photosystem II → Anti-PsbA (D1), Anti-PsbB (CP47)
Photosystem I → Anti-PsaC
Anti-RbcL (form I), Anti-RbcL (form I and form II)
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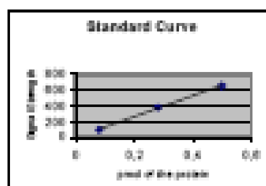
Please note that the same Global Antibody can be used for detection of the target protein in different species, from cyanobacteria to higher plants

Molar Quantitation of key photosynthetic proteins using Global Antibodies:

Step 1:

Immunoblotting:
Analyzed sample and related protein standard are separated on the gel. Followed by immunoblotting and detection using ECL.

Step 2:




Molar Quantitation:
Create a standard curve for estimation of quantity of the target protein in the analyzed sample by using AgriSera Protein Standard.

AgriSera Protein Standards: RbcL (Rubisco), PsaC, PsbA, PsbB, AtpB

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AgriSera also provides a custom in-house screening of samples for levels of key photosynthetic proteins as well as a service of custom antibody production. For more information and to browse our product portfolio, please visit our homepage: www.agrisera.com

# Detailed Conference Program

## GUEST SPEAKERS

**DR. ROBERTO BASSI**

**UNIVERSITY OF VERONA, ITALY**

The Function of Individual Xanthophyll Species in Light Harvesting and Photoprotection of the Higher Plant Chloroplast

**DR. CHARLES DISMUKES**

**PRINCETON UNIVERSITY**

Nature's Renewable Energy Blueprint: Future Fuel from Photosynthesis and Biomimics

**DR. KENNETH SAUER**

**UNIVERSITY OF CALIFORNIA, BERKELEY**

Photosynthetic Oxygen Evolution - Past, Present and Future

### CONFERENCE SCHEDULE

**Friday:** April 21, 2006

**4:00-6:00PM** ARRIVAL AND REGISTRATION

**5:00PM** COCKTAILS

**6:00PM** DINNER

**7:30PM** WELCOME AND OPENING REMARKS

**7:45PM** SESSION A: OXYGEN EVOLUTION, CHAIR DR. H. FRANK

- **Invited Lecture:** Dr. Kenneth Sauer: Photosynthetic Oxygen Evolution - Past, Present and Future
- **Presentation A1:** Victor S. Batista: QM/MM Model of the Oxygen-Evolving Complex of Photosystem II

**9:00PM** MIXER AND POSTER VIEWING

**Saturday:** April 22, 2006

**7:30-8:30AM** BREAKFAST

**8:45AM** **SESSION A: OXYGEN EVOLUTION (CONTINUED)**

- **Presentation A2:** James McEvoy: Analysis of Proposed Mechanisms of Water Splitting in Photosystem II

**9:00AM** **SESSION B: PHOTOSYSTEM I**
**CHAIR: DR. J. GOLBECK**

- **Presentation B1:** Mark Heinnickel: Identification of a Ferredoxin-like Protein (PshB) that binds the Terminal Electron Acceptors (FA and FB) in *Heliobacterium modesticaldum*
- **Presentation B2:** Wade Johnson: Quantification of in vivo supplemented anthraquinones incorporated into PS I complexes of synechocystis sp. pcc 6803 mutants lacking phylloquinone
- **Presentation B3:** Sreeja Parameswaran: On the origin of the 1656(+)/1637(-) cm-1 difference band in (p700+-p700) ftir difference spectra: low temperature ftir difference spectroscopy for the study of p700 in y(b718)t mutant cyanobacterial PS I particles
- **Presentation B4:** Art van der Est: The Role of Aspartate D556psaB in Electron Transfer through Phylloquine in PS I

**10:15AM** **COFFEE BREAK**

**10:45AM** **SESSION B: PHOTOSYSTEM I (CONTINUED)**

- **Presentation B5:** Priyangika Jayaweera: Time-Resolved Ftir Difference Spectroscopy Used to Study Photosystem I Particles with Different Quinones Occupying the A1 Binding Site

**11:00AM** **SESSION C**

- **Presentation C1:** Dr. O. Onder: Comparative Proteomics Reveals that the Amount of the Periplasmic Protease degp Becomes Crucial in the Absence of the Dithiol: Disulfide Oxidoreductase dsba
- **Presentation C2:** Dariusz Niedzwiedski: Femtosecond Time-Resolved Absorption Spectroscopy of Xanthophylls
- **Presentation C3:** Klaas J. van Wijk: Protein profiling of plastoglobules in chloroplasts and chromoplasts; a surprising site for differential accumulation of metabolic enzymes
- **Presentation C4:** Christian Fufezan: Influence of the redox potential of QA on photoinhibition.

**12:15PM** **LUNCH**

**1:30-4:00PM** **EXHIBITS AND FREE TIME**

**4:00PM** **POSTER SESSION**

**5:00PM** **COCKTAILS, POSTER SESSION CONTINUES**

**6:00PM** **DINNER**

**7:00PM** **SESSION D: LIGHT HARVESTING**
**CHAIR: DR. D. BRUCE**

- **Invited Lecture:** Dr. Roberto Bassi: The Function of Individual Xanthophyll Species in Light Harvesting and Photoprotection of the Higher Plant Chloroplast

**8:00PM** **MIXER AND POSTER VIEWING**

**Sunday:** April 23, 2006

**7:30-8:30AM** **BREAKFAST AND PRELIMINARY CHECK-OUT**

**8:45AM** **SESSION D: LIGHT HARVESTING (CONTINUED)**

- **Presentation D1**: Robert A. Niederman: Unusual properties of the light-harvesting 1 complex of purple bacteria: structural implications of nonconservative near-ir circular dichroism spectrum
- **Presentation D2:** Ruili Wang: Density functional theory based calculations for the study of the vibrational properties of chlorophyll a
- **Presentation D3:** Robielyn Ilagan: Spectroscopic Studies of the Main-form and High-salt Peridinin-chlorophyll a-protein from amphidinium carterae
- **Presentation D4:** Dion Durnford: The Evolution of the LHC Superfamily in Chl A/B-containing Organisms
- **Presentation D5:** Julia A. Maresca: Identification of a Gene Cluster Responsible for the Brown Phenotype In Green Sulfur Bacteria

**10:15AM** **COFFEE BREAK**

**10:30AM** **MEETING OF PI'S IN THE LECTURE HALL**

**10:45AM** **SESSION E**

- **Invited Lecture:** Dr. G.C. Dismukes: Nature's Renewable Energy Blueprint: Future Fuel from Photosynthesis and Biomimics

**11:45AM** **CLOSING REMARKS**

**12:00PM** **LUNCH**

**1:00PM** **DEPARTURE**

# Abstracts

## INVITED LECTURES

### Session A (Friday, 7:45 PM)

PHOTOSYNTHETIC OXYGEN EVOLUTION – PAST, PRESENT AND FUTURE

*Ken Sauer* - Dept. of Chemistry and Melvin Calvin Laboratory, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720-5230

Since the consolidation of Planet Earth 4.8 Ga (billion years ago), the gaseous atmosphere has had abundant oxygen during only the most recent 12% of the time. The increase happened with the arrival of rooted and stemmed plants, and it led to major consequences in the evolution of oxygen-tolerant invertebrates and vertebrates. Cyanobacteria, which had arisen by 2.7 Ga, developed the capacity to oxidize water to dioxygen, but the abundant supply of reducing compounds in the environment greatly restricted the concentration of $O_2$ in the atmosphere for another 2 billion years. It is perhaps surprising that the complex in Photosystem II in present day cyanobacteria bears a close resemblance to that in plants and algae that arose at 1.9-1.4 Ga, apparently by endosymbiosis of a cyanobacterium into a primitive non-photosynthetic eukaryote. This oxygen-evolving complex survives in modern plants and algae.

Investigations by Joseph Priestly in 1772 showed that plants evolve oxygen gas, which had only recently been "discovered" by Lavoisier, and Ingen-Housz confirmed in 1779 that light plays an essential role. Subsequent studies by Theodore Engelmann in 1882-3 showed that illuminating the green chloroplasts in plant cells led to the evolution of $O_2$. The requirement of two light reactions to transfer 4 electrons from water to produce an $O_2$ led to a minimal quantum requirement of 8, which was verified by experiments in the early 1960's. The Kok-Joliot cycle rationalized the storage of oxidizing equivalents in five states: $S_0$ -> $S_4$. It had previously been shown that manganese is an essential element for this reaction and that it occurs in PS II. Subsequent studies showed that a cluster of 4 Mn and 1 Ca is involved and that chloride plays an essential role – in both higher plants and cyanobacteria. The structure of PS II based on X-ray crystallography has been reported from several laboratories; however, the best resolution to date (3.0Å) falls short of atomic resolution. Although the general location of the $Mn_4Ca$ cluster is seen in the electron density maps, radiation damage from the incident X-rays resulted in chemical reduction of the Mn and disruption of the cluster. A less damaging approach using X-ray spectroscopy, which provides very high resolution, has been successful in elucidating a likely model for the structure of the cluster, its orientation in the PS II complex and its interaction with ligands provided by the protein environment.

To complete our knowledge of the structure and its mechanism of action will require additional research. Some of the objectives are to decrease the radiation damage while increasing the resolution for the crystallographic X-ray studies. Time-resolved spectroscopic methods need to be applied to elucidate the steps in the water oxidation mechanism, which are still largely unexplored. The manner in which water enters the cycle, protons are extracted, oxidation occurs, the O-O bond is formed and $O_2$ is released still involves many unanswered questions. It is the hope that our improved understanding of this mechanism will lead to the development of bio-informed applications for the conversion of solar energy into chemical fuels.

## Session D (Saturday, 7:00 PM)

THE FUNCTION OF INDIVIDUAL XANTHOPHYLL SPECIES IN LIGHT HARVESTING AND PHOTOPROTECTION OF THE HIGHER PLANT CHLOROPLAST.

*Roberto Bassi* - Dipartimento Scientifico e Tecnologico, Università di Verona (Italy),
*and* Laboratoire de Génétique et Biophysique des Plantes, Université Aix-Marseille II ( France).

The xanthophyll composition of higher plants and green algae is one of their best conserved biochemical characteristics. This suggests that each xanthophyll species has a special function despite the very similar characteristics of purified xanthophylls. In the dark, or in low light conditions, three xanthophyll species are found, namely lutein, violaxanthin and neoxanthin. Upon exposure to excess light, violaxanthin is transformed into zeaxanthin. In order to elucidate the function of xanthophyll species, we have undertook a systematic genetic analysis of the xanthophyll biosynthetic pathway in order to obtain, per each xanthophyll species, a mutant plant containing: (a) all xanthophylls but one and (b) a single xanthophyll species. These genotypes have been obtained for zeaxanthin and lutein while only partial success was reached for violaxanthin and neoxanthin. The results so far obtained support the view that each carotenoid has specialized function in light harvesting or different aspects of protection from photooxidation. Moreover, it appears that the function of carotenoid in photosynthesis can be understood in the framework of their binding to specific sites of antenna proteins belonging to the Lhc family, besides their intrinsic properties as lipid-free molecules.

References

Fiore A., Dall'Osto, L., Bassi R. and G. Giuliano (2006) Elucidation of the beta-carotene hydroxylation pathway in Arabidopsis thaliana reveals a fundamental role of epoxixanthophylls in photoprotection. Submitted

Dall'Osto,L. Caffarri,S. Bassi, R. (2005) A mechanism of non-photochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. The Plant Cell. 17(4):1217-32.

Havaux M, Dall'Osto L, Cuine S, Giuliano G, Bassi R. (2004) The effect of zeaxanthin as the only xanthophyll on the structure and function of the photosynthetic apparatus in Arabidopsis thaliana. J. Biol. Chem. 279, 13878–13888.

