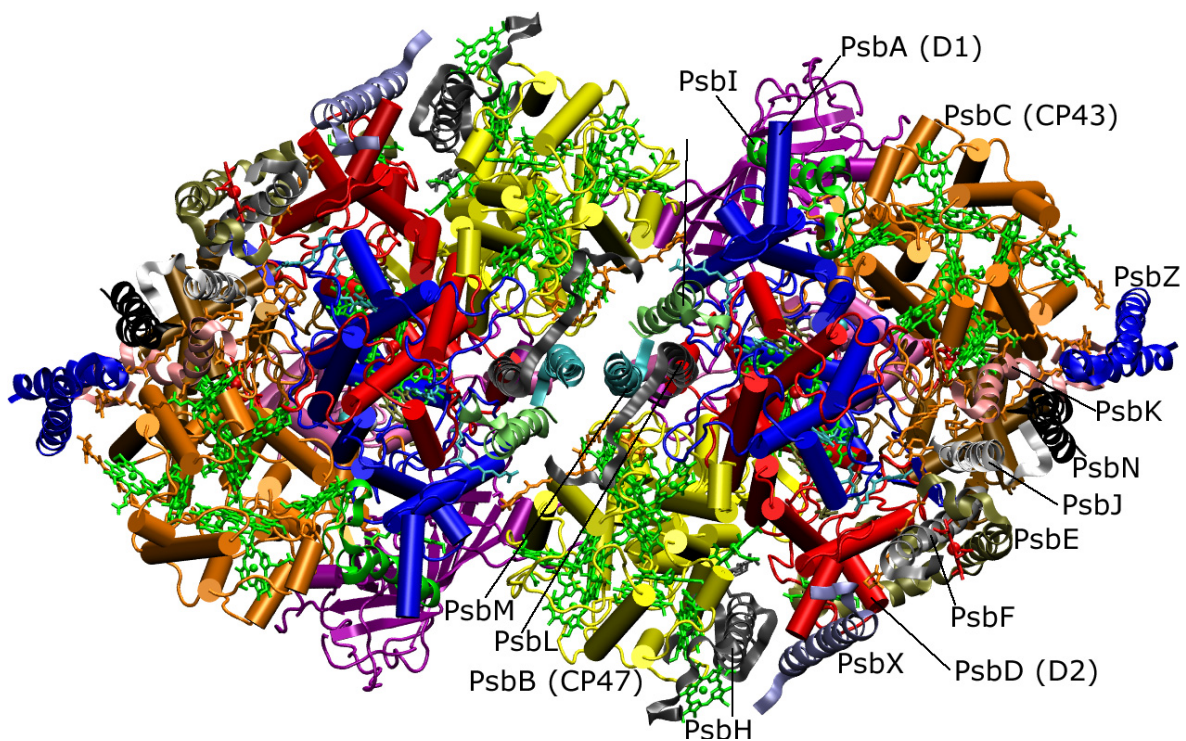


# Twenty Fourth Annual Eastern Regional Photosynthesis Conference



Swope Center  
Marine Biological  
Laboratory Woods Hole, MA  
April 20<sup>th</sup>-22<sup>nd</sup>, 2007

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

It is my great pleasure to welcome you to the 24<sup>th</sup> annual Eastern Regional Photosynthesis Conference. Unfortunately, several of our colleagues could not make it to Woods Hole this year, but they send their greetings.

If you have participated in previous meetings, you will recognize that the overall structure of this meeting has not changed. As in previous years, there are three sessions. These three sessions very loosely represent, or can be thematically grouped as: Antenna systems, Type II reaction centers and Type I reaction centers. In some cases my categorization decisions may appear arbitrary, but this is merely a reflection of the impossibility of categorizing the diversity of work being presented here into three groups.

This year we have four featured speakers. Two are "local" (regular participants at this meeting) two are from "outside". In addition, to encourage student participation, we have introduced a reduced registration rate for all students. We will also be giving awards for best student speaker and best student poster. All of this has been made possible by a successful fund raising campaign, headed by our co-chair, Wade Johnson. The several donors that have provided generous financial support are listed by logo on page 3, with further literature on pages 4-7.

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

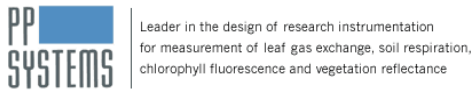
Gary Hastings, Chair ERPC-24. email: [ghastings@gsu.edu](mailto:ghastings@gsu.edu)

ERPC schedule for the coming years:

ERPC-25	18th-20th April, 2008	Wade Johnson, Chair.
ERPC-26	17th-19th April, 2009	
ERPC-27	23rd-25th April, 2010	

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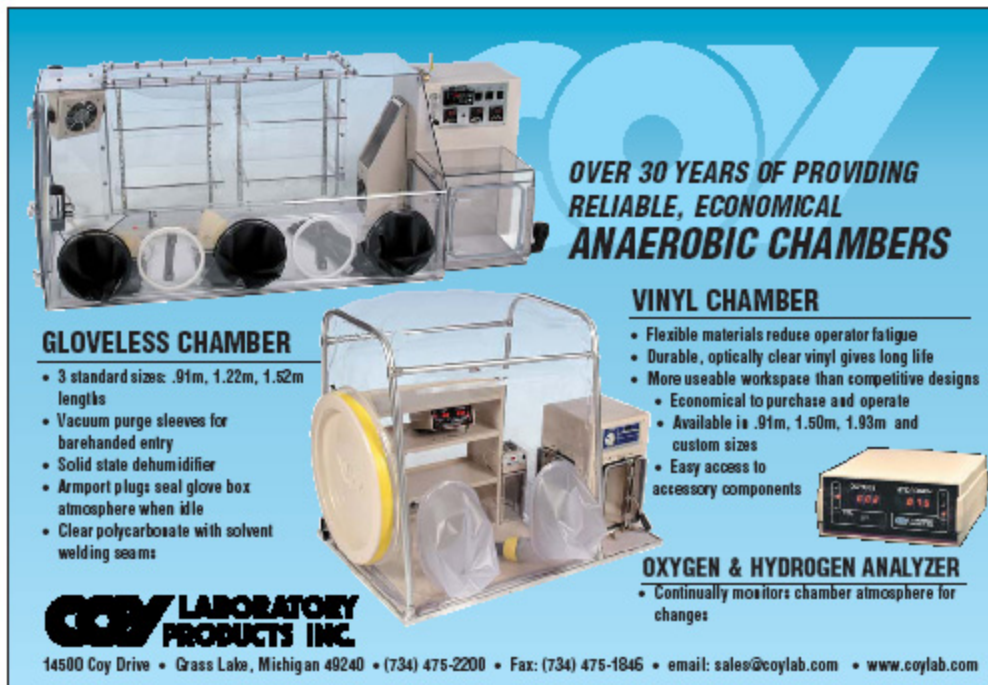


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Not all the light energy absorbed by a chlorophyll molecule is converted into photochemical production. A portion is dissipated as heat and still another portion is re-emitted as Chlorophyll Fluorescence. Because this is a competitive process, any change in one pathway will be reflected in the others. For instance, Chlorophyll Fluorescence will increase in proportion to photosynthetic inhibition and, conversely, it will decrease in proportion to a healthy, uninhibited photochemical pathway. It is therefore possible to study and ascertain the physiological state of the sample by measuring chlorophyll fluorescence.

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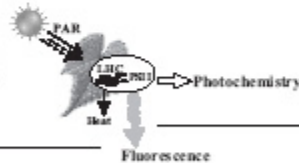
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## CONFERENCE SCHEDULE AT A GLANCE

SESSIONS WILL BE HELD IN THE **LILLY AUDITORIUM**.

### **Friday: April 20, 2007**

4 - 6PM	ARRIVAL AND REGISTRATION
5:00PM	COCKTAILS
6:00PM	DINNER
7:30PM	WELCOME AND OPENING REMARKS
7:45PM	INVITED LECTURE – PETRA FROMME
8:45PM	MIXER AND POSTER VIEWING

### **Saturday: April 21, 2007**

7:30-8:30AM	BREAKFAST
8:45AM	SESSION A – CONTRIBUTED PAPERS (Antennas)
10:15AM	COFFEE BREAK
10:45AM	SESSION B CONTRIBUTED PAPERS (Type II Systems)
12:15PM	LUNCH
1:30-4:00PM	EXHIBITS POSTERS AND FREE TIME
4:00PM	POSTER SESSION
5:00PM	COCKTAILS, POSTER SESSION CONTINUES
6:00PM	DINNER
7:30PM	INVITED LECTURE – ART VAN DER EST
8:30PM	INVITED LECTURE – KIRTHI TENNAKONE
9.30-11.30	MIXER AND POSTER VIEWING

### **Sunday: April 23, 2006**

7:30-8:30AM	BREAKFAST AND PRELIMINARY CHECK-OUT
8:45AM	SESSION C - CONTRIBUTED PAPERS (Type I Systems)
10:15AM	COFFEE BREAK
10:45AM	INVITED LECTURE – MARILYN GUNNER
11:45AM	CLOSING REMARKS
12:00-1:00PM	LUNCH

DEPARTURE



## Detailed Conference Program

### Guest Speakers

**Petra Fromme**, Arizona State University

Structure and function of Photosynthetic membrane proteins: New findings and surprises

**Art van der Est**, Brock University

Transient EPR studies of Electron Transfer in Photosynthetic Systems

**Kirthi Tennakone**, Institute for Fundamental Studies, Sri Lanka

Dye-sensitization: A viable route to solar energy conversion and storage

**Marilyn Gunner**, City College of New York

Connections between quinone affinity and electrochemistry in photosynthetic reaction centers

### CONFERENCE SCHEDULE

#### Friday: April 20, 2007

4:00- ARRIVAL AND REGISTRATION

6:00PM

5:00PM COCKTAILS

6:00PM DINNER

7:30PM WELCOME AND OPENING REMARKS

7:45PM Invited Lecture - Petra Fromme

Structure and function of Photosynthetic membrane proteins: New findings and surprises.

Chair: Art van der Est

8:45PM MIXER AND POSTER VIEWING

#### Saturday: April 21, 2007

7:30- BREAKFAST

8:30AM

8.45 SESSION A - CONTRIBUTED PAPERS. Chair: Jurgen Polle  
(18 minutes)

**Presentation A1:** Alan Derks:  $\beta$  PC lyase mutations alter PC absorbance and energy transfer within whole cells of the cyanobacterium *Synechococcus* sp. PCC 7002: Evidence for alternate chromophorylation?

**Presentation A2:** Mary Grace Galinato: Role of Cation Radicals of Xanthophylls in Nonphotochemical Quenching

**Presentation A3:** Joel Graham: Carotenoid Biosynthesis in *Synechococcus* sp. PCC 7002: The pathway to Myxoxanthophyll and Aromatic Xanthophylls.

**Presentation A4:** Hong Cong: Femtosecond Time-Resolved Absorption Spectroscopy of Open-Chain Carotenoids

**Presentation A5:** Sreeja Parameswaran: Calculated Electronic Spectra of Chlorophylls in Solution

10:15AM COFFEE BREAK

10:45 SESSION B - CONTRIBUTED PAPERS. Chair: Robert Carpentier  
(18 minutes)

**Presentation B1:** Derrick Kolling: Do the energetics of biological water oxidation limit  $O_2$  levels in the atmosphere?

**Presentation B2:** Perreault Francois: Photosystem II activity in xanthophyll-deficient mutants of *Chlamydomonas reinhardtii* being exposed to chromium effect

**Presentation B3:** Danian Carrieri: *In vivo* bicarbonate requirement for water oxidation activity of Photosystem II in the cyanobacterium *Arthrospira maxima*

**Presentation B4:** K.V. Lakshmi: Snapshots of Biological Proton-Coupled Electron Transfer: Electron-Nuclear Double Resonance Spectroscopy of Tyrosine Intermediates in Photosystem II\*

**Presentation B5:** Bob Niederman: Atomic Force Microscopy Reveals Unanticipated Electron Transfer Mechanisms and Multiple Patterns of Antenna Organization in Membranes of Purple Bacteria

12:15PM LUNCH

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

4:00PM POSTER SESSION

5:00PM COCKTAILS, POSTER SESSION CONTINUES

6:00PM DINNER

7:30PM Invited Lecture: Art van der Est  
Transient EPR studies of Electron Transfer in Photosynthetic Systems  
Chair: Doug Bruce

8:30PM      Invited Lecture: Dr. Kirthi Tennakone  
Dye-sensitization: A viable route to solar energy conversion and storage  
Chair: Gary Hastings

9:30PM      MIXER AND POSTER VIEWING

**Sunday: April 22, 2007**

7:30              BREAKFAST AND PRELIMINARY CHECK-OUT

-8:30AM

8:45    SESSION C - CONTRIBUTED PAPERS. Chair: Wade Johnson  
(18 minutes each)

**Presentation C1:** David Joly: Role of the plastoquinone pool in regulation of PSI energy dissipation.

**Presentation C2:** Bharat Jagannathan: Spectroscopic characterization of PscB, an iron-sulfur protein from the reaction center of *Chlorobium vibrioforme*.

**Presentation C3:** Besnier Nicolas: Anthocyanin effects on Photosystem II and I energy dissipation

**Presentation C4:** Carolyn Lubner: A Hybrid Biological/Organic Half-cell for Hydrogen Production

**Presentation C5:** Rajiv Luthra: Mutational Analysis of the EC3 Environment in PS I

10:15AM      COFFEE BREAK

10:45AM      SESSION E: Invited Lecture - Dr. Marilyn Gunner  
Connections between quinone affinity and electrochemistry in photosynthetic reaction centers  
Chair: John Golbeck

11:45AM              CLOSING REMARKS

12:00-1:00PM      LUNCH  
DEPARTURE

## Abstracts

### Invited Lectures

#### 1 Friday, 7.45 pm

#### NEW INSIGHTS INTO THE STRUCTURE AND FUNCTION OF PHOTOSYNTHETIC MEMBRANE PROTEINS

*Petra Fromme* - Department of Chemistry, Arizona State University, Tempe, AZ.

Oxygenic photosynthesis is the main biological process on earth where the light energy from the sun is converted into chemical energy. The primary step in this energy conversion, the light induced charge separation, is catalyzed by two distinct, membrane intrinsic protein complexes, photosystems I and II. Cyanobacterial Photosystem I consists of 12 protein subunits, to which 127 cofactors (chlorophylls, carotenoids, FeS clusters, quinones and lipids) are non-covalently bound. It catalyzes the light-driven electron transfer from plastocyanin to ferredoxin. In the talk, the structure and function of Photosystem I is discussed with special focus on the docking of the soluble electron carriers to PS I and the major changes that Photosystem I undergoes under iron deficiency.

The second part of the talk is focus on the ATP Synthase. We have recently grown crystals of an oligomeric form of subunit c from the spinach chloroplast enzyme diffracting as far as 2.5 angstroms. Though we are currently collecting experimental phases, the high symmetry of the oligomer allow for the determination of valuable structural insights from the native data. The native Patterson reveals the presence of 14-fold symmetry in the oligomer, confirming previous AFM studies. Though there are no known pigments in the membrane subunits of ATP synthase, these crystals possess a strong yellow color. The pigment analysis shows that the c-ring chloroplast ATP-synthase contains chlorophylls and carotenoids..

Acknowledgement: This work is supported by USDA (award 2003-35318-13573), NSF (award MCB-0417142) and NIH (award 1 R01 GM71619-01)



## 2 Saturday, 7.30 pm

### TRANSIENT EPR STUDIES OF ELECTRON TRANSFER IN PHOTOSYNTHETIC SYSTEMS

*Art van der Est* - Department of Chemistry, Brock University, St.Catharines, ON, L2S 3A1, Canada.

Light induced electron transfer in photosynthetic reaction centers generates a series of radical pairs with unusual spin polarization properties. The sudden separation of the two spins puts the system into an initial state that is far from equilibrium in much the same way as the preparation pulse in a pulsed NMR or EPR experiment. As the electron is passed along the chain of acceptors, the individual steps affect the spin system like a pulse sequence that encodes information about the magnetic parameters governing the spin system. This information can be read out in the spin polarization of subsequent radical pairs observed in transient EPR measurements. However, extracting this information is not straightforward and over the past several years we have developed strategies for simplifying the analysis by making approximations based on the known properties of photosynthetic RC's. More recently, we have been using these techniques to analyze the transient EPR spectra from modified Photosystem I complexes and obtain information about the energetics of the electron transfer. Of particular interest is the electron transfer from the chlorophyll  $A_0$  through phylloquinone to  $F_X$ . A decrease in the  $\Delta G$  of the  $A_0$  to PhQ step by either point mutations or quinone exchange slows the kinetics and causes corresponding changes in the spin polarization. In contrast, when  $\Delta G$  is increased by quinone exchange, no evidence for an increase in the rate of  $A_0$  to Q electron transfer is seen.

Electrostatic calculations<sup>1</sup> predict that the partial negative charges associated with aspartates D575<sub>PsaB</sub> and D566<sub>PsaB</sub> play a significant role in modulating the midpoint potentials of PhQ and  $F_X$ . To test this prediction, the side chains of these residues were changed from negatively charged (Asp) to neutral (Ala) and to positively charged (Lys). In the D575<sub>PsaB</sub> variants, the rate of electron transfer from  $A_{1A}$  to  $F_X$  is observed to decrease slightly according to the sequence D/A/K. In the D566<sub>PsaB</sub> variants, the opposite effect of a slight increase in the rate is observed according to the same sequence D/A/K. However, the changes in the rates are very small. The kinetic results as well as electrostatic calculations suggest that while the amino acid changes do shift the midpoint potentials of PhQ and  $F_X$  by different amounts, the difference in the shifts is small so that the overall effect on the rate of electron transfer is minimal.

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.

### **3 Saturday, 8.30 pm**

#### **DYE-SENSITIZATION: A VIABLE ROUTE TO SOLAR ENERGY CONVERSION AND STORAGE**

*Kirthi Tennakone* - Institute of Fundamental Studies, Kandy , Sri Lanka and Department of Physics, Georgia State University, Atlanta GA 30303.

Dye-sensitization, the process by which the excited pigment molecules anchored to a semiconductor surface injects electrons to the conduction band is the simplest way of utilizing molecular excitation energy to build a chemical potential. As the electron injection rate greatly exceeds the rate of geminate recombination, the separated charges could drive a current through an external circuit or initiate redox chemical reactions. The quantum efficiencies of original dye-sensitized photovoltaic devices based on plane electrodes rarely reached a few percent because of the insufficient light absorption cross-section. Thicker dye layers absorbing more light were found to be ineffective owing to concentration quenching and electrical insulation. This problem has been elegantly circumvented in the Gratzel cell, where a mesoporous film with a roughness factor of the order one thousand replaces the plane electrode. The optimized Gratzel cell in its present form converts sunlight to electricity at an efficiency of 10 -12%. A further increase in efficiency depends on finding ways to suppress recombination and broadening the spectral response up to near IR wavelengths. The main recombination processes limiting the efficiency are the interaction of electrons with acceptor species on the mesoporous surface and geminate back reaction that reduces the open-circuit voltage. It is suggested that these losses may be mitigated by insertion of tunneling barriers on the nanocrystalline semiconductor surface, molecular rectification and design of dyes that passivate the recombination centers on the surface. An option available for achieving panchromatic sensitization is application of more than one pigment with favorable electron and energy transfer properties.