## Session F (Sunday, 10:45 PM)

NATURE'S RENEWABLE ENERGY BLUEPRINT: FUTURE FUEL FROM PHOTOSYNTHESIS & BIOMIMICS

*Charles Dismukes* - Princeton University

Natural photosynthesis is intrinsically highly efficient, with maximum photon capture efficiency approaching 100% and energy conversion efficiency for light into $O_2$ and primary products ranging from 59% to > 27%. However, at incident solar intensity this high efficiency is never attained for conversion to biomass - significantly less than 1% typical – owing to the sophistication of phototrophs in converting sunlight into their own power sources for cellular needs. This talk will discuss strategies for harnessing this significantly larger fraction of energy production capacity than is available from conventional biomass, by the conversion of water into hydrogen using cyanobacteria and microalgae as more efficient fuel factories. Nature's blueprint for the catalytic site in natural photosynthesis that powers global $O_2$ production from water is emerging from basic research. The design of man-made catalysts that use this blueprint has begun to yield promising results for solar-based methods for direct solar water splitting into $H_2$ and $O_2$.

# PRESENTATIONS

## Presentation A1 (Friday, 8:45 PM)

QM/MM MODEL OF THE OXYGEN-EVOLVING COMPLEX OF PHOTOSYSTEM II

*Victor S. Batista*

Yale University, Department of Chemistry, P.O. Box 208107, New Haven, CT 06520-8107

This talk introduces structural models of the Oxygen-Evolving Complex (OEC) of Photosystem II (PSII) in the dark-stable S1 state, as well as in the reduced S0 and oxidized S2 states, with complete ligation of the metal-oxo cluster by amino-acid residues, water, hydroxide and chloride. The models are developed according to state-of-the-art Quantum Mechanics / Molecular Mechanics (QM/MM) hybrid methods, applied in conjunction with the X-ray crystal structure of PSII from the cyanobacterium Thermosynechococcus elongatus, recently reported at 3.5 angstrom resolution. Manganese and calcium ions are ligated consistently with standard coordination chemistry assumptions, supported by biochemical and spectroscopic data. Furthermore, the calcium-bound chloride ligand is found to be bound in a position consistent with pulsed Electron Paramagnetic Resonance (EPR) data obtained from acetate-substituted PSII. Ligation of protein ligands includes monodentate coordination of D1-D342, CP43-E354 and D1-D170 to Mn(1), Mn(3) and Mn(4), respectively; $\eta^2$ -coordination of D1-E333 to both Mn(3) and Mn(2); and ligation of D1-E189 and D1-H332 to Mn(2). The resulting QM/MM structural models are consistent with available mechanistic data and also compatible with X-ray diffraction models and Extended X-ray Absorption Fine Structure (EXAFS) measurements of PSII. It is, therefore, conjectured that the proposed QM/MM models are particularly relevant to the development and validation of catalytic water-oxidation intermediates.

## Presentation A2 (Saturday, 8:45 AM)

ANALYSIS OF PROPOSED MECHANISMS OF WATER SPLITTING IN PHOTOSYSTEM II

*James P. McEvoy, Ranitendranath Tagore, Gary W. Brudvig*

Department of Chemistry, Yale University, PO Box 208107, New Haven, CT 06520-8107, U.S.A.Email: gary.brudvig@yale.edu

The mechanism of water oxidation by the oxygen-evolving complex (OEC) of photosystem II (PSII) remains unclear, although many different theories have been proposed. One point of contention has been whether substrate waters are incorporated into the OEC as terminal or as bridging ligands to manganese and/or calcium. In our lab we have recently measured, using mass spectroscopy, the rates of exchange of μ-O and terminal water ligands in the complex $[L_2Mn_2^{III/IV}(\mu\text{-}O)_2(H_2O)_2](NO_3)_3$, where L = 4′-mesityl-2,2′:6′,2″-terpyridine, and the rates of exchange of μ-O and μ-acetate ligands in $[L'_2Mn_2^{III/IV}(\mu\text{-}O)_2(\mu\text{-}OAc)](ClO_4)_2$ and $[L'_2Mn_2^{IV/IV}(\mu\text{-}O)_2(\mu\text{-}OAc)](ClO_4)_3$, where L′ = bis(2-pyridyl)ethylamine. This is the first time that ligand exchange rates have been measured in such high-valent manganese compounds, which serve as model complexes for the OEC. Wydrzynski *et al.* have used mass spectroscopy to measure the rates of exchange of substrate water molecules in the OEC.[1] We have found that the μ-O ligands in our model complexes exchange more slowly (between $\leq 10^{-8}$ $s^{-1}$ and *ca.* $10^{-3}$ $s^{-1}$) than do the substrate water molecules in the OEC (between *ca.* $10^{-2}$ $s^{-1}$ and $\geq 10^{2}$ $s^{-1}$). We therefore conclude that substrate water molecules are likely to be incorporated into the OEC as terminal, rather than bridging, ligands to manganese and/or calcium. This work is supported by the NIH.

(1) Hillier, W.; Wydrzynski, T. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4882-4889.

## Presentation B1 (Saturday, 9:00 AM)

IDENTIFICATION OF A FERREDOXIN-LIKE PROTEIN (PshB) THAT BINDS THE TERMINAL ELECTRON CLUSTERS ($F_A$ AND $F_B$) IN *HELIOBACTERIUM MODESTICALDUM*†

*Mark Heinnickel, Gaozhong Shen, and John H. Golbeck*[1,2]

[1]Department of Biochemistry and Molecular Biology, [2]Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA

Although Heliobacteria are known to contain Type I homodimeric photosynthetic reaction centers (HbRC), the subunit composition and the cofactor complement of these complexes are poorly understood. In particular, a PsaC-type protein could not be identified in the major photosynthesis gene cluster in *Heliobacillus mobilis,* even though $F_A$ and $F_B$-like iron-sulfur clusters have long been demonstrated in isolated complexes by EPR spectroscopy. Previously, we found that a bound ferredoxin can be removed from *Heliobacterium modesticaldum* reaction centers using 1 M KCl (Heinnickel, M., Shen, G., Agalarov, R. and Golbeck, J. H. (2005) *Biochemistry 44*, 9950-9960). This protein, named PshB, could be isolated from the resulting HbRC cores by ultrafiltration over a 30-kDa cut-off membrane. Analysis of the filtrate by SDS PAGE showed a major band at <8 kDa that was weakly stained with Coomassie brilliant blue and strongly stained with silver. EPR studies were consistent with the presence of two magnetically-interacting Fe/S clusters that resemble the terminal electron acceptors $F_A$ and $F_B$. The N-terminal sequence of PshB strongly resembles Fd2, a ferredoxin-like protein that was previously cloned from *Heliobacillus mobilis* (Hatano, A., Seo, D., Kitashima, M., Sakurai, H and Inoue, K. (2004) in Proceedings of the 13th International Congress on Photosynthesis (A. van der Est and D. Bruce, eds), S2 P83). Based the nucleotide sequence, primers were designed for PCR amplification of the comparable protein from *Heliobacterium modesticaldum*. The PCR product was cloned and sequenced, and shown to encode a 5.5 kDa protein containing two [4Fe-4S] cluster binding sites. We overexpressed the His-tagged protein in *Escherichia coli*, purified the protein by affinity chromatography, and reconstituted the protein with Fe/S clusters. The resulting Fe/S protein has an optical absorbance spectrum and an EPR spectrum similar to the PshB protein purified from *H. modesticaldum*. The His-tagged Fe/S protein and HbRC cores were mixed and passed over a nickel affinity column. The Fe/S protein and the HbRC core co-eluted after addition of imidazole. The Fe/S protein and the HbRC core also co-eluted after chromatography on a G-75 gel filtration column. EPR spectroscopy showed that $F_B$ could be reduced by illumination at 20 K. Room temperature optical kinetic spectroscopy showed that the characteristic kinetics derived from the $P798^+ [F_A/F_B]^-$ backreaction were restored. We conclude that the His-tagged Fe/S protein can be rebound to HbRC cores to produce a functional HbRC compex, and is therefore equivalent to PshB in *Heliobacterium modesticaldum.* The ability to affinity-purify reconstituted HbRC complexes using His-tagged PshB makes it possible to obtain highly purified reaction centers. Although the HbRC has been reported to contain only a homodimer of PshA, we find that HbRC cores contain several other polypeptides in addition to PshA and PshB.

†Supported by a grant to J.H.G. from the U.S. Department of Energy (DE-FG02-98ER20314).

## Presentation B2 (Saturday, morning)

QUANTIFICATION OF IN VIVO SUPPLEMENTED ANTHRAQUINONES INCORPORATED INTO PS I COMPLEXES OF *SYNECHOCYSTIS SP.* PCC 6803 MUTANTS LACKING PHYLLOQUINONE.

*Shelley Reppert*[1], *Eric Drago*[1], *John Golbeck*[2], *Art Van der Est*[3] *and T. Wade Johnson*[1]

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Phylloquinone (vitamin $K_1$) acts as a secondary electron acceptor between $A_o$ and the Fe-S clusters of photosystem I in higher plants and cyanobacteria. In the phylloquinone-less mutants (*menA* and *menB*) of the cyanobacterium *Synechocystis* sp. PCC 6803, plastoquinone occupies the $A_1$ site and functions in electron transport. Because of reduced electron transfer efficiency, plastoquinone containing mutants are incapable of growing at high light. Here we report experiments in which we supplemented the growth medium of the mutant cells with various 9,10-anthraquinones (AQ). Interestingly, both of the *menB* and *menA* mutants appear to utilize AQ. For both mutants, most AQ supplements restore some capability to grow at high light and result in doubling times similar to wild type. Quantitative HPLC of the extracted PS I complex pigments indicate that AQ is incorporated in approximately a 1:75 AQ:Chl ratio. The function of quinone in the $A_1$ site of the *menB* mutant was monitored by transient EPR and P700 back reaction kinetics. This is the first example of a non-phytylated quinone being utilized by PS I *in vivo*.

## Presentation B3 (Saturday, morning)

ON THE ORIGIN OF THE 1656(+)/1637(-) $CM^{-1}$ DIFFERENCE BAND IN ($P700^{+}$-P700) FTIR DIFFERENCE SPECTRA: LOW TEMPERATURE FTIR DIFFERENCE SPECTROSCOPY FOR THE STUDY OF P700 IN Y(B718)T MUTANT CYANOBACTERIAL PS I PARTICLES.

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The primary electron donor species in Photosystem I (PS I) is called P700, which consists of a heterodimer of a chlorophyll *a* ($P_A$) and a chlorophyll *a'* ($P_B$) molecules. $P_A$ and $P_B$ are asymmetrically bound, with $P_A$ being involved in a hydrogen (H) bond network involving several amino acids and a water molecule. $P_B$ appears to be free from H-bonding. ThrA739 on PsaA is involved in the H-bond network around $P_A$, and may be suitably positioned to form an H-bond to the $13^1$ keto C=O oxygen of $P_A$. The homologous residue on PsaB is TyrB718, which appears not to be involved in H-bonding to $P_B$. In an attempt to symmeterize P700, site directed mutagenesis was used to change TyrB718 to Thr. PS I particles containing the mutation are referred to as Y(B718)T mutant particles.

We have obtained a ($P700^{+}$-P700) FTIR difference spectrum using Y(B718)T mutant PS I particles at 77 K. The low temperature FTIR difference spectrum does not suggest the formation of a strong H-bond to the $13^1$ keto C=O oxygen of $P_B$, as was envisioned when making the mutant. The difference spectrum does show a very large alteration in the differential signal at 1656(+)/1637(-) $cm^{-1}$, however. This differential signal was assigned in wild type ($P700^{+}$-P700) spectra to the $13^1$ keto C=O of $P_A$. Since the mutation was made near $P_B$, this previous hypothesis is unlikely. Alternative models as to the origin of the 1656(+)/1637(-) $cm^{-1}$ signal will be discussed.