Dye-sensitization can also be adopted to design visible light sensitive photocatalysts for splitting water. Although sacrificial photo-reduction of water can be demonstrated, oxygen evolution presents difficulties owing to dye photo-

degradation and the low probability of multi-electron transfer. It is suggested that advanced dye-sensitized solar energy conversion and storage devices may be developed borrowing ideas from natural photosynthesis and the physics of low dimensional semiconductors. Studies on dye-sensitization could also enlighten our understanding of the dynamics of charge and energy transfer in photosynthesis.

#### **4 Sunday, 10.45 am**

### **RELATIONSHIP BETWEEN QUINONE ELECTROCHEMISTRY AND BINDING AFFINITY AND KINETICS IN $Q_A$ AND $Q_B$ SITES OF BACTERIAL REACTION CENTERS.**

*Marilyn Gunner* - Physics Department City College of New York

In type II reaction centers the primary quinone electron acceptor ( $Q_A$ ) is reduced only to the semiquinone while the secondary quinone ( $Q_B$ ) is reduced first to  $Q_B^-$ , then transiently forms QH before being reduced to  $QH_2$ . The quinone electrochemistry will be compared in solution and in the  $Q_A$  and distal and proximal  $Q_B$  sites in the protein by measurement and simulation. The electrochemistry is changed when oxidized and reduced state has a different affinity for the protein. The semiquinone formed in  $Q_A$  and  $Q_B$  sites is a high energy intermediate which the protein must keep bound for milliseconds. This can be accomplished by stabilizing the semiquinone thermodynamically, raising the  $E_m$  or kinetically but slowing on and off rates. The quinone on and off rates have been measured. It is found that the protein may be able to keep the high energy semiquinone bound to the  $Q_A$  site without stabilizing it thermodynamically by slowing both on and off rates for anionic quinones.

Supported by CREES 2003-02123.

## Presentations

### Presentation A1 (Saturday, 8.45 am)

**$\beta$  PC LYASE MUTATIONS ALTER PC ABSORBANCE AND ENERGY TRANSFER WITHIN WHOLE CELLS OF THE CYANOBACTERIUM SYNECHOCOCCUS SP. PCC 7002: EVIDENCE FOR ALTERNATE CHROMOPHORYLATION?**

*Allen Derks and Doug Bruce*

Department of Biological Sciences, Brock University, St. Catharines, ON, L2S 3A1, Canada.

Phycocyanin (PC) is the primary phycobiliprotein component of the phycobilisome rod. Phycobilisomes are the major light harvesting complex of cyanobacteria and primarily associate with photosystem II (PSII). The lyases responsible for chromophorylation of the PC  $\beta$  subunit (CpcB) have been recently identified in the cyanobacterium *Synechococcus* sp. PCC 7002 (Shen et al (2006) JBC 281: 17768-78; Gaozhong Shen, personal communication). Null mutants in which the lyase(s) responsible for phycobilin attachment at the cysteine residues of  $\beta$ 153,  $\beta$ 83, and both  $\beta$ 153 and  $\beta$ 83 (CpcTV<sup>-</sup>, CpcSU<sup>-</sup>, CpcSUT<sup>-</sup>, respectively) were investigated spectroscopically. The mutations did specifically target PC as determined from analyzing absorbance of whole cells at room temperature and 10K, and from analyzing whole cell steady state 77K emission spectra. The null mutations did not completely inhibit phycobilin attachment to the CpcB binding domains, but phycobilin placement within the binding domains was altered in such a way as to modify protein-chromophore and chromophore-chromophore interactions. Fluorescence emission spectra indicate disruption of PC excitation energy transfer. A hypothesis in which alternate lyases are able to act on the  $\beta$  binding domains is presented.

Special thanks to Gaozhong Shen, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802 USA for his generous gift of the cyanobacteria cultures.



## Presentation A2 (Saturday, 9.03 am)

### Role of Cation Radicals of Xanthophylls in Nonphotochemical Quenching

Mary Grace I. Galinato, Dariusz Niedzwiedzki, Cailin Deal,  
Robert R. Birge and Harry A. Frank

*Department of Chemistry, University of Connecticut, Storrs, CT 06269-3060, USA.*

Nonphotochemical quenching (NPQ) is an adaptation mechanism exhibited by higher plants and some algae whereby excess absorbed light energy is dissipated to avoid photo-induced damage to the photosynthetic apparatus. NPQ has many components, the most rapid of which denoted qE, and referred to as feedback-regulated quenching. For qE to occur, the chloroplast thylakoid lumen must be acidified, the PsbS protein must be present, and the xanthophyll, violaxanthin must be enzymatically de-epoxidated to zeaxanthin. Recently, a transient absorption signal in the near-IR region thought to be associated with the formation of a zeaxanthin cation radical in high-light illuminated thylakoids, was reported and invoked as a key component in the molecular mechanism of qE.<sup>1</sup> The present work examines the steady state spectra of a number of xanthophyll cation radicals in solution which will be useful in guiding the identification of these species in biological preparations. The data show that the absorption spectra of the cation radicals red-shift with increasing number (N) of  $\pi$ -electron conjugated carbon-carbon double bonds. Surprisingly, zeaxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene, all of which have N=11 exhibit cation radical signals at different wavelengths. Quantum computations are being done to determine the factors that cause this spectral behavior.

(1) Holt, N. E.; Zigmantas, D.; Valkunas, L.; Li, X. P.; Niyogi, K. K.; Fleming, G. R. *Science* 2005, 307, 433.

## **Presentation A3 (Saturday, 9.21 am)**

### **Carotenoid Biosynthesis in *Synechococcus sp.* PCC 7002: The pathway to Myxoxanthophyll and Aromatic Xanthophylls.**

Joel E. Graham and Donald Bryant

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

All of the carotenoids of *Synechococcus sp.* PCC 7002 were identified, including a new aromatic xanthophyll,  $\chi,\chi$ -caroten-18,18'-dioic acid, now called synechoxanthin. Synechoxanthin is a member of a family of  $\chi,\chi$ -carotenes found in a variety of cyanobacteria. Phylogenetic analysis was used to identify the genes of the carotenoid biosynthetic pathway in this organism. Among the new genes identified were two genes involved in the biosynthesis of synechoxanthin, "*crtU*" and *cruH*, lycopene cyclases *cruA* and *cruP*, and two new genes in the myxoxanthophyll pathway, *cruF*, *cruG*. CruF and CruG are respectively a 1'-hydroxylase and 2'-glycosyltransferase. Knockout mutants were used to verify the function of each of these genes as well as to identify intermediates in the pathway. Spectroscopic and chemical methods were used to identify individual compounds. These analyses were used to propose a comprehensive pathway of carotenoid biosynthesis in *Synechococcus sp.* PCC 7002.

## Presentation A4 (Saturday, 9.39 am)

### Femtosecond Time-Resolved Absorption Spectroscopy of Open-Chain Carotenoids

Hong Cong,<sup>1</sup> Dariusz Niedzwiedzki,<sup>2</sup> George N. Gibson<sup>1</sup> and Harry A. Frank<sup>2</sup>

<sup>1</sup>*Department of Physics, University of Connecticut, Storrs, CT 06269-3046, USA;*

<sup>2</sup>*Department of Chemistry, University of Connecticut, Storrs, CT 06269-3060, USA;*

Many of the spectroscopic features and photophysical properties of carotenoids are interpreted using a three-state model where the strong visible absorption 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. Quantum calculations have suggested additional excited singlet states, one of which is denoted  $S^*$ , lie either between or in the vicinity of  $S_1$  and  $S_2$ , and spectroscopists have reported evidence for these states using ultrafast laser methodologies. In this work we present the results of an ultrafast time-resolved spectroscopic investigation of all-*trans* geometric isomers of HPLC-purified neurosporene (N=9), spheroidene (N=10), rhodopin glucoside (N=11), rhodovibrin (N=12), and spirilloxanthin (N=13) in acetone and CS<sub>2</sub> 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 the excited singlet states of each of these molecules, and suggests  $S^*$  is associated with a twisted conformational structure, the yield of which is increased in molecules having longer conjugation lengths.

## **Presentation A5 (Saturday, 9.57 am)**

### **Calculated Electronic Spectra of Chlorophylls in Solution**

Sreeja Parameswaran and Gary Hastings

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

Chlorophylls and bacteriochlorophylls play a fundamental role in photosynthesis. They are used to capture and funnel solar energy to the reaction center. They are also the primary units used to convert excitation energy into chemical products. Knowledge of their function in various environments, from solution, to photosynthetic light harvesting complexes, to reaction center protein complexes, is therefore of considerable importance. As a first step in developing a quantitative model of chlorophylls in various environments we have been using time dependent density functional theory to calculate the electronic properties of several chlorophyll structures in various solvents. Up until now most studies have been limited to isolated pigments in the gas phase. In particular, we have used time dependant density functional methods [B3LYP/6-31G(d)] in conjunction with the polarizable continuum model, to obtain fully optimized structures of chlorophylls *a*, *b*, *c*<sub>1</sub>, *c*<sub>2</sub>, *d* and bacteriochlorophylls *a*, *b*, *c*, *d*, *e* and *g*, in both polar and non-polar solvents. The calculated wavelengths for the Q<sub>y</sub>, Q<sub>x</sub> and Soret bands of the different bacteriochlorophylls and chlorophylls were found to agree well with experimental spectra.

We have also used QM/MM methods to calculate the electronic properties of chlorophyll-*a* in the presence of 40 explicitly added solvent molecules. Solvent induced electronic band shifts calculated using this explicit solvent model are compared to the results obtained using the polarizable continuum model. In this way the applicability of the polarizable continuum model is assessed.



## **Presentation B1 (Saturday, 10.45 am)**

### **Do the energetics of biological water oxidation limit O<sub>2</sub> levels in the atmosphere?**

**Derrick R. J. Kolling<sup>1</sup>, Tyler S. Brown<sup>2</sup>, Gennady Ananyev<sup>3</sup>, and G. Charles Dismukes<sup>4</sup>**

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We investigated the effects of elevated O<sub>2</sub> pressure on reversing the O<sub>2</sub> production reaction by photosynthetic organisms and on the rate of damage to photosynthesis. We used the intrinsic chlorophyll fluorescence yield of the Photosystem II: oxygen-evolving-complex to monitor the rate of turnover at high O<sub>2</sub> pressure. We show that O<sub>2</sub> production by oxygenic phototrophs (plants, cyanobacteria, and algae) occurs unabated at O<sub>2</sub> pressures at least 50-fold above ambient, in contradiction to earlier reports (Ref. 1 and 2). High O<sub>2</sub> partial pressure accelerates the irreversible damage of cells via superoxide radicals, formed upon photoreduction of O<sub>2</sub> by Photosystem I. We conclude that the fundamental water oxidation/O<sub>2</sub> evolution chemistry in contemporary oxygenic phototrophs is not reversible at elevated O<sub>2</sub> pressure and would not have limited the attainable O<sub>2</sub> pressure in Earth's prehistoric atmospheric. Rather, oxidative damage caused by PSI-photoinduced O<sub>2</sub> radicals is a probable cause for limiting the rise of atmospheric O<sub>2</sub> in the Carboniferous period.

### **References**

1. Clausen and Junge (2004) *Nature* 430: 480-3
2. Clausen, Junge, Dau, and Haumann (2005) *Biochemistry* 44: 12775-9

## Presentation B2 (Saturday, 11.03 am)

### Photosystem II activity in xanthophyll-deficient mutants of *Chlamydomonas reinhardtii* being exposed to chromium effect

Ait Ali Nadia<sup>1</sup>, **Perreault François**<sup>2</sup>, Didur Olivier<sup>3</sup>, Juneau Philippe<sup>4</sup> and Radovan Popovic<sup>5</sup>  
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The Photosystem II activity and energy dissipation were investigated when algae *Chlamydomonas reinhardtii* genotypes were exposed to chromium effect. The exposure to Cr of two *C. reinhardtii* mutants having non functional xanthophylls cycle, as *npq1* zeaxanthin deficient and *npq2* zeaxanthin accumulating, induced strong inhibition of Photosystem II electron transport. Photosystem II functions of *C. reinhardtii* mutants were investigated under different light intensities after algal cultures have been exposed to Cr effect. To determine Cr toxicity and light intensity effect on PSII functional properties we investigated the change of energy dissipation via Photosystem II electron transport, non photochemical regulated and non regulated energy dissipation according to Kramer et al. (2004). We used experimental evidence to explain dependency between Cr inhibitory effect of Photosystem II and light induced photoinhibition in algae deficient in xanthophyll cycle. When algal mutants missing xanthophylls cycle were exposed to Cr effect and to high light intensity the energy dissipation via non regulated mechanism takes the most important pathway. Therefore, the mutants *npq1* and *npq2* having non functional xanthophylls cycle were more sensitive to Cr inhibitory effect.

## Presentation B3 (Saturday, 11.21 am)

### *In vivo* bicarbonate requirement for water oxidation activity of Photosystem II in the cyanobacterium *Arthrospira maxima*

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While the presence of inorganic carbon in the form of (bi)carbonate has been known to be important for photosynthesis at Photosystem II (beyond its importance as a substrate for carbon fixation in the Calvin-Benson-Bassham cycle), the vast majority of studies on this "bicarbonate effect" have been limited to *in vitro* studies of isolated thylakoid membranes. Here we report an *in vivo* requirement for bicarbonate that is both reversible and selective for this anion for efficient Photosystem II activity in the cyanobacterium *Arthrospira (Spirulina) maxima*, originally isolated from highly alkaline soda lakes. Using a non-invasive internal probe of Photosystem II charge separation (variable fluorescence), fluorescence measurements of charge (Q<sub>a</sub>) reoxidation, and measuring flash oxygen yields electrochemically, we report the largest bicarbonate effect on PSII activity ever observed, which is due to the requirement for bicarbonate at the water oxidizing complex. Separation of this donor side bicarbonate effect from an acceptor side bicarbonate effect was observed. We expect the atypical way in which *Arthrospira* manages intercellular pH, sodium, and inorganic carbon concentrations relative to other cyanobacteria is responsible for this strong *in vivo* bicarbonate requirement.

## Presentation B4 (Saturday, 11.39 am)

### Snapshots of Biological Proton-Coupled Electron Transfer: Electron-Nuclear Double Resonance Spectroscopy of Tyrosine Intermediates in Photosystem II\*

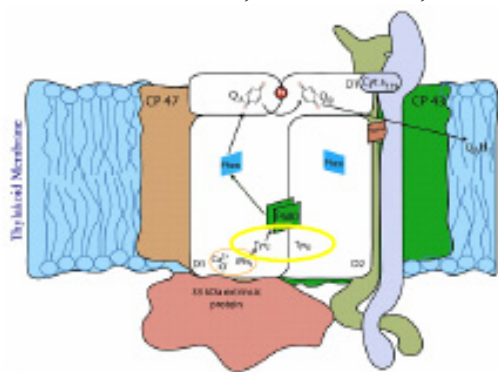
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The solar water-splitting protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in Nature by using light energy to drive the catalytic oxidation of water. Proton-coupled electron transfer (PCET) reactions, which are exquisitely tuned by smart protein matrix effects, are central to this water-splitting chemistry. PSII contains two symmetrically placed tyrosine residues,  $Y_D$  and  $Y_Z$ , one on each subunit of the heterodimeric core (Figure 1). The functions of these symmetry-related tyrosines are quite distinct, a versatility provided by their distinct local environments in PSII.  $Y_Z$  is kinetically competent and has been proposed to be directly involved in the PCET reactions of water oxidation. In contrast, the  $Y_D$  PCET redox poises the catalytic  $Mn_4$  cluster and may electrostatically tune the adjacent monomeric redox-active chlorophyll and  $\beta$ -carotene in the secondary ET pathway of PSII. This study focuses on disentangling individual steps of the photo-induced PCET events that lead to the formation of the redox-active tyrosyl radical,  $Y_D^\bullet$ , in PSII. Owing to the cryogenic turnover properties of PSII, it is possible to photooxidize  $Y_D$  at 1.8 K where both the proton and protein motions are limited. Annealing this state at a slightly higher temperature allows proton movement and relaxation of the protein environment. It is our hypothesis that the electron transfer, proton movement and associated conformational changes of the  $Y_D$ -binding pocket are the individual steps of the PCET reaction. Together, these reactions result in highly-efficient unidirectional proton and electron transfer at  $Y_D$ . Using pulsed high-frequency EPR and  $^1H$  and  $^2H$  ENDOR spectroscopy, we elucidate the sequence of events that lead to PCET at the  $Y_D$  site of PSII. These studies provide direct ‘*snapshots*’ of functional PCET intermediates and, for the first time, make it possible to detail the mechanism of PCET in biological energy transduction.

\*Work at RPI was supported by Rensselaer New Faculty Research Funds. Work at ANL was supported by the U. S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, under contract DE-AC02-06CH11357.



**Figure 1.** The arrangement of the protein subunits and cofactors of PSII with the sequence of the primary electron-transfer (ET) events leading to water oxidation shown by black arrows. Highlighted are the two symmetrically placed redox-active tyrosine residues,  $Y_D$  and  $Y_Z$  that participate in the proton coupled electron transfer reactions of PSII.

## Presentation B5 (Saturday, 11.57 am)

### Atomic Force Microscopy Reveals Unanticipated Electron Transfer Mechanisms and Multiple Patterns of Antenna Organization in Membranes of Purple Bacteria

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Recent atomic force microscopy (AFM) studies have provided highly informative surface views of the intracytoplasmic membrane (ICM) of purple photosynthetic bacteria at submolecular resolution. The resulting AFM topographs reveal multiple, species-dependent patterns of organization for their light-harvesting (LH) complexes. These vary from the highly ordered linear arrays of dimeric LH1-reaction center (RC) core complexes in *Rhodobacter sphaeroides*, with the peripheral LH2 antenna either interspersed between them or arranged in large clusters, to less orderly arrangements in several other purple bacteria, where randomly organized, monomeric LH1-RC complexes coexist with large, paracrystalline LH2 domains. Neither the ATP synthase nor the cytochrome *bc*<sub>1</sub> complex was observed in any of these images and they may localize at the poles of ICM vesicle out of view of the flat regions imaged by AFM. This does not support conventional models which place the cytochrome *bc*<sub>1</sub> complex in close association with RCs. Instead, the observed arrangements of LH2 and core complexes may specifically control quinol escape from the RC, with short-range diffusion within disordered regions of the membrane promoting passage of quinone, while the exclusion from large, ordered fields of LH2 antenna may also augment the flow of quinone species. Further details of these models will be discussed.