This work was supported by the National Research Initiative of the USDA Cooperative State Research Education and Extension Service grant number 2004-35318-14889, and NSF grant number DBI:0352324 to GH

## Presentation B4 (Saturday, morning)

### THE ROLE OF ASPARTATE D556PSAB IN ELECTRON TRANSFER THROUGH PHYLLOQUINE IN PS I

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An examination of the redox potential of the cofactors in PSI and PSII, reveals that PSI is optimized to generate a strong reducing potential on the stromal side of the thylakoid membrane while PSII is tailored to generate the very positive potential needed to oxidize water. From this point of view it is surprising that PSI contains a quinone acceptor since semiquinones are generally relatively weak reductants. Thus, an intriguing question is how the very negative reduction potential of phylloquinone in PS I is achieved. Recently, a number of explanations have been put forward based on specific features of the PhQ environment. All of them also invoke differences in the electrostatic environments of the two quinones and suggest a more positive midpoint potential for the A-branch quinone. We have are investigating these factors by making selective changes to the environments of the two quinones designed to alter the structural elements proposed to be responsible for the high potential of PhQ. One proposal is that the negative charge on aspartate $D556_{PsaB}$ makes a large contribution to the PhQ redox potential and that there is a shift in the protonation state equilibrium when $PhQ_A$ is reduced . If this is true changing the $pK_a$ of the side chain of the residue at position PsaB-556 should dramatically alter the electron transfer kinetics. Replacing the aspartate with alanine and lysine generates a series of residues in this position that span almost the entire range of possible $pK_a$ values. Lysine in particular would be expected to cause a large stabilization of the reduced quinones and should lead to much slower forward electron transfer. Both mutants do indeed slow forward electron transfer. However, the slowing is relatively minor and no evidence of an additional kinetic phase from the B-branch is found in the transient EPR. We conclude therefore that although $D556_{PsaB}$ does play a role in determining the potential of PhQ and the ET rate, it is not the dominant factor.

## Presentation B5 (Saturday, morning)

TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY USED TO STUDY PHOTOSYSTEM I PARTICLES WITH DIFFERENT QUINONES OCCUPYING THE $A_1$ BINDING SITE

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A phylloquinone (PhQ) molecule (2-methyl, 3-phytyl, 1, 4-naphthoquinone) occupies the $A_1$ binding site in photosystem I. Previously we have obtained $A_1^-/A_1$ FTIR difference spectra using labeled and unlabeled photosystem I (PS I) particles, and proposed assignments for many of the bands in the spectra (Sivakumar, Wang, and Hastings (2005) *Biochem*istry 44, 1880-1893). To further investigate the $A_1$ binding site, and to test our proposed assignments, we have obtained $A_1^-/A_1$ FTIR DS using *menB* mutant PS I particles. In *menB* mutant PS I (and men*D* and *E*), plastoquinone-9 (PQ-9) occupies the $A_1$ binding site. Under repetitive laser flash illumination of *menB* PS I particles at 77 K it is found that the P700 triplet state ($^3$P700) is formed in a fraction of the particles. Infrared spectral signatures that are not due to $^3$P700 are also observed. We suggest that these spectral signatures are associated with PQ-9 anion formation. We test this hypothesis by calculating the vibrational spectra of PhQ and PQ-9.
In *menB* PS I, externally added PhQ can displace the PQ-9 molecule that occupies the $A_1$ binding site. In this way we have reincorporated PhQ back into the $A_1$ binding site. We find that the $A_1^-/A_1$ FTIR difference spectrum obtained using the PhQ reincorporated *menB* photosystem I particles is almost identical to that obtained using WT PS I particles. This PhQ reincorporation strategy allowed us to place specifically labeled PhQ into the $A_1$ binding site, and here we report $A_1^-/A_1$ FTIR difference spectra obtained using PS I particles with $^{18}$O label PhQ occupying the $A_1$ binding site. In this way we are able to "unambiguously" identify bands associated with the C=O modes of neutral and reduced PhQ in $A_1^-/A_1$ FTIR difference spectra.

This work was supported by the National Research Initiative of the USDA Cooperative State Research Education and Extension Service grant number 2004-35318-14889 to GH.

RW was supported by a fellowship from the Molecular Basis of Disease Program at Georgia State University.

## Presentation C1 (Saturday, 11:00 AM)

### COMPARATIVE PROTEOMICS REVEALS THAT THE AMOUNT OF THE PERIPLASMIC PROTEASE DEGP BECOMES CRUCIAL IN THE ABSENCE OF THE DITHIOL:DISULFIDE OXIDOREDUCTASE DSBA

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In Gram negative bacteria, DsbA is a major dithiol:disulfide oxidoreductase that is involved in the formation of disulfide bonds during the oxidative protein folding in the periplasm. In the absence of DsbA, its substrate proteins lack their disulfide bonds, do not fold properly, and are degraded rapidly by periplasmic proteases. *Rhodobacter capsulatus* DsbA-null mutants are proficient in photosynthesis, but they exhibit severe pleiotropic phenotypes extending from motility defects to increased sensitivity to lysis and oxidative stresses. Remarkably, their respiratory growth abilities in enriched growth medium at 35ºC are also impaired, however, they can revert frequently to overcome this defect.

In this study, to gain insight into the molecular basis of this striking phenotype, a combined biochemical and genetic approach was used. The extracytoplasmic proteomes of *R. capsulatus* from a wild type, a DsbA-null mutant, and a DsbA-null revertant were examined using 2D-gel electrophoresis coupled to mass spectrometric identifications. Comparative analyses of the data indicated that most of the differentially expressed proteins in both DsbA-null mutant and in its revertant *versus* the wild-type strain shared a common profile. Among the several hundreds of protein spots tracked, only four exhibited an increase in the DsbA mutant, followed by a decrease to the wild type levels in the revertant strain. All of these four spots were identified by mass spectrometry to correspond to DegP. DegP is a periplasmic serine protease that is essential for the removal of misfolded proteins, which is a crucial process for cell survival. Proteomic approach revealed that *R.capsulatus* DsbA-null mutants overproduce DegP, and consequently are temperature sensitive in enriched medium, demonstrating that overproduction of DegP is deleterious for the respiratory growth of DsbA-null mutants. On the other hand, genetic approach indicated that respiratory growth defect of DsbA-null mutant can be bypassed by decreasing, but not by completely abolishing the activity of DegP, as some DegP activity is needed in the absence of DsbA for cell survival.

**Acknowledgements**
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## Presentation C2 (Saturday, morning)

FEMTOSECOND TIME-RESOLVED ABSORPTION SPECTROSCOPY OF XANTHOPHYLLS

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Many of the photophysical properties of xanthophylls and their role in energy transfer to chlorophyll are understood in terms of two low-lying excited states: $S_1$ ($1^1A_g^-$), into which absorption from the ground state is forbidden by symmetry; and $S_2$ ($1^1B_u^+$), which accounts for the strong visible coloration of the molecules. However, recent studies of open chain carotenoids using femtosecond time-resolved transient absorption spectroscopy have suggested that other "dark" states may also be associated with the mechanism by which these molecules are deactivated. One such state, denoted S*, has eluded characterization, and several models exist seeking to explain its spectroscopic characteristics. We have performed femtosecond time-resolved transient absorption spectroscopic experiments on all the major carotenoids from higher plants: β-carotene (N=11) and the xanthophylls, zeaxanthin (N=11), lutein (N=10), violaxanthin (N=9), and neoxanthin (N=9). The data have been analyzed using single wavelength and global fitting methods, the results of which are consistent with a kinetic model involving the formation and decay of $S_2$ ($1^1B_u^+$), vibrationally-hot $S_1$ ($1^1A_g^-$), vibrationally-relaxed $S_1$ ($1^1A_g^-$), and S*, with distinct dynamics. The data reveal the inherent spectral properties and ultrafast dynamics of each of the pigments and address the molecular features of xanthophylls that control S* formation in solution. The results provide evidence for the origin of the S* state and are expected to be beneficial to researchers employing ultrafast time-resolved spectroscopic methods to investigate the mechanisms of energy transfer in intact thylakoid membranes, isolated pigment-protein complexes, and whole photosynthetic organisms.

This work is supported by the National Science Foundation (MCB-0314380 and MRI-0320403) and the University of Connecticut Research Foundation. TPo thanks the Czech Ministry of Education for financial support (grants No. MSM6007665808 and AV0Z50510513).

## Presentation C3 (Saturday, morning)

PROTEIN PROFILING OF PLASTOGLOBULES IN CHLOROPLASTS AND CHROMOPLASTS; A SURPRISING SITE FOR DIFFERENTIAL ACCUMULATION OF METABOLIC ENZYMES

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Plastoglobules (PGs) are oval or tubular, lipid-rich structures present in all plastid types, but their specific functions are unclear. PGs contain quinones, -tocopherol and lipids and in chromoplasts also carotenoids. It is not known if PGs contain any enzymes or regulatory proteins. Here, we determined the proteome of PGs from chloroplasts of stressed and unstressed leaves of *Arabidopsis thaliana*, as well as from pepper (*Capsicum annuum)* fruit chromoplasts using mass spectrometry. Together, this showed that the proteome of chloroplast PGs consists of seven fibrillins, providing a protein coat and preventing coalescence of the PGs, and an additional 25 proteins likely involved in metabolism of isoprenoid derived molecules (quinones, tocochromanols), lipids and carotenoid cleavage. Four unknown ABC1 kinases were identified, possibly involved in regulation of quinone mono-oxygenases. Most proteins have not been observed earlier, but have predicted N-terminal chloroplast transit peptides and lack trans-membrane domains, consistent with localization in the PG lipid monolayer particles. Quantitative differences in PG composition in response to high light stress and degreening were determined by differential stable-isotope labeling using formaldehyde. More than 20 proteins were identified in the PG proteome of pepper chromoplasts and include four enzymes of carotenoid biosynthesis and several homologues of proteins observed in the chloroplast PGs. Our data strongly suggest that PGs in chloroplasts form a functional metabolic link between the inner envelope and thylakoid membranes and play a role in breakdown of carotenoids and oxidative stress defense, whereas PGs in chromoplasts are also an active site for carotenoid conversions.

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## Presentation D1 (Sunday, 8:45 AM)

UNUSUAL PROPERTIES OF THE LIGHT-HARVESTING 1 COMPLEX OF PURPLE BACTERIA: STRUCTURAL IMPLICATIONS OF NONCONSERVATIVE NEAR-IR CIRCULAR DICHROISM SPECTRUM

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In *Rhodobacter sphaeroides*, radiant energy harvested by the peripheral light-harvesting 2 complex (LH2) is transferred to a core light-harvesting 1 complex (LH1), which surrounds and interconnects the dimeric reaction center (RC) complex and funnels excitations to the RC-BChl “special pair,” where they are trapped in a transmembrane charge separation. In contrast, the photosynthetic unit of *Rhodospirillum rubrum* lacks LH2 and consists of only LH1-RC core structures. The LH1 complex has a number of unusual structural and spectroscopic properties (Westerhuis et al., J. Phys. Chem. B 103:7733, 1999), which have been extensively investigated. Among these is the weak and nonconservative circular dichroism (CD) of the 875-nm $Q_Y$ absorption band of BChl (Bolt et al., Photochem. Photobiol. 34:653, 1981). By using the overall structural framework of the LH1 complex as a basis for modeling of observed carotenoid-induced alterations in both LH1 absorption and CD spectra, a detailed explanation for the origin of this enigmatic spectral signature has recently emerged (Georgakopoulou et al., J. Phys. Chem. B 110:3354, 2006). Strains, in which, the carotenoids of the *Rba. sphaeroides* wild-type LH1 complex were changed from spheroidene and spheroidenone (conjugated double bonds, $n$ = 10 and 11, respectively) to neurosporene ($n$ = 9), and the colorless carotenoid precursor phytoene ($n$ = 3), were provided by J.D. Olsen. They were constructed by complementing neurosporene- and phytoene-producing strains, lacking the LH and RC complexes, with an LH1-only plasmid. An isolated *Rsp. rubrum* wild-type LH1 complex containing spirilloxanthin ($n$ = 13), and a complex in which the carotenoidless *Rsp. rubrum* G9 LH1 was reconstituted with neurosporene, were also examined. These altered carotenoid compositions resulted in small shifts in $Q_Y$ absorption, which were accompanied by strong effects on the $Q_Y$ CD signal, and correlated with the extent of carotenoid conjugation and carotenoid/BChl ratios. Accordingly, in the *Rb. sphaeroides* LH1 complex with a carotenoid/BChl ratio of 1:1, a nonconservative CD spectrum is observed in the presence of the wild-type spheroidene-type carotenoids, while the CD signal was weaker and more conservative in the neurosporene and phytoene-containing strains. Although detergent affects that reversed the +/- signature were observed in the CD spectra of the *Rsp. rubrum* LH1 preparations, the signal in the wild-type remained nonconservative, while a nearly conservative CD was seen in the neurosporene-containing complex. Thus, the redder absorbing carotenoids, of increasing $n$, distort the conservative nature of the $Q_Y$ CD signal significantly, rendering it almost completely nonconservative, especially at higher carotenoid/BChl ratios. An upper exciton component arising from B875 BChl ring of LH1, previously established for the B850 BChls of LH2, was also observed in the LH1 CD signals as a small negative band at ~770 nm, and as a shoulder in the absorption spectra of a purified LH1 complex from a *Rba. sphaeroides* green strain. Since changes from a circular pigment arrangement to the open elliptical LH1 structures seen in structural studies had only small affects on modeled absorption and CD spectra, it is concluded that these various LH1 arrangements are functionally competent in excitation energy transfer between the LH2 and RC complexes.