## **Presentation C1 (Sunday, 8.45 am)**

### **Role of the plastoquinone pool in regulation of PSI energy dissipation**

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Several mechanisms are known to reversibly control energy dissipation and photochemical activity of photosynthetic samples by modulating heat dissipation of photosystem II (PSII). These mechanisms are important for light adaptation and photoprotection of the photosynthetic apparatus. However, the contribution of photosystem I (PSI) to the regulative mechanisms is rarely discussed in the literature, because its activity is thought to be only indirectly controlled by the regulation of PSII. In the present work, we have studied the effects of exogenous PQ on fluorescence, photochemical activity and heat dissipation of PSI. Addition of decyl-plastoquinone (dPQ) and PQ-2 strongly quenched the fluorescence emission spectra of PSI submembrane fractions. They also retarded P700 photooxidation measured under limiting actinic light irradiances. Photoacoustic measurements showed that addition of dPQ increased heat dissipation and decreased the photochemical capacity of PSI. These results show that exogenous oxidized PQ efficiently quench Chl excited state in PSI antennas and change the balance between Chl deexcitation pathways. Most interestingly, reduction of the endogenous PQ pool in whole thylakoid membranes increased PSI fluorescence by 65%, indicating the importance of the redox state of the PQ pool on PSI energy dissipation.

## Presentation C2 (Sunday, 9.03 am)

### Spectroscopic characterization of PscB, an iron-sulfur protein from the reaction center of *Chlorobium vibrioforme*

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*Chlorobium vibrioforme* is a green-sulfur bacterium that performs anaerobic photosynthesis using Type I reaction centers, *i.e.* iron-sulfur clusters serve as the terminal electron acceptors. The iron-sulfur clusters are harbored in a protein denoted PscB, which is bound to the reaction center on the cytoplasmic side of the membrane. Based on the amino acid sequence and the presence of two [4Fe-4S] cluster binding motifs, PscB is likely to be a dicluster ferredoxin. However, the EPR spectrum of the dithionite-reduced protein is axial in nature, indicative of only a single [4Fe-4S]<sup>1+</sup> cluster ( $S = 1/2$ ). Weak resonances are observed around  $g = 4$  to  $8$  that are attributed to higher spin states ( $S = 3/2, 7/2$ ). This is in contrast to PsaC, its counterpart in Photosystem I, and PshB in *Heliobacterium modesticaldum*, wherein a complex EPR spectrum is obtained due to magnetic interaction upon the reduction of the two terminal [4Fe-4S] ( $S = 1/2$ ) clusters. Spin quantitation of chemically reduced PscB yields 1.7 spins/polypeptide. Reconstitution of the protein with selenide instead of sulfide results in an EPR spectrum with intense resonances around  $g = 4$  to  $8$  which are characteristic of ferredoxins that contain two [4Fe-4Se] clusters. It is interesting that while one iron-sulfur cluster-binding motif is the traditional sequence of CxxCxxCxxxCP, the other is a unique sequence of CxxCxxCxxxxxCP, which may be responsible for the unusual EPR spectra of PscB. Conversion of the second cysteine of each cluster binding motif into an Asp (C140D and C179D) results in a 50% reduction of  $S = 1/2$ , [4Fe-4S]<sup>1+</sup> clusters. The C179D variant contains a [3Fe-4S] cluster as indicated by a broad, asymmetric EPR resonance at  $g = 12$  in the reduced protein. However, a reduced [3Fe-4S] cluster is not detected in the C140D variant. It appears as if PscB contains two [4Fe-4S] clusters in a mixed-spin system, with about 15% of the clusters in a high-spin state. To our knowledge, this is the first detailed investigation of the magnetic properties of an iron-sulfur protein with this unique cluster-binding motif. *Funded by the National Science Foundation.*



## Presentation C3 (Sunday, 9.21 am)

### Anthocyanin effects on Photosystem II and I energy dissipation

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Anthocyanin as a pigment absorbing light do not participate directly in photosynthesis since this pigment is not associated with photosynthetic apparatus within chloroplast. We investigated effect of anthocyanin on Photosystem II and I energy dissipation by using red leaf of *Setcreasea pallida* accumulating high concentration of anthocyanin (grown under strong light intensity) and green leaf of *Setcreasea pallida* not having anthocyanin (grown under low light intensity) in epidermis cell layer. Functions of Photosystem II and I were measured by using DUAL-Pulse Amplitude Modulated fluorometer. We investigated anthocyanin effect on Photosystem II and I effective quantum yields, quantum yields of energy dissipation and quantum yields of non-regulated energy dissipation. When leaf having anthocyanin was exposed to high light intensity, we found for Photosystem II and I effective quantum yield to be two time higher compared to leaf with less anthocyanin content. However, regulated and non-regulated energy dissipation values for both Photosystems were evidently smaller in leaf having high content of anthocyanin. The result of this study permitted to propose a model of anthocyanin regulatory role in Photosystem II and I energy dissipation process.

## Presentation C4 (Sunday, 9.39 am)

### A Hybrid Biological/Organic Half-cell for Hydrogen Production

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We describe the design, fabrication, characterization, and optimization of a biological/organic hybrid electrochemical half-cell that couples Photosystem I (PS I), which efficiently captures and stores energy derived from sunlight, with either a [FeFe]-H<sub>2</sub>ase or a [NiFe]-H<sub>2</sub>ase, which can generate a high rate of H<sub>2</sub> evolution with an input of reducing power. Using a method that does not depend on inefficient solution (diffusion) chemistry, the challenge is to deliver the highly reducing electron from PS I to the H<sub>2</sub>ase rapidly and efficiently *in vitro*. To this end, we have designed a covalently bonded molecular wire that will connect the [4Fe-4S] clusters of the two enzymes. The result is that the low-potential electron can be transferred without loss and at high rates directly from PS I to the H<sub>2</sub>ase enzyme. The *PS I-molecular wire-H<sub>2</sub>ase* complex will be tethered to a gold electrode through a baseplate of cytochrome *c*<sub>6</sub>, which will additionally serve as a conduit of electrons from the gold to PS I. Cytochrome *c*<sub>6</sub> will be covalently bonded to the electrode through a self-assembling monolayer of functionalized alkane thiols. The device should be capable of transferring at least 1000 electrons per second from PS I to the H<sub>2</sub>ase to carry out the reaction:  $2\text{H}^+ + 2\text{e}^- + 2\text{h}\nu \rightarrow \text{H}_2$ . In the long run, it may be possible to eliminate the need for the H<sub>2</sub>ase enzyme by replacing it with Au or Pt nanoparticles. *Funded by the DOE H<sub>2</sub> Program.*

## Presentation C5 (Sunday, 9.57 am)

### MUTATIONAL ANALYSIS OF THE EC3 ENVIRONMENT IN PS1

Rajiv Luthra<sup>1</sup>, Johan van Tol<sup>2</sup>, Fabrice Rappaport<sup>3</sup>, Alexander Petrenko<sup>1</sup>, Louis-Claude Brunel<sup>2</sup>, Kevin Redding<sup>1</sup>

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Photosystem I (PS1) has two branches of cofactors (A and B), which are potential electron transfer pathways from P<sub>700</sub> to the FeS cluster F<sub>X</sub>. Each branch consists of two chlorophylls (ec2 and ec3) and a phylloquinone (PhQ). In the green alga *Chlamydomonas reinhardtii*, we have mutated to Phe the Tyr residue that donates a H-bond to each ec3 Chl's 13<sup>1</sup>-keto oxygen (PsaA-Y692F and PsaB-Y672F). This H-bond may stabilize the ec3<sup>-</sup> chlorophyll anion radical after charge separation. Hence breaking it could destabilize ec3<sup>-</sup>, making electron transfer less favorable down that branch. The PsaA-Y692F mutation increased the amplitude of the faster kinetic component (assigned to forward electron transfer from PhQ<sub>B</sub>) and decreased the amplitude of the slower kinetic component (assigned to electron transfer from PhQ<sub>A</sub>), while the PsaB-Y672F mutation had the opposite result. High-field EPR spectra of the "A<sub>0</sub>" anion radical, photo-accumulated after double-reduction of PhQ, are similar spectrum in both mutants and display similar upshifts ( $\sim 10^{-4}$ ) in g<sub>xx</sub> and g<sub>yy</sub>. The upshift in g<sub>xx</sub> is predicted from the loss of a H-bond, but the shift in g<sub>yy</sub> may arise from coupling between ec2 and ec3.

## Posters

### Poster 1

#### Entropy production and the Second Law in photosynthesis

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At physiological levels of light intensity, absorption of a photon of energy  $h\nu$  by an ensemble of photopigments at ambient temperature  $T$  is accompanied by a gain of photopigment entropy  $\Delta S_p = h\nu / T$ . This intuitively reasonable fact is used routinely in theories of solar conversion efficiency, but the demonstration of its validity, presented here, is not well documented. Recently an assertion that the primary photochemistry of photosynthesis can violate the Second Law of thermodynamics in certain efficient systems has been put forward by Jennings *et al.*, who maintain their position strongly despite an argument to the contrary by Lavergne. We observe that an incorrect value of  $\Delta S_p$  was used in the calculation of Jennings *et al.* and show that no violation of the Second Law occurs, regardless of the photosynthetic efficiency.