## Presentation D2 (Sunday, morning)

DENSITY FUNCTIONAL THEORY BASED CALCULATIONS FOR THE STUDY OF THE VIBRATIONAL PROPERTIES OF CHLOROPHYLL *A*

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The vibrational properties of neutral and cation forms of several chlorophyll-*a* (Chl-*a*) model molecules were investigated using hybrid density functional calculations at the B3LYP/6-31G(d) level. Higher level calculations using the B3LYP/6-31G(df,p) and B3LYP/6-31+G(d) methods were also undertaken to test the appropriateness of the chosen basis.
The Chl-*a* model systems studied differed in the number of carbonyl (C=O) functional groups, with the most complicated species containing three C=O groups ($13^3$ and $17^3$ ester C=O's and a $13^1$ keto C=O). To model H-bonding a water molecule was placed in the vicinity of $13^1$ keto C=O group. The harmonic vibrational properties of fully $^2$H, $^{15}$N and $^{13}$C isotopically labeled neutral Chl-*a* were also calculated. From these calculations infrared absorption spectra and "cation minus neutral" infrared difference spectra were produced.
Although an oversimplification, it is shown that the calculated vibrational properties of a Chl-*a* model molecule containing only the $13^1$ keto C=O is a useful model for pyro-Chl-*a*, as the calculated and experimental cation minus neutral FTIR difference spectra are similar.
In neutral model Chl-*a* molecules that contain both the $13^1$ keto and $13^3$ ester C=O groups our calculations predict considerable coupling between the keto and ester C=O modes. This coupling is reduced for the cation state, however. These predictions are inconsistent with experiment results. These calculations presume a molecule in the gas phase, however. H-bonding to the $13^1$ keto C=O group decreases the coupling between the keto and ester C=O vibrations but it also alters the intensity of the C=O modes in a way that is not observed experimentally. For model molecules containing all three carbonyl groups the $17^3$ ester C=O has the highest vibrational frequency, as is observed experimentally. Calculated isotope induced band shifts also closely agree with experimentally observed band-shifts for Chl-*a in vitro*.

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## Presentation D3 (Sunday, morning)

SPECTROSCOPIC STUDIES OF THE MAIN-FORM AND HIGH-SALT PERIDININ-CHLOROPHYLL A-PROTEIN FROM *AMPHIDINIUM CARTERAE*

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The main-form (MFPCP) and high-salt (HSPCP) peridinin-chlorophyll *a*-proteins from the dinoflagellate *Amphidinium carterae* were investigated using steady-state and ultrafast transient absorption spectroscopy at 10 K. Pigment analysis has demonstrated previously that the MFPCP contains eight peridinins and two chlorophyll *a* (Chl), whereas the HSPCP has six peridinins and two Chl.[1] The absorption spectra from both complexes exhibit several distinguishing features that become even more evident at 10 K. In particular, the $Q_y$ transitions of the Chls bound in the HSPCP complex are split into two well-resolved bands indicating structural heterogeneity in the Chl binding sites. In addition, the HSPCP complex shows more pronounced vibrational structure in the peridinin absorption region than does the MFPCP complex. Ultrafast, time-resolved, transient absorption spectroscopy has been used to measure the rate and efficiency of energy transfer from peridinin-to-Chl in both complexes. The complexes were excited in the peridinin absorption band at 530 nm and the transient absorption spectra were monitored in the region 500 nm to 750 nm. A global fitting analysis was carried out on the transient absorption data to obtain detailed information regarding the kinetic behavior of the complexes. The data are consistent with a model whereby both $S_2$ and $S_1$ states of peridinin transfer energy to both Chls, and subsequently the high energy Chl transfers energy to the low energy Chl. The rates and efficiencies of energy transfer will be presented. The study provides insight into the molecular factors that control energy transfer in this class of photosynthetic light-harvesting complexes.

This work is supported by a grant to HAF from the National Institutes of Health (GM-30353) and the University of Connecticut Research Foundation. The ultrafast laser was purchased by funds from the National Science Foundation (MRI-0320403).

(1) Sharples, F. P.; Wrench, P. M.; Ou, K.; Hiller, R. G. *Biochem. Biophys. Acta* **1996**, *1276*, 117.

## Presentation D4 (Sunday, morning)

THE EVOLUTION OF THE LHC SUPERFAMILY IN CHL *A/B*-CONTAINING ORGANISMS

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The Light-harvesting complexes (LHCs) of higher plants and green algae have essential roles in light harvesting and photoprotection. Though the functional diversity of the individual LHC proteins are well described in plants, the extent of this family in the majority of green algal groups is unknown. In order to examine the evolution of the Chl *a*/*b* antennae system and to infer functional capabilities, we initiated several expressed sequence tag (EST) projects from a taxonomically broad range of Chl a/b-containing protists as part of the Protist EST Program. Several green algae with primary plastids from diverse groups were examined, including the Ulvophyceae (*Acetabularia acetabulum*), the Mesostigmatophyceae (*Mesostigma* viride) and the Mamiellales (*Micromonas* sp.), as well as representatives from the euglenophytes (*Euglena gracilis*) and chlorarachniophytes (*Bigelowiella natans*), whose plastids evolved secondarily from green algae. The evolution of the LHC superfamily in Chl a/b-containing organisms with primary and secondary plastids will be discussed with the aim of inferring structural features and functional capabilities of the LHC antennae system during the diversification of green algae.

## Presentation D5 (Sunday, morning)

IDENTIFICATION OF A GENE CLUSTER RESPONSIBLE FOR THE BROWN PHENOTYPE IN GREEN SULFUR BACTERIA

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Two pigment phenotypes exist among green sulfur bacteria (GSB): brown species produce bacteriochlorophyll (BChl) *e* as their antenna pigment and isorenieratene as the primary carotenoid, and green species synthesize chlorobactene as the principal carotenoid and BChl *c* or *d* for the antenna. These pigment pairs are invariant, but both brown and green species appear in all major phylogenetic groups of GSB, suggesting that coordinated gene acquisition or loss may have occurred several times. Because brown species are able to live at much lower light intensities than green species, and in environments more enriched in green wavelengths, identification of the genes underlying these pigment phenotypes is important for understanding adaptation to different environments. The genome of the model organism *Chlorobium* (*Chl.*) *tepidum*, which produces BChl *c* and chlorobactene, has been used to predict and verify the BChl and carotenoid biosynthetic pathways in GSB. Comparison of this genome with the recently sequenced genomes of 3 brown- and 5 green-colored GSB has enabled identification of a 6.5 kb gene cluster responsible for isorenieratene and tentatively for BChl *e* biosynthesis. In order to test the roles of these newly identified genes, we are transferring these genes into *Chl. tepidum*. Identification of this gene cluster and confirmation of the functions of its products may explain both why the BChl *e*/isorenieratene pair is more effective under certain light conditions and how a phenotype that relies on genes in unrelated biosynthetic pathways may have been transferred among different organisms.

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# Posters

## Poster 1

PROTEIN DYNAMICS OF PHOTOSYSTEM II; CHANGING PROTEIN CONFORMATION AFFECTS REACTION CENTER FUNCTION.

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Molecular dynamics (MD) simulations were done to study photosystem II (PSII) structure and function. Structural information obtained from MD simulations were combined with *ab initio* computations of chromophore excited states. These MD - based calculations accurately predicted the experimental absorbance spectrum. Our calculations correctly assigned the energy levels of experimentally determined reaction center chromophores as well as the lowest energy core antenna chlorophyll. The primary and secondary quinone electron acceptors, $Q_A$ and $Q_B$, exhibited independent changes in position over the duration of the simulation. $Q_B$ fluctuated between two binding sites that were similar to the proximal and distal sites previously observed in light and dark adapted RC, respectively, from purple bacteria. Kinetic models were used to characterize the relative influence of chromophore geometry, site energies and electron transport rates on the efficiency of PSII. The fluctuating energy levels of antenna chromophores had a larger impact on calculated quantum yield than did their relative positions. Variations in electron transport rates arising from the changing distances between electron transport cofactors had the most significant effect on calculated efficiency and were sufficient to explain the experimentally observed multicomponent decay of excitation in PSII. The implications of our results are discussed in the context of competing evolutionary selection pressures for RC structure and function.

## Poster 2

LIGAND REPLACEMENT AND HYDROGEN BOND PERTURBATION OF CHLD1 OF PHOTOSYSTEM II; ALTERED SPECTRAL AND KINETIC PROPERTIES

*Rachel Cohen*

E. I du Pont de Nemours & Co

To establish the pathway of electron transfer in the PS II reaction center, it is necessary to distinguish between the chlorophylls, the absorbance spectra of which are very similar. To modify their spectra, site-directed mutations were constructed that alter the pigment spectra. There has been growing indirect evidence that chlorophyll $Chl_{D1}$ is the site of primary electron transfer in PS II at low temperature. In order to investigate the participation of $Chl_{D1}$ directly at low and ambient temperatures, two sets of site- directed mutations were constructed at sites close to $Chl_{D1}$. These are located at D1-T179, which is likely hydrogen bonded to a water molecule that serves as the axial ligand to the $Mg^{2+}$ of $Chl_{D1,}$ and at D2-H197 which is the axial ligand to the special pair chlorophyll, $P_{B,}$ and which is also hydrogen bonded to a water molecule hydrogen bonded to the $C_9$=0 of $Chl_{D1}$. D1-Thr179 was replaced with His, Ala. Lys, and Gln. D2-His197 was replaced by Gln, Glu, Asn, Ala, Lys, and Leu. The low temperature (5 and 77K) $P^+$-P and the $^3P$-$^1P$ difference spectra show that the D-T179 mutations shift the absorption maximum of $Chl_{D1}$. In the D1-T179H mutant the shift is to the red and in the D1-T179E mutant the shift is to the blue. These results show that the triplet state is exclusively localized on $Chl_{D1}$ in both the D1-T179 mutants and the wild type. There is no CP43 and CP47 triplet detectable on the ms time scale.

In the D1-H197Q mutations, the triplet-singlet difference spectrum (at 5K) show what is likely two overlapping bands with maxima around 675 and 681 nm as opposed to a single peak at 683nm for the wild-type. Both, the $P^+$-P and the $^3P$-$^1P$ difference spectra in the D2-H197Q mutant show that the $Chl_{D1}$ absorbance spectrum is shifted to the blue. These results indicate that the D2-H197Q mutation has likely perturbed the hydrogen bond to the $C_9$=0 of $Chl_{D1}$ shifting its absorbance spectrum and raising the energy of the triplet state. In this mutant, the triplet state is shared between $Chl_{D1}$ and $P_A$.

## Poster 3 & Presentation C4

INFLUENCE OF THE REDOX POTENTIAL OF $Q_A$ ON PHOTOINHIBITION

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Strong light creates a stressful condition for plants that can lead to the destruction of the photosynthetic apparatus. This phenomenon is known as photoinhibition. The role of the primary quinone electron acceptor of photosystem II (PSII) during photoinhibition was investigated in vivo and in vitro. We created two mutants, A249S and L267I, in the D2 subunit of photosystem II in *Thermosynechococcus elongatus* by site-directed mutagenesis. Both mutations are located within the binding pocket of the primary quinone acceptor ($Q_A$). While the L267I mutant exhibits characteristics similar to the wild type, the A249S shows an accelerated photoinhibition. This is accompanied by an increase in the singlet oxygen production of the A249S mutant compared to the wild type. Measurements of the thermoluminescence and the rate of the fluorescence yield decay indicate that the radical pair [$S_2Q_A^{-\bullet}$] is destabilized, most probably due to a shift in the redox potential of $Q_A$. Measurement of the redox potential of $Q_A$ show that the A249S mutation has indeed induced a shifted in the redox potential by approximately -60 mV. The last disagreements with the hypothesis that the redox potential of $Q_A$ plays an important role during photoinhibition could be ruled out as the redox potential of $Q_A$ was not altered by the usage of a herbicide but by the mutation A249S.

## Poster 4

A CONSERVED TYROSINE RESIDUE IN THE CYTOCHROME $bc_1$ COMPLEX HELPS TO REGULATE THE HIGH OXIDATION POTENTIAL OF THE [2Fe-2S] CLUSTER PRIOR TO HYDROQUINONE OXIDATION.

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The hydroquinone oxidation reaction at the cytochrome $bc_1$ complex appears to be engineered to prevent the naturally rapid reduction of oxygen that would be expected in simple solution chemistry. Studies trying to rationalize how this reaction is promoted with such efficiency, i.e. without leakage of electrons to $O_2$, have worked out that the oxidation potential of the reaction initiating [2Fe-2S] cluster is transiently very high only when all of the proper molecular ducks are in a row. Specifically it is thought that only when an oxidized [2Fe-2S] cluster, a reduced quinone (hydroquinone) and an oxidized $b_L$ heme (the $II^o$ electron acceptor) come together is the potential of the [2Fe-2S] cluster high enough to overcome the energetic barrier dictated by the highly unstable semiquinone intermediate. The efficiency with which the reactivity of this reaction is harnessed seems to imply that some sort of a "gate" is involved in regulating the oxidation event. Here we describe the mutation of a single tyrosine residue in the cytochrome *b* subunit at the hydroquinone oxidation related site that fails to limit the formation of the higher potential form(s) of the [2Fe-2S] cluster in the steady state. What the consequences of this persistently higher [2Fe-2S] cluster oxidation potential have on the oxidation of hydroquinone and its efficiency will be discussed.
Funding for this project came from the National Institute of Health (F. D.) as well as the American Heart Association (J. W. C.).

## Poster 5

ORNITHINE LIPID IS ESSENTIAL FOR THE PRESENCE OF *C*-TYPE CYTOCHROMES IN *RHODOBACTER CAPSULATUS*

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The *c*-type cytochromes are hemoproteins that are subunits or physiological partners of electron transport chain components, like the cytochrome $bc_1$ complex or the $cbb_3$-type cytochrome *c* oxidase. Their heme moieties are covalently attached to the corresponding apocytochromes via a complex post-translational maturation process. During our studies of cytochrome biogenesis, we uncovered a novel class of mutants that are unable to produce ornithine lipid and that lack several *c*-type cytochromes. Molecular analyses of these mutants led us to the ornithine lipid biosynthesis genes of *Rhodobacter capsulatus*. Herein, we have characterized these mutants, and established the chemical structure of this non-phosphorus membrane lipid from *R. capsulatus*. Ornithine lipids are known to induce potent host immune responses, including B-lymphocyte mitogenicity, adjuvanticity and macrophage activation. Yet, despite their widespread occurrence in Eubacteria, and the diverse biological effects they elicit in mammals, their physiological role in bacterial cells remained hitherto unknown. Our findings now show that ornithine lipids are rather crucial in bacteria for the presence under specific conditions of some extracytoplasmic proteins such as the *c*-type cytochromes, allowing us to attribute them for the first time a novel and important biological function.

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to S. A-S.)

## Poster 6

A FUNCTIONAL HYBRID BETWEEN THE CYTOCHROME *BC*$_1$ COMPLEX AND ITS PHYSIOLOGICAL MEMBRANE ANCHORED ELECTRON ACCEPTOR CYTOCHROME *C*$_Y$ IN *RHODOBACTER CAPSULATUS*

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The membrane integral ubihydroquinone ($QH_2$): cytochrome (cyt) *c* oxidoreductase (or the cyt *bc*$_1$ complex) and its physiological electron acceptor, the membrane-anchored cytochrome $c_y$ (cyt $c_y$), are discrete components of photosynthetic and respiratory electron transport chains of purple non-sulfur, facultative phototrophic bacteria of *Rhodobacter* species. In *Rhodobacter capsulatus*, it has been observed previously that, depending on the growth condition, absence of the cyt *bc*$_1$ complex is often correlated with a similar lack of cyt $c_y$ (Jenney, F. E., et al. (1994) Biochemistry **33**, 2496-2502), as if these two membrane-integral components form a non-transient larger structure. To probe whether such a structural super complex can exist in photosynthetic or respiratory membranes, we attempted to genetically fuse cyt $c_y$ to the cyt *bc*$_1$ complex. Here we report successful production, and initial characterization, of a functional cyt *bc*$_1$-$c_y$ fusion complex that supports photosynthetic growth of an appropriate *R. capsulatus* mutant strain. The three-subunit cyt *bc*$_1$-$c_y$ fusion complex has an unprecedented bis-heme cyt $c_1$-$c_y$ subunit instead of the native mono-heme cyt $c_1$, is efficiently matured and assembled, and can sustain cyclic electron transfer *in situ*. The remarkable ability of *R. capsulatus* cells to produce a cyt *bc*$_1$-$c_y$ fusion complex supports the notion that structural super complexes between photosynthetic or respiratory components occur to ensure efficient cellular energy production.

**Acknowledgements**
This work was supported by NIH GM38237 and DOE ER9120053.

## Poster 7

QUANTITATIVE ANALYSIS OF THE O-J-I-P CHLOROPHYLL FLUORESCENCE RISE REVEALS THE APPARENT ACTIVATION ENERGIES OF EACH KINETIC STEP

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Fluorescence induction (FI) has been studied for a long period of time. When dark-adapted photosynthetic samples are excited with actinic light, fluorescence increases from its initial level ($F_0$ or O) to its maximal level ($F_m$ or P), passing through two intermediate steps (J and I). Most studies agree that O-J rise is related to photosystem II (PSII) primary acceptor ($Q_A$) reduction, but several discordant theories have been enounced for J-I and I-P rises. One limitation with FI studies is that most work done to date uses only qualitative data analysis by visually comparing traces to observe the effects of different chemicals or treatments. While this method is useful to observe major changes in the induction traces, the accurate characteristics of the induction phases are almost impossible to evaluate. A quantitative approach is then needed in FI studies.
To achieve this, we used a simple mathematical approach to extract amplitudes, half-times, and activation energies ($E_A$) for each kinetic step. The effects of two well-known chemicals, DCMU and decyl-plastoquinone (dPQ), were studied using this quantitative analysis. DCMU is known to block electron transfer in PSII beyond the primary electron acceptor, $Q_A$, while dPQ can act as endogenous reducible quinones and then increase the PQ pool size. Our results indicate a different bioenergetics origin for each kinetic step of the FI rise, as they have different apparent $E_A$. The origin of each step will be discussed.

## Poster 8

IRON-RESPONSIVE DIFFERENTIAL EXPRESSION OF PHOTOSYNTHESIS AND NITROGEN FIXATION GENES IN THE MARINE DIAZOTROPH TRICHODESMIUM ERYTHRAEUM IMS101

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Marine cyanobacteria in the genus of Trichodesmium are prominent primary producers and suppliers of “new” nitrogen in the tropical and subtropical oceans where the Fe concentration is thought to be limiting their productivity. In an effort to understand how the photosynthesis and nitrogen fixation machinery acclimate to iron availability, we performed Fe deprivation and reconstitution experiments using axenic culture of Trichodesmium erythraeum IMS101. Here we show that the major constituents of Photosystem I (PSI), PSII, cytochrome (Cyt) f and phycobilisome are differentially regulated by Fe availability. Upon Fe depletion, where the idiA and isiA are induced, all the measured transcripts including nifH, psaA, psaC, psbE, petB, petC and apcA decrease. The photochemical efficiencies of the two photosystems also decline during this phase of acclimation. However, when Fe is supplemented, expression of idiA and isiA decreases, whereas that of the above nif and ps genes increases. Furthermore, we found that under short-term iron stress there is a transcriptional exchange in the expression of a family of three psbA genes encoding two distinct PSII D1 proteins. The normally predominant psaAI gene encoding D1:1 is replaced with the psbAII and psbAIII identically encoding D1:2. This transcriptional interchange is fully reversible once cells are Fe reconstituted.
We suggest that the observed functional and compositional alterations represent cellular response enabling orchestrated gene expression under iron deficiency and acclimation capacity when iron was available.

## Poster 9

THERMODYNAMICS OF PHOTOSYNTHESIS AND THE STORAGE OF ENERGY

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The thermodynamics of photosynthesis have been much discussed in the past but recent articles have shown some confusion on the subject. The aim of this comment is to clarify this state of affairs as much as is possible.

Assuming the light is absorbed, considerations of thermal machines are not relevant to the efficiency of photosynthesis since it is an (almost) isothermal photochemical process. The efficiency of converting the energy of the absorbed photon to free energy of products is limited only by kinetics: the ratio of loss channels to the product channel. If the losses are negligible, the efficiency can be >98%.

Since the early steps may not be in thermal equilibrium because of changes in the protein, the free energy of these steps is not well-defined. The observed large changes in enthalpy on the <μs time scale in photobiological processes suggests that negative entropy can be stored in the protein structure to be used as positive entropy to drive slower reactions. Because the photosynthetic system is "open" one can explain the observed effects of energy storage including the vexing order-producing or "emerging" properties without recourse to violations of the 2nd Law.

## Poster 10

PROGRESS TOWARD HIGH THROUGHPUT STRUCTURE-FUNCTION ANALYSIS OF THE PSBS PROTEIN: PROPERTIES OF PSBS-SILENCED *NICOTIANA BENTHAMIANA*.

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The 22-kilodalton product of the nuclear *psbS* gene is essential for expression of photoprotective nonphotochemical quenching of fluorescence yield (NPQ) in eukaryotic photosystem II. The mechanistic basis for this requirement is a mystery. We are attempting to develop a high throughput system for *in vivo* evaluation of psbS structural variants in *Nicotiana benthamiana* using virus-induced gene silencing (VIGS) and transient expression. This new system is a competitive as well as complementary alternative to the traditional approach of generating stable transgenic lines in Arabidopsis. *Nicotiana benthamiana psbS* sequences were inserted into a Tobacco Rattle Virus cassette embedded in a binary vector and propagated in Agrobacterium. Injection of *N. benthamiana* leaves with the Agrobacterium suspension led to systemic infection culminating in a *de facto* deletion phenotype (NPQ-deficient) in newly developed leaf tissue. Several physiological properties of this system are described. The wide range of variation in expression of *psbS* in these plants allowed a first-time investigation of the quantitative relationships linking NPQ and psbS and zeaxanthin levels. Furthermore, we have succeeded, using Agrobacterium-mediated transient expression, in expressing the orthologous Arabidopsis *psbS* gene in *N. benthamiana* leaves in which endogenous *psbS* was silenced. Preliminary results indicate surprisingly that the capacity for zeaxanthin to *activate* psbS-dependent NPQ is lost in this system. This would seem to suggest that activation of NPQ by zeaxanthin depends on a highly specific interaction between psbS and some other component(s) of photosystem II. Structural changes in these proteins would necessarily have occurred in parallel during the evolution of land plants.

## Poster 11

### A STRUCTURE-FUNCTION STUDY OF THE PSBS PROTEIN OF PHOTOSYSTEM II BASED ON PHYLOGENY AND SITE-DIRECTED MUTAGENESIS

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The 22-kiloDalton protein, psbS, in plants is essential for expression of photo-protective thermal dissipation of excess absorbed quanta often referred to as nonphotochemical quenching of chlorophyll fluorescence yield (NPQ). Amino acid sequence data for 60 psbS proteins from 43 species was compiled using cDNA and expressed sequence tag nucleotide sequences in publicly accessible internet databanks as well as by isolating and characterizing genomic sequences. A phylogenetic analysis of psbS sequences are similar to phylogenetic trees derived using conventional taxonomic criteria. Alignment analysis reveals numerous regions of psbS are highly conserved consistent with their involvement in intrinsic aspects of the NPQ mechanism. Two such regions resemble a known chlorophyll-binding motif in the photosystem II light-harvesting proteins LHCII and CP29. Site-directed mutagenesis was employed to block possible ion-bonding to two chlorophyll molecules by replacing putative glutamate and arginine ligands with non-binding valine and leucine in psbS from *Arabidopsis thaliana*. The capacity to form NPQ was severely restricted in leaves from *Arabidopsis* lacking psbS due to deletion of the *psbS* locus as well as in transformants of this deletion line that expressed either of the mutant forms of psbS described. The results are consistent with, but do not prove, that chlorophylls bound to psbS participate in the NPQ mechanism.