## Poster 2

### **FTIR Difference Spectroscopy for The Study of Specifically Labeled Quinones in The $A_1$ Binding Site in PS I**

Priyangi Banderanayake and Gary Hastings

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Phylloquinone (PhQ) (2-methyl, 3-phytyl, 1,4-naphthaquinone) is the secondary electron acceptor ( $A_1$ ) in photosystem I (PS I). Previously we have obtained ( $A_1^- - A_1$ ) FTIR difference spectra (DS) using labeled and unlabeled cyanobacterial PS I particles at 77K, and proposed assignments for many of the bands in the spectra [Sivakumar et al., *Biochem.* (2005)]. In menB mutant PS I particles plastoquinone-9 ( $PQ_9$ ) occupies the  $A_1$  binding site. However, externally added PhQ will displace  $PQ_9$  and reoccupy the binding site, and we have found that ( $A_1^- - A_1$ ) FTIR DS obtained using menB mutant PS I particles with reincorporated PhQ are almost identical to that obtained using WT PS I particles. [Banderanayake et al., *Biochem.* (2006)]. Using this PhQ reincorporation strategy has allowed us to place specifically labeled quinones in to the  $A_1$  binding site, which is extremely useful as it allows us to distinguish between band associated with quinone or protein modes in the spectra. Here we report ( $A_1^- - A_1$ ) FTIR DS obtained using menB PS I with PhQ, 1,4-NQ, and 2-methyl-1,4-NQ incorporated. We also report on spectra obtained using menB PS I with  $^{18}O$  labeled 2-methyl 1,4-NQ,  $^{13}C_1$  and  $^{13}C_4$  labeled 2-methyl-1,4-NQ occupying the  $A_1$  binding site.

We have also used density functional theory based vibrational frequency calculations to try to calculate what isotope induced shifts could be expected for different isotope labeled quinones in both solution and the  $A_1$  binding site.

## Poster 3

### Novel mathematical formulation of oxygen evolution in photosystem II using a Langmuir adsorption isotherm

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Oxygen evolution was investigated in isolated photosystem II (PSII) particles irradiated with white light of intensities ( $I$ ) of 20 to about 4000 micromol of photons/m<sup>2</sup>.s. We showed that in steady-state conditions the oxygen evolution varies with  $I$  according to the hyperbolic expression  $OE_{th} = OE_{th(max)}I / (L_{1/2} + I)$  (**eq 1**), where  $OE_{th}$  is the theoretical oxygen evolution,  $OE_{th(max)}$  is the maximum oxygen evolution, and  $L_{1/2}$  is the light intensity giving  $OE_{th(max)}/2$ . This mathematical relationship was derived using a **Langmuir adsorption isotherm** where, according to a Turro's photochemistry hypothesis, the interactions of the photons with the chlorophylls (Chl) in the PSII reaction center are assumed to be heterogeneous reactions in which the light is represented as a stream of particles instead of an electromagnetic wave. In accordance with this approximation, the Chl molecules (P680) are taken as the adsorption surfaces, i.e., the heterogeneous catalysts, and the incident photons as the substrate. We demonstrate in this work that **eq 1** (*Langmuir equation*) is a reliable interpretation of the photon-P680 interaction and the subsequent electron transfer from the excited state P680 (P680\*) to the oxidized pheophytin (Phe), then from Phe<sup>-</sup> to the primary quinone Q<sub>A</sub>. We conclude that **eq 1** is a novel mathematical formulation of energy and electron transfer in photosystem II. (*Work supported by a grant to MF from NSERC Canada*)

## Poster 4

### **Perturbation in electron donation from Tyr<sub>Z</sub> to P680<sup>+</sup> and Q<sub>A</sub><sup>-</sup> to Q<sub>B</sub> by spermine and spermidine**

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Polyamines like putrescine, spermine, and spermidine play a major role in protecting thylakoid membrane integrity during chilling, osmotic, UV-B, and photoinhibitory stress conditions. It is widely believed that they are associated with plant growth and stress response. However, in the present study, both spermine and spermidine are shown to elicit strong inhibitory effects in photosystem II (PSII). The interaction of spermine and spermidine with PSII submembrane fractions resulted in complete inhibition of oxygen evolution. With polyamine treatment, the extrinsic polypeptides associated with the oxygen evolving complex (OEC) were released and the Mn<sub>4</sub>Ca complex became dysfunctional. Consequently, the electron transfer from Tyr<sub>Z</sub> to P680<sup>+</sup> was impaired on one hand and plastoquinone (PQ) reduction strongly declined on the other. The above inhibition of light-induced reduction of PQ was partially restored by the artificial electron donor diphenylcarbazide as detected by chlorophyll fluorescence induction measurements. Thermoluminescence measurements confirmed that charge recombination between Q<sub>A</sub> or Q<sub>B</sub> with the S<sub>2</sub> state of the Mn<sub>4</sub>Ca-cluster was abolished. Also, the dark decay of chlorophyll fluorescence after a single turn-over flash indicated that the rate of Q<sub>A</sub><sup>-</sup> reoxidation was strongly retarded.



## Poster 5

### THE USE OF LIGHT EMITTING DIODES (LED) TO SIMPLIFY PHOTOACOUSTIC MEASUREMENTS

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Photoacoustics is a very good method to rapidly determine the thermal efficiencies of photosynthetic systems. Both enthalpy and volume changes are readily determined on the ns-us time scale. However the pulsed YAG-OPO lasers used are unstable, unreliable and expensive. The use of pulsed LED's to provide the short pulses of light of varying wavelength promises to revolutionize the field as they are very stable, reliable and of modest cost. We show that available red LED's provide sufficient energy in a few microsecond pulse to obtain data on whole cells using a resonant ceramic piezoelectric detector for sensitivity. However the 1000 fold longer pulse of light and the resonant detector introduce complications in the data analysis that now must be resolved. Still, the present data could be used to determine the standard "PI" curve of photosynthesis and has led to the discovery of a remarkable slow oscillation in the response of *Porphyridium cruentum* to saturating light.

## Poster 6

### **GENERATION AND CHARACTERIZATION OF ONE NEW CHLOROPHYLL DEFICIENT MUTANT 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 that are impacted in their cellular content of light-harvesting chlorophyll proteins. These Chl-deficient mutants have less Chl per cell and often ratios of Chl *a* to Chl *b*. To generate new chlorophyll-deficient mutants for analysis of their defects in LHC biosynthesis, we generated about 500 transformants of *C. reinhardtii* by random nuclear DNA insertional mutagenesis. Transformants were screened for aberrant coloration and by Chl fluorescence emission. Several new Chl-deficient mutants were identified through our screening process. One of these mutants called *ls1* was light sensitive, but *ls1* still grew photoautotrophically. In summary, we present a preliminary analysis of our new *ls1* mutant of *Chlamydomonas reinhardtii*.

## Poster 7

### **The oxidation state of the manganese cluster modulates the kinetics of chlorophyll fluorescence induction**

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The oxygen evolving complex (OEC) provides electrons to the primary electron donor of photosystem II (PSII), P680. Previous works demonstrated that changes in the initial population of the S-states induced by flashes affected PSII fluorescence. A popular way to study PSII is fluorescence induction (FI) because it is fast, reliable and non invasive. There are three apparent steps in the FI curve of thylakoid membranes denoted O-J, J-I and I-P, which are generally related to peak accumulation of the different reduced species in PSII. A recent study has confirmed that O-J is related to the accumulation and equilibrium of  $Q_A^-$  while I-P is related to plastoquinone pool reduction. However, while the origin of J-I remains unclear, it was observed that the amplitude of O-J ( $A_{O-J}$ ) and J-I ( $A_{J-I}$ ) are closely related. In the present work, we analyzed the effects of the initial S-state population on FI of thylakoid membranes pre-illuminated with a sequence of flashes. The results indicate that the intensity of  $A_{J-I}$  correlates with the  $S_2+S_3$  population and that  $A_{O-J}$  pattern is exactly the opposite. Both amplitudes follow a period four oscillation, related to the different S-states. From these observations, we suggest that both O-J and J-I are related to  $Q_A$  reduction but they represent two different bottleneck, strongly regulated by the S-state, prior to the complete closure of the reaction center.

## Poster 8

### Optical Spectroscopic Studies of Modified Peridinin

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Peridinin is a highly-substituted C<sub>37</sub> carotenoid found in marine dinoflagellates. It is the primary light harvesting component of the peridinin-chlorophyll-complex (PCP) from *Amphidinium carterae*. An unusual property of peridinin is that its S<sub>1</sub> excited state lifetime is dependent on solvent polarity. This has been attributed to the presence of an intramolecular charge transfer state, S<sub>ICT</sub>, in the excited state manifold of peridinin.<sup>1</sup> The precise nature of this state and its role in light-harvesting is not yet entirely clear. In order to explore this issue, chemically modified peridinins were synthesized and the spectroscopic properties and dynamic behavior of the molecules were studied at room temperature in methanol solution by steady-state absorption, fluorescence, and ultrafast transient absorption spectroscopic techniques. Low temperature (77 K) absorption and fluorescence experiments were also carried out for the molecules dissolved in EPA (5:5:2 v/v/v ether: isopentane: ethanol). The data reveal a number of differences between the behavior of peridinin and the modified molecules including substantial blue shifts in both steady-state and fluorescence emission spectra of the modified peridinins. Ultrafast transient absorption spectroscopy was done to determine the dynamics of the excited states of the molecules. Global fitting of the data reveal three kinetic components in the Evolution Associated Difference Spectra (EADS). The data are helping elucidate the structural features that control the spectroscopic properties and dynamics of peridinin.

(1) Bautista, J. A.; Connors, R. E.; Raju, B. B.; Hiller, R. G.; Sharples, F. P.; Gosztola, D.; Wasielewski, M. R.; Frank, H. A. *J. Phys. Chem. B* 1999, *103*, 8751-8758.