# Poster 12

## MECHANISTIC INFLUENCES ON THE EFFICIENCY OF PHOTOSYNTHETIC WATER OXIDATION

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Important aspects of how photosynthetic organisms produce oxygen by the oxidation of water remain unresolved. Among these are the range of catalytic chemistries across the diverse species of prokaryotes and eukaryotes, and many mechanistic features that determine the efficiency of the reaction *in vivo*.
A number of longstanding questions surround the principles of operation of the Kok S-state cycle of photointermediates. Notably, the origin of S-state miss probability, i.e. the likelihood that the water oxidation center will not extract an electron upon photochemical excitation, remains unclear. The unresolved origin of photochemical misses exists alongside the newly discovered possibility that S-state photointermediates can be produced in the dark by reversing the S-state cycle using elevated $O_2$ backpressure. Here we apply improved fluorescence and amperometric techniques to investigate system-wide influences on the miss-probability and the character of the water oxidation cycle under high $O_2$ back pressure in whole cells of the cyanobacterium *Arthrospira maxima*. We introduce a Clark-type $O_2$ concentration cell designed to measure flash induced $O_2$ concentrations as low as $\geq 2$ nanomolar and with 0.1s time resolution. This tool resolves flash induced oscillations in $O_2$ concentration from 70 single turnover flashes; the data represents the longest sustained period-four oscillation pattern yet recorded for an observable modulated by the water oxidation cycle.
Non-linear regression analysis of this data shows a decrease in the miss probability with flash number, the character of which suggests the influence of two distinct $Q_A^-$ reoxidation kinetics on the miss probability. High pressure oxygen on whole cells of *Arthrospira maxima* leads to no appreciable changes in the S-state cycle, as monitored using the period four oscillations in chlorophyll-a variable fluorescence. These results differ from previous observations for PS II-enriched spinach membrane particles [Clausen, J., W. Junge, et al. (2005) *Biochemistry* **44** (38): 12775-12779.], providing a possible explanation for the abnormally fast turnover for water splitting observed in *A. maxima*.

## Poster 13

ANALYSIS OF PROPOSED MECHANISMS OF WATER SPLITTING IN PHOTOSYSTEM II

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The mechanism of water oxidation by the oxygen-evolving complex (OEC) of photosystem II (PSII) remains unclear, although many different theories have been proposed. One point of contention has been whether substrate waters are incorporated into the OEC as terminal or as bridging ligands to manganese and/or calcium. In our lab we have recently measured, using mass spectroscopy, the rates of exchange of μ-O and terminal water ligands in the complex $[L_2Mn_2^{III/IV}(\mu\text{-}O)_2(H_2O)_2](NO_3)_3$, where L = 4′-mesityl-2,2′:6′,2″-terpyridine, and the rates of exchange of μ-O and μ-acetate ligands in $[L'_2Mn_2^{III/IV}(\mu\text{-}O)_2(\mu\text{-}OAc)](ClO_4)_2$ and $[L'_2Mn_2^{IV/IV}(\mu\text{-}O)_2(\mu\text{-}OAc)](ClO_4)_3$, where L′ = bis(2-pyridyl)ethylamine. This is the first time that ligand exchange rates have been measured in such high-valent manganese compounds, which serve as model complexes for the OEC. Wydrzynski *et al.* have used mass spectroscopy to measure the rates of exchange of substrate water molecules in the OEC.[1] We have found that the μ-O ligands in our model complexes exchange more slowly (between $\leq 10^{-8}$ $s^{-1}$ and *ca.* $10^{-3}$ $s^{-1}$) than do the substrate water molecules in the OEC (between *ca.* $10^{-2}$ $s^{-1}$ and $\geq 10^{2}$ $s^{-1}$). We therefore conclude that substrate water molecules are likely to be incorporated into the OEC as terminal, rather than bridging, ligands to manganese and/or calcium. This work is supported by the NIH.

(1) Hillier, W.; Wydrzynski, T. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4882-4889.

# Poster 14

## SEARCH FOR THE INTERMEDIATE AND TERTIARY ELECTRON ACCEPTORS OF THE PHOTOSYNTHETIC REACTION CENTER IN *HELIOBACTERIUM MODESTICALDUM*

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Type I homodimeric photosynthetic reaction centers (RCs), especially those in Heliobacteriaceae, remain the least understood of all photosynthetic systems. What is known with certainty concerns only the early light-induced reactions. The primary electron donor, $P798^+$, is a dimer of Bchl *g*' (the $13^2$ epimer that now appears to be a common feature of all Type I RCs) and the cation is delocalized over both molecules of Bchl *g*'. The primary acceptor is Chl *a* 670, an $8^1$-hydroxy Chl *a* molecule. Forward electron transfer from $A_0^-$ occurs in 600 ps at room temperature and if blocked, charge recombination between $P798^+$ and $A_0^-$ occurs in 17 ns, repopulating the triplet state of P798. An early EPR study reported signals similar to $F_A$ and $F_B$ in Photosystem I and an optical study hinted at the presence of $F_X$, but no single study or technique has shown the presence of all three Fe/S clusters. The problem of the terminal electron acceptors is compounded by the fact that a gene harboring $F_A$ and $F_B$ FeS clusters comparable to PsaC in cyanobacteria or PscB in *C. tepidum* has not been identified in *Heliobacillus mobilis.*

Given our ability to isolate a simplified RC from the mild thermophile *Heliobacterium modesticaldum*, we considered it timely to reinvestigate the role of these cofactors in forward electron transfer. We have recently identified a PsaC-like protein, PshB, in *H. modesticaldum* (Hb) that contains two [4Fe-4S] clusters analogous to $F_A$/$F_B$ in Photosystem I (Heinnickel, M., Shen, G., Agalarov, R., Golbeck, J.H. (2005) *Biochemistry 44,* 9950-9960). We have also identified an EPR signal at *g* = 4-5 from a S = 3/2 ground state [4Fe-4S] cluster that is derived from $F_X$ in HbRC cores (which have been stripped of PshB) (Heinnickel, M., Agalarov, R., Svensen, N., Krebs, C., Golbeck, J. H. *Biochemistry, in press*). What remains unknown is whether a quinone functions as an electron transfer cofactor between $A_0$ and $F_X$. Previous EPR (Brok, M., Vasmel, H., Horikx, J. T. C., Hoff, A. J. (1986) *FEBS Letters 194,* 322-326) and ENDOR studies (Muhiuddin, I. P., Rigby, S. E., Evans, M. C., Amesz, J., Heathcote, P. (1999) *Biochemistry 38,* 7159-7167) have assigned a resonance around *g* = 2 to a semi-quinone radical in photoaccumulated HbRCs, but optical kinetic experiments in which the quinone has been extracted have shown no change in charge recombination kinetics (Kleinherenbrink, F. A. M., Ikegami, I., Hiraishi, A. Otte, S. C. M., Amesz, J. (1993) *Biochim Biophys Acta 1142,* 69-73). To further probe the function of the quinone, we purified HbRC cores using DEAE anion exchange chromatography, and we extracted the menaquinone in lyophilized HbRC cores using dry organic solvents. We find that the extracted HbRCs retain the ability to donate electrons from P798 to $F_X$ and have unaffected charge recombination kinetics of $F_X^-$ with $P798^+$, thereby providing no evidence for the participation of an extractable quinone in forward electron transfer.

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## Poster 15

P700$^+$ REDUCTION IN LEAVES OF *CUCUMIS SATIVUS* L. FOLLOWING CHILLING-INDUCED PHOTOINHIBITION

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Photosystem I (PSI) is preferentially destroyed in *Cucumis sativus* L. during weak irradiances at low temperatures. The primary event occurring during PS I inhibition is the destruction of terminal iron-sulphur acceptors such as $F_X$, $F_B$ and $F_A$. Subsequent degradation of the geneproducts, *psa*A and *psa*B of the reaction centers (RC) results in the disassembly of PSI complex. In this phenomenon, the reactive oxygen species play a key role. Among the mechanisms of the protection against chilling-induced photoinhibition (CIP), a temporary acceleration of the cyclic PSI electron transport, stromal donation of electrons and charge recombination reactions in the acceptor side of PSI mainly involve the re-reduction of oxidized P700 with distinct kinetics. In previous studies relevant to CIP of PSI, the redox state of the P700 is measured often by monitoring leaf absorbance changes at 820 nm in leaf segments excited with saturating white or far-red light. In those measurements, a large fraction of P700$^+$ reduced in the time-scale of less than about 15 ms due to the linear electron transfer from photosystem II (PSII) is usually undetected during the long measurement regimes. This restricts the true estimates of the photooxidation of P700 and photoinhibitory damage to PSI. In the present study, we evaluated the oxidation kinetics of P700 in *C. sativus* experienced CIP using inhibitor(s) that block electron transport at the acceptor side of PSII, cytochrome $b_6$/f complex and cyclic electron transport around PSI. In leaf segments which were not exposed to CIA, excitation with 50-ms white pulse resulted in the two-exponential rise of $\Delta A_{820}$. About 90% of the total amplitude of $\Delta A_{820}$ was related to the rapid initial rise phase ($t_{1/2}$ ~ 17 ms). When the electron transfer beyond $Q_A$ was inhibited with diuron, photooxidation of P700 was increased by about 60%. While 70-75% increase in the amplitude of $\Delta A_{820}$ was found if the cyclization of electrons to P700$^+$ through intersystem chain and re-oxidation of plastoquinol was prevented by either stigmatellin or DBMIB. On the other hand, a strong inhibition of the above mentioned routes and PSI charge recombination with DBMIB together with methyl viologen amplified the detectable P700$^+$ by more than two folds relative to the one found in leaves not subjected to any inhibitor treatment. Similar trend was found with the use of either white or far-red light. Using this criterion, chilling dependent photoinhibitory damage to PSI was estimated. These results will be presented in detail.

# Poster 16

## THE ROLE OF ASPARTATE $D556_{PSAB}$ IN ELECTRON TRANSFER THROUGH PHYLLOQUINE IN PS I

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An examination of the redox potential of the cofactors in PSI and PSII, reveals that PSI is optimized to generate a strong reducing potential on the stromal side of the thylakoid membrane while PSII is tailored to generate the very positive potential needed to oxidize water. From this point of view it is surprising that PSI contains a quinone acceptor since semiquinones are generally relatively weak reductants. Thus, an intriguing question is how the very negative reduction potential of phylloquinone in PS I is achieved. Recently, a number of explanations have been put forward based on specific features of the PhQ environment. All of them also invoke differences in the electrostatic environments of the two quinones and suggest a more positive midpoint potential for the A-branch quinone. We have are investigating these factors by making selective changes to the environments of the two quinones designed to alter the structural elements proposed to be responsible for the high potential of PhQ. One proposal is that the negative charge on aspartate $D556_{PsaB}$ makes a large contribution to the PhQ redox potential and that there is a shift in the protonation state equilibrium when $PhQ_A$ is reduced (*1*). If this is true changing the $pK_a$ of the side chain of the residue at position PsaB-556 should dramatically alter the electron transfer kinetics. Replacing the aspartate with alanine and lysine generates a series of residues in this position that span almost the entire range of possible $pK_a$ values. Lysine in particular would be expected to cause a large stabilization of the reduced quinones and should lead to much slower forward electron transfer. Both mutants do indeed slow forward electron transfer. However, the slowing is relatively minor and no evidence of an additional kinetic phase from the B-branch is found in the transient EPR. We conclude therefore that although $D556_{PsaB}$ does play a role in determining the potential of PhQ and the ET rate, it is not the dominant factor.

1. Ishikita, H., and Knapp, E. W. (2003) Redox potential of quinones in both electron transfer branches of photosystem I, *J. Biol. Chem.* 278, 52002-52011.

# Poster 17

## THE MOLECULAR DETAILS UNDERLYING PHYLLOQUINONE FUNCTION IN PHOTOSYSTEM I

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One of the research goals in our lab is to gain a molecular level understanding of the intermediate phylloquinone (PhQ) electron acceptor, $A_1$, in cyanobacterial photosystem I. To study $A_1$ in PS I we are using time-resolved infrared difference spectroscopy, in combination with various strategies that allow us to modify either the $A_1$ pigment itself or its binding site. To complement/extend these experimental studies we are also using computational methods to calculate the properties of PhQ in a variety of environments.
Recently we have obtained $A_1^-/A_1$ FTIR difference spectra using labeled and unlabeled photosystem I particles. Comparison of these spectra allowed us to assign bands in the spectra to specific molecular bonds of $A_1$ or the binding site. To test some of these assignments we have obtained $A_1^-/A_1$ FTIR DS using *menG* mutant PS I particles, in which the PhQ in the $A_1$ binding site is replaced with a slightly different analogue (a methyl group on the quinone ring is changed to a hydrogen atom). We find that specific bands in the wild type $A_1^-/A_1$ FTIR DS shift in specific ways in the *menG* mutant spectra. To test if such shifts are reasonable we have calculated the infrared difference spectra for both PhQ and its analogue in a variety of environments. We find that the calculated and experimental spectra agree well, confirming our previously suggested band assignments.

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RW was supported by a fellowship from the Molecular Basis of Disease Program at Georgia State University.

## Poster 18

QUANTIFICATION OF IN VIVO SUPPLEMENTED ANTHRAQUINONES INCORPORATED INTO PS I COMPLEXES OF *Synechocystis sp.* PCC 6803 MUTANTS LACKING PHYLLOQUINONE

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Phylloquinone (vitamin $K_1$) acts as a secondary electron acceptor between $A_o$ and the Fe-S clusters of photosystem I in higher plants and cyanobacteria. In the phylloquinone-less mutants (*menA* and *menB*) of the cyanobacterium *Synechocystis* sp. PCC 6803, plastoquinone occupies the $A_1$ site and functions in electron transport. Because of reduced electron transfer efficiency, plastoquinone containing mutants are incapable of growing at high light. Here we report experiments in which we supplemented the growth medium of the mutant cells with various 9,10-anthraquinones (AQ). Interestingly, both of the *menB* and *menA* mutants appear to utilize AQ. For both mutants, most AQ supplements restore some capability to grow at high light and result in doubling times similar to wild type. Quantitative HPLC of the extracted PS I complex pigments indicate that AQ is incorporated in approximately a 1:75 AQ:Chl ratio. The function of quinone in the $A_1$ site of the *menB* mutant was monitored by transient EPR and P700 back reaction kinetics. This is the first example of a non-phytylated quinone being utilized by PS I *in vivo*.

## Poster 19

BIOGENESIS OF THE BOUND IRON-SULFUR CLUSTERS IN PHOTOSYSTEM I

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*Research Objectives:* Our research program aims at elucidating the biochemical steps in the regulation and assembly of the bound iron-sulfur (Fe/S) clusters in Photosystem I (PS I) of cyanobacteria and plants. Our previous work showed that the *sufR* gene codes for a protein that functions as a transcriptional regulator of the *sufABCDSE* regulon, which encodes Fe/S cluster assembly proteins (Wang, T., Shen, G., Balasubramanian, R., McIntosh, L., Bryant, D. A., and Golbeck, J. H. (2004) 'The *sufR* gene (*sll0088* in *Synechocystis* sp. strain PCC 6803) functions as a repressor of the sufBCDS operon in iron-sulfur cluster biogenesis in cyanobacteria'. *J Bacteriol 186*, 956-967.) The ability of SufR to function as a repressor is proposed to depend on the presence of a Fe/S cluster, which in turn may be sensitive to the levels of reactive oxygen species (ROS) in the cell. Oxidative stress results in damage to Fe/S clusters, including those in PS I, thereby requiring their replacement. Fe/S biogenesis in eukaryotes is not well understood, especially in plants, where the plastid is a major contributor to Fe/S cluster assembly. Our approach involves a mechanistic analysis of the *suf* regulon and its regulation by the repressor SufR in response to iron and oxidative stress.

*Research Results:* SufA, IscA and Nfu have been proposed to function as scaffolds in the assembly of Fe/S clusters. To investigate the roles of these proteins in photosynthetic organisms, single and double null-mutant strains of *Synechococcus* sp. PCC 7002 were constructed by insertional inactivation of genes homologous to *sufA*, *iscA,* and *nfu*. Demonstrating the non-essential nature of their products, the *sufA, iscA,* and *sufA iscA* mutants grew photoautotrophically with doubling times that were similar to the wild type under standard growth conditions. In contrast, attempts to inactivate the *nfu* gene only resulted in stable merodiploids. These results demonstrate that Nfu, but not SufA or IscA, is the essential Fe/S scaffold protein in cyanobacteria. When cells were grown under iron-limiting conditions, the *iscA* and *sufA* mutant strains exhibited less chlorosis than the wild type. This is a likely consequence of increased siderophore production; *sidA* and *sidC* transcript levels, which code for enzymes involved in siderophore biosynthesis, were higher in the mutant strains than in wild type. Under iron-sufficient growth conditions, *isiA* transcript levels, a marker for iron limitation in cyanobacteria, as well as transcript levels of genes in both the *suf* and *isc* regulons were significantly higher in the *iscA* mutant than in the wild type. In other words, the *iscA* deletion mutant acts as if it is under iron stress even when it is grown under iron replete conditions. Under photosynthesis-induced redox stress conditions, the transcript levels of the *suf* genes are notably higher in the *sufA* mutants than in wild type. We conclude that the growth phenotypes and mRNA abundance patterns of the mutant strains contradict the proposed scaffold function for the SufA and IscA proteins in generalized Fe/S cluster assembly and instead suggest that they play regulatory roles in iron homeostasis and the sensing of redox stress in cyanobacteria. SufA and IscA may instead function as sensors of iron-sulfur cluster availability in a complex signal transduction pathway in cyanobacteria.

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## Poster 20

### GENERATION AND CHARACTERIZATION OF A NEW CLASS OF CHLOROPHYLL DEFICIENT MUTANTS OF THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*.

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In oxygenic photosynthesis of plants and green algae light is absorbed through chlorophyll-protein complexes that are located in photosynthetic membranes of chloroplasts. The unicellular green alga *Chlamydomonas reinhardtii* is a model system for research on oxygenic photosynthesis, in part because its photosynthetic apparatus is similar to that in higher plants. As in higher plants chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) are associated with the photosystems. Chl *b* is associated specifically with the light-harvesting complex proteins (LHC) that make up the auxiliary antenna of the photosystems.

For higher plants and *C. reinhardtii* a number of Chl-deficient mutants are known. These Chl-deficient mutants have less Chl per cell and increased ratios of Chl *a* to Chl *b*. We generated transformants of *C. reinhardtii* by random nuclear DNA insertional mutagenesis. Transformants were screened for aberrant coloration and by Chl fluorescence emission. Three new Chl-deficient mutants have drastically reduced levels of Chl per cell and at the same time similar Chl *a* to Chl *b* ratios as the wild type. Although cells of these new mutants are light sensitive, they grow photoautotrophically. In summary, we present a new class of Chl deficient mutants of the alga *Chlamydomonas reinhardtii.*

## Poster 21

AN EXAMINATION OF THE EVOLUTION AND DIVERSIFICATION OF LIGHT-HARVESTING COMPLEXES IN *EUGLENA GRACILIS*

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Light-harvesting complexes (LHCs) are a superfamily of chlorophyll and carotenoid-binding proteins, present in all photosynthetic eukaryotes that are responsible for the capture of light energy and its transfer to the photosynthetic reaction centres. The antennae system itself is composed of distinct LHC proteins that are primarily associated either with photosystem I (LHCI) or with photosystem II (LHCII as well as the minor PSII-associated antenna that includes the CP29 and CP26 proteins). *Euglena gracilis* is a chlorophyll *a*/*b*-containing organism that acquired its plastid secondarily from a green alga. During the transition from symbiont to plastid, the photosynthetic structural and regulatory proteins were transferred to the host nuclear genome. In *Euglena*, this process resulted in the generation of large, concatenated Lhc genes that are expressed as polyproteins, and post-translationally processed into individual units in the chloroplast. This organism provides a unique opportunity to study the diversity and evolution of the Lhc multi-gene family following a plastid acquisition event. There are two main objectives to this project: 1) to examine the diversification and evolution of LHC proteins in the euglenophyte *Euglena gracilis*, and 2) to examine the structural diversity of the LHC polyproteins, and to infer the functional significance of this organization.

As a part of the Protist EST Program (PEP), over 25 000 Expressed sequence tags (ESTs) have been sequenced and analysed in an effort understand the evolution of the Lhc gene family in *Euglena*. While phylogenetic analyses indicate that *E. gracilis* LHC proteins can be divided into LHCI, LHCII, and LHC relatives, *E. gracilis* lacks the LHC orthologs common to most green algae and plants. With a few exceptions, the majority of the LHCs were determined to be polyprotein-encoding mRNAs that contain extensive paralogy at both the inter- and intra-polyprotein level of the LHCs. This paralogy may have led to the large LHC diversity encountered, with more than 40 individual LHCs classified thus far. Additionally, the presence of nearly identical paralogs within the polyproteins indicates that the polyproteins may have originated in order to ensure the proper stoichiometry of the antenna proteins within the thylakoid membrane.

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## Poster 22

MECHANISM OF THE DOWN REGULATION OF PHOTOSYNTHESIS BY BLUE LIGHT IN THE CYANOBACTERIUM *SYNECHOCYSTIS* SP PCC 6803.

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A down regulation of photosynthetic light harvesting, manifested as blue-light induced fluorescence quenching, has previously been described in cyanobacteria We have investigated the molecular mechanism of this regulation by characterizing the changes in excitation energy transfer through the phycobilin pigments to chlorophyll with steady-state and time-resolved fluorescence excitation and emission spectroscopy. Quenching was investigated in both a photosystem II - less mutant and DCMU poisoned wild-type *Synechocystis* sp. PCC 6803. The action spectra for blue-light induced quenching was identical in both cell types and was dominated by a band in the blue region peaking at 480 nm. Fluorescence quenching, and its dark recovery, was inhibited by the protein cross-linking agent glutaraldehyde, which could maintain cells in either the quenched or unquenched state. Both room temperature and 77K fluorescence emission spectra revealed that fluorescence quenching was associated with phycobilin emission. Quenching was characterized by a decrease in the emission of allophycocyanin and phycobilisome terminal emitters relative to phycocyanin. Global analysis of the room temperature fluorescence decay kinetics revealed that phycocyanin and photosystem I decay components were unaffected by quenching while decay components originating from allophycocyanin and phycobilisome terminal emitters were altered. Our data support a regulatory mechanism involving a protein conformational change and/or change in protein-protein interaction which quenches excitation energy at the core of the phycobilisome.

## Poster 23

PROPOSED ROLE FOR THE HOMOLOGS OF *BCHQ* AND *BCHR* IN GREEN SULFUR BACTERIA

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Green sulfur bacteria (GSB) make bacteriochlorophylls (BChls) that are methylated at the C-8$^2$ and C-12$^1$. The degree of methylation varies depending on the light intensity and on the species of GSB. Around 90% of the BChl in GSB is methylated at the C-12$^1$ position, so most of the variation in methylation happens at the C-8$^2$ position. In *Chl. tepidum* two genes, *bchQ* and *bchR*, have been shown to encode the enzymes responsible for the methylation of the C-8$^2$ and C-12$^1$ carbons respectively. BchQ in *Chl. tepidum* produces C-8$^2$ propyl and *iso*-butyl derivatives of BChl *c*; however, no *neo*-pentyl substituent has ever been detected in *Chl. tepidum*. The two genes group with other BChl biosynthesis genes in *Chl. tepidum* and in the other GSB whose genomes have been sequenced. From these genomes we have found paralogs of *bchQ* in *Chl. phaeobacteroides* and *Pelodyction phaeoclathratofrome* and of *bchR* in *Chl. chlorochromatii.* These observations are consistent with the detection of more highly methylated BChl species in these organisms. We propose that, even though BchQ is responsible for doubly methylating the C-8$^2$ carbon of BChl *c* in *Chl. tepidum,* another gene product is required to produce the *neo*-pentyl form of BChl *c*.