## Poster 9

### Femtosecond Time-Resolved Absorption Spectroscopy of Open-Chain Carotenoids

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Many of the spectroscopic features and photophysical properties of carotenoids are interpreted using a three-state model where the strong visible absorption 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. Quantum calculations have suggested additional excited singlet states, one of which is denoted  $S^*$ , lie either between or in the vicinity of  $S_1$  and  $S_2$ , and spectroscopists have reported evidence for these states using ultrafast laser methodologies. In this work we present the results of an ultrafast time-resolved spectroscopic investigation of all-*trans* geometric isomers of HPLC-purified neurosporene (N=9), spheroidene (N=10), rhodopin glucoside (N=11), rhodovibrin (N=12), and spirilloxanthin (N=13) 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 the excited singlet states of each of these molecules, and suggests  $S^*$  is associated with a twisted conformational structure, the yield of which is increased in molecules having longer conjugation lengths.

## Poster 10

### Ultrafast Time-resolved Absorption Spectroscopy of Isomers of Spheroidene and Spirilloxanthin at 77 K

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The strong visible absorption of carotenoids responsible for their yellow-orange coloration is associated with an  $S_0 (1^1A_g^-) \rightarrow S_2 (1^1B_u^+)$  transition. The lowest-lying singlet state,  $S_1 (2^1A_g^-)$ , is a state into which absorption from the ground state is forbidden according to both symmetry ( $g \rightarrow u$ ) and pseudoparity ( $- \rightarrow +$ ) selection rules. A transition from the ground state,  $S_0 (1^1A_g^-)$ , to another state, denoted  $S_3 (1^1A_g^+)$ , becomes allowed when the symmetry ( $g \rightarrow u$ ) selection rule is relaxed upon *trans*  $\rightarrow$  *cis* isomerization. The resulting absorption band appears in the UV region and is termed a “*cis*-peak”. In this work, we present the results of an ultrafast time-resolved spectroscopic comparison of all-*trans* and central-*cis* geometric isomers of two open-chain carotenoids isolated from photosynthetic bacteria, spheroidene ( $N = 10$ ) and spirilloxanthin ( $N = 13$ ). The molecules were studied in EPA (5:5:2 v/v/v ether: isopentane: ethanol) glasses at 77 K to enhance the spectral resolution. Analysis of the data using global fitting techniques has revealed the ultrafast dynamics of the excited states and spectral changes associated with their decay. The goal of this work is to understand the role geometric isomerization plays in controlling the photophysics of carotenoids.

## Poster 11

### **Photoprotection, photoinhibition and recovery in the lichen, *Parmelia sulcata*: A fluorescence study.**

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Mechanisms of photoprotection and recovery from photoinhibition in the lichen *Parmelia sulcata* were investigated via room temperature chlorophyll a (Chl a) fluorescence analysis. Lichens exhibit a decreased fluorescence yield and lack of variable fluorescence in the desiccated state. These yield changes in desiccated lichens are associated with a very short fluorescence lifetime indicative of a fluorescence quenching mechanism which serves to protect against photodamage. An immediate increase in variable fluorescence,  $F_v/F_m$ , was observed upon hydration of desiccated samples. This increase appeared to reach a plateau after two minutes. The fluorescence emission spectra showed 685nm and 720-750nm peaks in desiccated samples, with the broad 720-750nm peak being the larger of the two. An increase in the amplitude of the 685nm peak relative to that at 720-750nm was observed immediately following the addition of water. However, both peaks increased upon hydration and both peaks exhibited variable fluorescence and a similar sensitivity to photoinhibition in hydrated samples. Picosecond fluorescence decay kinetics from both emission peaks showed very similar increases in lifetime upon transition from  $F_0$  to  $F_m$ . A five minute treatment of 4200  $\mu\text{mol}/\text{m}^2/\text{s}$  actinic light to hydrated samples induced photoinhibition (decrease in  $F_v/F_m$ ) which recovered in three to five minutes in the dark. The dark recovery was inhibited in samples soaked for 2h in a 0.07M solution of the eukaryotic protein synthesis inhibitor cycloheximide. The same high light treatment of desiccated samples had no effect on the rehydration kinetics or final  $F_v/F_m$  of the rehydrated samples. We discuss the possibility that the novel long wavelength fluorescence emission associated with PSII in *P. sulcata* is indicative of a photoprotective low energy quenching chlorophyll species.



## Poster 12

### KINETIC SIMULATIONS OF CHLOROPHYLL AND CAROTENOID CATION RADICAL DYNAMICS IN PHOTOSYSTEM II .

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When the electron-donation pathway from the water-oxidizing complex in PSII is inhibited, chlorophyll (Chl), beta-carotene (Car) and cytochrome b<sub>559</sub> serve as alternative electron donors in a photoprotection mechanism. We simulated the dynamics of cation radicals in PSII to analyze possible pathways of cation radical formation. Simulations were based on pairwise rates of cation radical transfer between cofactors calculated using the “Dutton ruler” from the recent X-ray crystal structure of PSII (2AXT.pdb) and the published set of Chl and Car redox potentials (Ishikita et al., 2005). We aimed to reproduce with our simulations the following experimental data: 0.9 ms P<sup>+</sup>Q<sub>A</sub><sup>-</sup> recombination time, 5% single flash cation radical yield, cation radical quenching by reduced cytochrome b<sub>559</sub> and formation of one Chl and two Car cation radicals. We analyzed two possible pathways of cation radical formation: a pathway involving chlorophyll Chl<sub>Z</sub>/D1 and a pathway involving chlorophyll Chl<sub>Z</sub>/D2. Results of our simulations show that only the pathway of cation radical formation: P<sub>680</sub><sup>+</sup> → Pheo/D1 → Car101/D1 → Chl<sub>Z</sub>/D1 → Chl41/CP43 → Chls/CP43 → [Chl47, Car111, Car112]/CP43 is consistent with the target data. This result provides new insight into the alternate electron-donation pathways to P<sub>680</sub><sup>+</sup>.

## Poster 13

### The SufR Transcriptional Repressor and the *suf* Regulon in Cyanobacteria

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The biogenesis of the Fe/S clusters in Photosystem I is a highly regulated process. In cyanobacteria, the *sufR* gene is an open reading frame located directly upstream of the conserved *sufBCDS* genes. SufR has two interesting features: i) an N-terminal domain that contains a helix-loop-helix motif characteristic of DNA-binding proteins, and ii) a C-terminal domain that contains four conserved Cys residues that provide ligands for an Fe/S cluster. EPR and Mössbauer spectroscopic analyses showed the presence of a stable [4Fe-4S] cluster in recombinant SufR. Chemical analyses indicated the presence of ~2 atoms of iron and labile sulfide per monomer. Gel-filtration showed that both apoSufR and holoSufR exist as a homodimer. Thus, one [4Fe-4S] cluster is coordinated by the SufR homodimer. Results of gel-retardation analysis demonstrated that SufR binds to the DNA fragment of the regulatory region between *sufR* and the *sufBCDS* operon and its binding affinity is dependent on the presence of, and redox state of, its bound [4Fe-4S] cluster. Two SufR binding sites with high affinity and low affinity were identified through DNaseI footprint analysis. The operator sequence contains two perfect inverted repeats consisting of CAAC N6 GTTG separated by 26 base pairs. The presence of two binding sites with different affinities indicates that SufR functions as a negative transcriptional regulator in control the expression of the *sufBCDS* operon. *Supported by the USDA.*

## Poster 14

### **Investigating Photochemistry and Exciton Transfer using Mutations to Pigment and Co-factor Ligands in Photosystem II**

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Specific point mutations to histidine residues functioning as axial ligands to chlorophylls in CP47 have been selected for study based on each pigments presumed importance to exciton transfer. Chlorophylls which may serve as exciton transfer conduits have been targeted with H469Q, H466Q and H455Q mutations. While the chlorophyll cited in pervious studies as red-shifted and therefore responsible for the 695nm PSII fluorescence emission peak at low temperature has been targeted with a H114Q mutation. We have also utilized the co-factor influencing H198Q and Q130E mutations in D1 that have previously been reported to effect PSII electron transfer inside the reaction center. Each mutation has also been produced in his-tagged form to facilitate measurements on isolated PSII complexes. Characterization using steady state and time resolved measurements is ongoing at this time; while molecular dynamic simulations of selected mutants using the most recent x-ray crystal structure of PSII as well as the isolation of his-tagged PSII complexes are to be performed in the near term.