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## Poster 24

STEADY-STATE AND FEMTOSECOND TIME-RESOLVED OPTICAL SPECTROSCOPIC STUDIES OF PERIDININ DERIVATIVES

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Peridinin is a highly substituted carotenoid characteristic of the Peridiniales group of dinoflagellates whose structure features an unusual $C_{37}$ carbon skeleton rather than the typical $C_{40}$ system present in most carotenoids. One of its most surprising properties is the fact that the lifetime of the lowest excited singlet state is strongly dependent on solvent environment ranging from 7 ps in the strongly polar solvent, trifluoroethanol, to 172 ps in the nonpolar solvents, cyclohexane and benzene. This behavior is highly anomalous for carotenoids which generally show very little dependence of their $S_1$ spectral properties and lifetimes on solvent environment. The findings suggest that the environment of protein-bound peridinin may modulate its light-harvesting efficiency *in vivo*. This study is aimed at uncovering the molecular features of peridinin that control its excited state properties and dynamics. We have begun an investigation of synthetically-modified peridinins and report here our findings on PerAcEs-Z compared to natural peridinin. The spectroscopic properties and dynamic behavior of HPLC-purified PerAcEs-Z in methanol and hexane were studied by steady-state absorption, fluorescence, fluorescence excitation, and transient absorption spectroscopy at room temperature. Low temperature (77K) absorption and fluorescence experiments of PerAcEs-Z in EPA (5:5:2 v/v/v ether: isopentane: ethanol, EPA) were also done. The investigation shows differences in absorbance maximum, vibrational structure, fluorescence emission maximum and dynamics compared to natural peridinin. The data will be discussed in terms of specific structural features that control the spectroscopic and dynamic behavior of peridinin.

This work is supported by grants to HAF from the National Institutes of Health (GM-30353) and the University of Connecticut Research Foundation.

# Poster 25

## FEMTOSECOND TIME-RESOLVED ABSORPTION SPECTROSCOPY OF OPEN CHAIN CAROTENOIDS

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Many of the spectroscopic features and photophysical properties of carotenoids are explained using a three-state model where the strong visible absorption of the molecules is associated with an $S_0$ ($1^1A_g^-$) $\rightarrow$ $S_2$ ($1^1B_u^+$) transition, and the lowest lying singlet state, $S_1$ ($2^1A_g^-$), is a state into which absorption from the ground state is forbidden by symmetry. However, semi-empirical and *ab-initio* quantum calculations have suggested that additional excited singlet states may lie between $S_1$ and $S_2$, and spectroscopists have reported evidence for these states using ultrafast laser methodologies. One such state, denoted S*, was invoked by van Grondelle and coworkers[1] to account for the ultrafast dynamics of the carotenoid, spirilloxanthin, in solution and in the LH1 complex from *Rhodospirillum rubrum* being different at different probe wavelengths, and in the LH1 complex leading to triplet state formation. Initially, S* was thought to be formed only in the very long (N=13) spirilloxanthin molecule. However subsequent studies on β-carotene, lycopene, rhodopin glucoside, zeaxanthin and spheroidene have suggested that S* may occur more commonly. The primary spectroscopic characteristics of S* are that it has a broad transition with a maximum in the wavelength region between the $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_n$ absorption bands, and that it decays in several picoseconds. An alternative view of the origin of S* has been published by Wohlleben et al[2] who argue that, in solution, S* is a vibrationally-excited, "hot" ground state populated by a combination of impulsive Raman scattering of the $S_0 \rightarrow S_2$ pump pulse and internal conversion from $S_1$. In this work we present the results of a systematic, ultrafast, time-resolved spectroscopic investigation of *cis* and *trans* geometric isomers of HPLC-purified spirilloxanthin (N=13), rhodopin glucoside (N=11), and spheroidene (N=10) in acetone and $CS_2$ solutions at room temperature and in EPA (5:5:2 v/v/v ether: isopentane: ethanol) at 77 K. Analysis of the data using global fitting techniques reveals the inherent spectral properties and ultrafast dynamics of each of these molecules and addresses the structural features controlling S* formation in solution.

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## Poster 26

ULTRAFAST TIME-RESOLVED TRANSIENT ABSORPTION SPECTROSCOPY OF LHCIIb AND CP MONOMERS FROM DARK-ADAPTED AND ILLUMINATED SPINACH THYLAKOIDS

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Nonphotochemical quenching (NPQ), a phenomenon occurring in LHCII antenna complexes of higher plants, constitutes an adaptation mechanism whereby excess energy is dissipated under conditions of high light stress. NPQ has many components, one of which is denoted qE and is sometimes referred to as high-energy or feedback-regulated quenching. For qE to occur, the PsbS protein must be present, the chloroplast thylakoid lumen must be acidified, and the xanthophyll, violaxanthin, must be enzymatically de-epoxidated to zeaxanthin. Recently, a transient absorption signal associated with the formation of a zeaxanthin radical cation in thylakoids active in qE was reported and implicated as the key component in the process of excess energy dissipation.[1] The present work seeks to verify this observation and to examine its origin using ultrafast, time-resolved, optical spectroscopy on LHCIIb and CP monomer (LHCIIb+CP) samples prepared from both dark-adapted and illuminated thylakoids from spinach. Ultracentrifugation on a sucrose density gradient allowed the separation of the various pigment-protein complexes, and high-performance liquid chromatography (HPLC) identified the pigment composition of the preparations. The sample containing the LHCIIb+CP monomers prepared from the illuminated thylakoids showed an increase in zeaxanthin content at the expense of violaxanthin compared to the dark-adapted material. Preliminary ultrafast transient absorption spectroscopic experiments on the dark-adapted and illuminated LHCIIb+CP samples using probe wavelengths in the IR region revealed distinct spectral lineshapes. The data will be discussed in terms of the spectral profiles expected to be formed upon excitation of the xanthophylls into the $S_2$ ($1^1B_u^+$) state and subsequent decay either by radiationless deactivation or cation radical formation.

This work is supported by the National Science Foundation (MCB-0314380 and MRI-0320403) and the University of Connecticut Research Foundation.

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## Poster 27

HYPERFINE SUBLEVEL CORRELATION SPECTROSCOPY (HYSCORE): DETERMINING THE ELECTRONIC STRUCTURE OF MONOMERIC CHLOROPHYLL A

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In the recent decade, a plethora of high resolution techniques for EPR spectroscopy have been developed. These techniques have allowed the study of the electronic structure of radical species that have heretofore been unattainable. The measurement of the nitrogen hyperfine coupling constants in the chlorophyll a cation radical has been hampered in the past by the presence of overlapping peaks caused by contributions from multiple nuclei. Pulsed EPR spectroscopy permitted an estimation of these couplings, but no definitive values have been measured. By using a combination of pulsed EPR, hyperfine sublevel correlation spectroscopy (HYSCORE), and spectral simulations, we have determined these coupling constants and present here the electronic structure of the radical. The electronic structure of the radical species *in vitro* can provide insight into the role of the cofactor *in vivo*.

## Poster 28

THE EFFECT OF CPCTSUV MUTANTS ON STATE TRANSITION FUNCTION IN THE CYANOBACTERIUM SYNECHOCOCCUS SP. PCC 7002

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Phycobilisomes (PBSs) are large pigment-protein super-complexes that serve as the major light-harvesting complex in cyanobacteria and usually facilitate in energy migration towards photosystem II (PSII) reaction centers (RCs). PBSs are composed of (i) an allophycocyanin containing core near the cytoplasmic thylakoid surface which energetically couples with PSII RCs (and to some degree PSI) and (ii) phycocyanin (C-PC) containing disks stacked into rods which radiate outwards from the core serving to capture and funnel photon energy towards the PBS core. Bilin chromophores of the disks are arranged in such a way as to collect and funnel photon energy from disk to disk towards the core. Mutations of the cpcTSUV genes in *Synechococcus* sp. PCC 7002 has yielded strains missing bilin attachment at βCys83 (CpcSU-), βCys153 (CpcTV-), and at both βCys83 and βCys153 (CpcSUT-). These alterations are shown to disrupt energy flow through the rods and cause changes in cellular pigment composition. State transitions are a rapid physiological adaptation of the photosynthetic light-harvesting apparatus resulting in changes in the distribution of excitation energy between PSII and PSI RCs. Under conditions in which PSII becomes limiting excitation energy is diverted from PSI to PSII (the state1 transition). PBS excitation energy accounts for the majority of energy redistribution observed in state transitions. The mechanism of this redistribution may involve PBSs that are only transiently bound to PSII which can decouple from PSII, migrate across the thylakoid membrane, and become energetically coupled to PSI. The C-PC bilin mutants and a rodless mutant (CpcBAC-) are shown to vary from WT cells in state transition kinetics and energy redistribution. Room temperature PAM fluorescence kinetic traces show there to be differences in state1 transition dependent PSII photochemical efficiency (Fv/Fm) and 77k fluorescence emission spectra show there to be shifts in PBS and Chla excitation energy transfer.

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| ERPC | Year | Chair | Speakers | |
|---|---|---|---|---|
| 1 | 1984 | Blankenship | Bogorad<br>Dutton<br>Huber | Harvard<br>U. Penn<br>N.C. State |
| 2 | 1985 | Brudvig | Blankenship<br>Youvan | Amherst<br>Cold Spring Harbor |
| 3 | 1986 | Frank | Mauzerall<br>McCarty<br>Prince | Rockefeller<br>Cornell<br>Exxon |
| 4 | 1987 | Owens | Beale<br>Bryant<br>Carpentier | Brown<br>Penn State<br>Trois-Rivieres |
| 5 | 1988 | Bruce | Gantt<br>Holzwarth<br>Marrs | U. Maryland<br>Muelheim<br>DuPont |
| 6 | 1989 | Redlinger | Guest<br>Horton<br>Ort | Indiana U.<br>Rob. Hill Inst., Sheffield<br>Illinois |
| 7 | 1990 | Diner | Brudvig<br>Daldal<br>Warncke | Yale<br>Univ. Penn<br>Univ. Penn |
| 8 | 1991 | Niederman | Biggins<br>Knox<br>Lam | Brown<br>Rochester<br>Rutgers |
| 9 | 1992 | Peterson | Berry<br>Drake<br>Gibbs | Carnegie Inst. Wash.<br>Smithsonian Edgewater, MD<br>Brandeis |
| 10 | 1993 | Biggins | Blankenship<br>Hind<br>Lorimer | Arizona<br>Brookhaven National Lab<br>DuPont |
| 11 | 1994 | De Paula | Neitherman<br>Therien | Rutgers<br>U. Penn |
| 12 | 1995 | Gunner | Armstron<br>Berry<br>Owens | Boston College<br>SUNY - Buffalo<br>Cornell |
| 13 | 1996 | McDermott | DePaula<br>Greenbaum<br>LeConte/Falzone | Haveford<br>Oak Ridge<br>Penn State |
| 14 | 1997 | Knox | Beck<br>Golbeck<br>Moser | Vanderbilt<br>Penn State<br>U. Penn |
| 15 | 1998 | Daldal | Frank<br>Van der Est<br>Berry | U. Con<br>Free University - Berlin<br>U.C. Berkeley |
| 16 | 1999 | Beck | Sension<br>Merchant<br>Dismukes | U. Michigan<br>U.C.L.A<br>Princeton |
| 17 | 2000 | Gogel | Diner<br>Hu<br>Bauer | DuPont<br>U. Toledo<br>Indiana U. |

| ERPC | Year | Chair | Speakers | |
|---|---|---|---|---|
| 18 | 2001 | Golbeck | Krauss<br>Falkowski<br>Klimov | Humboldt. U.<br>Rutgers<br>Russian Academy of Sciences |
| 19 | 2002 | Bryant | Beale<br>Carpentier<br>Golbeck | Brown<br>Trois-Rivieres<br>Penn State |
| 20 | 2003 | Haddy | Barber<br>Blankenship<br>Brudvig<br>Biggins | Imperial College London<br>Arizon State Univ.<br>Yale<br>Dry Creek Ridge Winery |
| 21 | 2004 | van der Est | Barber<br>Cogdell<br>Cramer<br>Bruce | Imperial College London<br>Univ. Glasgow<br>Purdue<br>Brock |
| 22 | 2005 | Punnett | Nelson<br>Evans<br>Bowes | Tel Aviv University<br>Univ. College London<br>Florida |
| 23 | 2006 | Polle | Sauer<br>Bassi<br>Dismukes | UC Berkeley<br>University of Verona<br>Princeton University |