**Poster 15****Modeling Photosystem II Water Oxidase using Synthetic Manganese Cluster Complexes**

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A tetranuclear manganese complex ( $Mn_4$ ), held together by oxo ( $O^{2-}$ ) atom bridges, is thought to be the active site for the conversion of water to dioxygen in plant and bacterial Photosystem II (PSII). A calcium ion is closely associated with  $Mn_4$ . Many tetranuclear oxo-bridged manganese aggregates have been synthesized *in vitro* and some of them provide insight into the properties of the biological cluster. We are interested in preparing compounds that mimic the physical and chemical properties of PSII  $Mn_4$ . One of the long range goals of this work is to develop a water oxidation catalyst, which would be incorporated into artificial photosynthetic devices. Some X-ray absorption studies of PSII reveal that  $Mn\bullet\bullet\bullet Mn$  distances lengthen during the PSII  $S_2$  to  $S_3$  transition. We have observed that one of our tetranuclear complexes, namely the ‘dimer-of-dimers’  $[(Mn_2O_2)_2(tphpn)_2](ClO_4)_4$ , undergoes a dramatic structural change when it is oxidized by one electron. We suggest that a similar rearrangement may occur in PSII  $Mn_4$  when it is oxidized to  $S_3$  or to  $S_4$ . When  $[(Mn_2O_2)_2(tphpn)_2](ClO_4)_4$  is reduced by one electron, a hyperfine-rich EPR spectrum appears. This spectrum strongly resembles that of the PSII  $S_0$  state. We propose that when the structure of this latter species is elucidated, we may have an accurate synthetic analog of PSII  $Mn_4$  for the first time. The second major topic to be examined will be ligand exchange chemistry of the adamantane-shaped  $[Mn_4O_6(bpxa)_4](ClO_4)_4$  and reaction characteristics of these exchange products. The tridentate nitrogen donor ligand *bpxa* can be exchanged for a host of other tridentate ligands. With the proper choice of ligands, we can access a  $Mn_4(IV_3,V)$  oxidation state, whereas the original complex is isolated in the oxidation state  $Mn_4(IV_4)$  and can be oxidized no further. In the higher oxidation state, the manganese oxo core is more reactive. Under electrospray ionization conditions, we have mass spectral data that are consistent with loss of dioxygen, which is of course the product of water oxidation in photosynthesis. Finally, we will present recent results from our continuing efforts aimed at preparing carboxylate-rich synthetic mimics for the PSII water oxidase active site manganese cluster as well as results of oxygen atom transfer reactions, which are confirmed to involve manganyl ( $L_xMn=O$ ) species.

## Poster 16

### Electron transfer in cyanobacterial Photosystem I: Characterization of site-directed mutants in the vicinity of the phylloquinones

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The phylloquinone molecules denoted as A<sub>1A</sub> and A<sub>1B</sub> in Photosystem I (PS I) are protein-bound intermediates that re-oxidize the primary electron acceptors, A<sub>0A</sub><sup>-</sup> and A<sub>0B</sub><sup>-</sup> and reduce the first [4Fe-4S] cluster, F<sub>X</sub>. The midpoint potentials of A<sub>1A</sub>/A<sub>1B</sub> are ca. -750 to -800 mV, which are by far the most reducing quinones in all of biology. The unusually negative reduction midpoint potentials of the phylloquinones in PS I are due largely to the influence of the protein environment. The 2.5 Å X-ray crystal structure indicates that the phylloquinones are H-bonded to Leu722<sub>PsaA</sub> and Leu706<sub>PsaB</sub> and are  $\pi$ -stacked with Trp697<sub>PsaA</sub> and Trp677<sub>PsaB</sub>. This work is designed to modify the protein environment by site-directed mutagenesis in order to influence the midpoint potential of the quinones. The data from time-resolved optical experiments indicate that the kinetics of forward electron transfer from A<sub>1A</sub> to F<sub>X</sub> and from A<sub>1B</sub> to F<sub>X</sub> is not altered in case of the L706C<sub>PsaB</sub> variant. Preliminary experiments on the L706W<sub>PsaB</sub> variant reveal the absence of phylloquinone and low amounts of PS I. Ongoing studies include transient EPR spectroscopy to corroborate the kinetic data. A spin label will be incorporated in the L706C<sub>PsaB</sub> and L722C<sub>PsaA</sub> variants to determine the degree to which both electron transfer pathways are active. In addition, the  $\pi$ -stacked Trp is being altered on both branches to study its effect on the rate of electron transfer. *Funded by the National Science Foundation.*

## Poster 17

### Effect of cell cycle on Photosystem II in the unicellular green alga *Chlamydomonas reinhardtii*

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The Photosystem II activity of the green alga *Chlamydomonas reinhardtii* was investigated when cell cycle was in different state as G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M. Algae growth was maintained under continues light or light/dark cycle (16h/8h) to obtain homogenize cell cycle. The flow cytometry method was use to determined the cycle state of alga population. Photosystem II activity was investigated by using fluorescence parameters indicating Photosystem II quantum yield, Photosystem II antenna size, plastoquinones pool size and pH dependent non-photochemical quenching were measured with Pulse Amplitude Modulated Fluorometer and Plant Efficiency Analyzer systems. Our results indicated for Photosystem II antenna size per reaction center and Photosystem II quantum yield to be higher during the G<sub>2</sub>/M cell cycle phase. However, pH dependent non-photochemical quenching and plastoquinones pool size didn't show variation during diverse state of the cell cycle. Those results were used to interpret the dependency of Photosystem II functional and structural properties to cell cycle during the growth of algae culture. Such study may explain frequent variability of results obtained by using algae cultures caused by not taking in account of cell cycle state.

## Poster 18

### **Anthraquinone *In Vivo* Incorporation into PS I complexes of *Synechocystis* sp. PCC 6803 Phylloquinone Lacking Mutants**

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In the phylloquinone-less mutants (*menA* and *menB*) of the cyanobacterium *Synechocystis* sp. PCC 6803, plastoquinone (PQ) occupies the A<sub>1</sub> site, in place of phylloquinone, and functions in electron transport. Because of reduced electron transfer efficiency, plastoquinone containing mutants are incapable of growing at high light intensities. Here we report experiments in which the growth medium of the mutant cells was supplemented with various 9,10-anthraquinones (AQ). Both of the *menB* and *menA* mutants appear to utilize AQ because at high light growth capability was restored. This suggests the AQ is incorporated into the A<sub>1</sub> site unmodified.

Quantitative HPLC of the extracted PS I complex pigments indicate that AQ is incorporated in approximately a 1:100 AQ:Chl ratio and roughly 1:1 AQ:PQ.

The A<sub>1</sub> site quinone of the *menB* mutant was monitored by P<sub>700</sub><sup>+</sup> back reaction kinetics and spin polarized transient X-band EPR. The results support that the AQ's are active in the A<sub>1</sub> site. Back reaction data suggests that both PQ and AQ are present. Spin polarized transient EPR reveals that reaction centers derived from mutants supplemented *in vivo* with substituted AQ's produce signals similar to reaction centers where AQ has been replaced *in vitro*. The P<sub>700</sub><sup>+</sup>AQ<sup>-</sup> lifetimes are faster than PhQ and PQ, consistent with AQ having a lower redox potential.

This is the first example of a non-phytylated quinone being utilized by PSI *in vivo*.



## Poster 19

### Genetic Tools for Investigating the Role of PsbS in NPQ

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A combination of stable transgenic lines and virus-induced-gene silencing (VIGS) is used to analyze the role of psbS in nonphotochemical quenching (NPQ). A series of Arabidopsis lines deleted for the endogenous *psbS* locus (*npq4-1*) and each containing a stable *CaMV35S-psbS* construct were generated. The differing levels of psbS produced allowed us to examine the quantitative relationship between the [psbS] and the amplitude of the rapid (qE) phase of NPQ. Surprisingly, qE was significantly correlated to the product of the concentration of psbS and, not zeaxanthin, but antheraxanthin. This result lays the foundation for future analysis of NPQ in stable transgenic or VIGS altered plants. In an accompanying poster, the utility of VIGS is discussed for the study of NPQ in *Nicotiana benthamiana*. Agrobacterium-mediated transient expression of Arabidopsis *psbS* in *N. benthamiana* leaf tissue silenced for endogenous psbS showed a modest level of NPQ complementation and underpins future structure/function analysis. We have also initiated experiments with VIGS in Arabidopsis. Our eventual goal is to alter the composition of photosystem II antennae complexes through a combination of T-DNA insertion lines and subsequent VIGS of *AtLhcb* loci and determine their effect upon NPQ and potential interaction with psbS.

## Poster 20

### **Photosynthetic Properties of *Nicotiana benthamiana* Blocked for Expression of *psbS***

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Recent advances in virus-induced gene silencing (VIGS) in solanaceous species and *Arabidopsis* constitute a powerful and rapid means to assess the function of any nuclear-encoded protein. As a step toward understanding the mechanism of photoprotective quenching of excess excitation (nonphotochemical quenching, NPQ) in photosystem II (PSII) we employed the tobacco rattle virus (TRV) vector system to suppress accumulation of the essential 22-kilodalton protein, *psbS*, in leaves of *Nicotiana benthamiana*. A wide range of photosynthetic properties were compared in uninfused plants and in plants exposed to the VIGS vector containing the *psbS* gene or an extraneous sequence as a control. As expected, the rapid (qE) phase of NPQ was specifically abolished in leaves carrying TRV-*psbS*. However, chlorophyll content, linear electron transport rate (based on CO<sub>2</sub> exchange), energy use in PSII (based on fluorescence), and photosystem I (PSI) function (based on the *in vivo* absorbance signal at 810 nm) were only modestly affected by the VIGS system. The 810-nm kinetic signal was deconvoluted using a model developed recently in the Tartu laboratory that assumes rapid electrochemical equilibration among the PSI donor pools. Thus, VIGS is relatively non-perturbing insofar as effects of silencing of *psbS* expression resemble effects of deletion of this gene observed previously in *Arabidopsis*.

## Poster 21

### A Hybrid Biological/Organic Half-cell for Hydrogen Production

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We describe the design, fabrication, characterization, and optimization of a biological/organic hybrid electrochemical half-cell that couples Photosystem I (PS I), which efficiently captures and stores energy derived from sunlight, with either a [FeFe]-H<sub>2</sub>ase or a [NiFe]-H<sub>2</sub>ase, which can generate a high rate of H<sub>2</sub> evolution with an input of reducing power. Using a method that does not depend on inefficient solution (diffusion) chemistry, the challenge is to deliver the highly reducing electron from PS I to the H<sub>2</sub>ase rapidly and efficiently *in vitro*. To this end, we have designed a covalently bonded molecular wire that will connect the [4Fe-4S] clusters of the two enzymes. The result is that the low-potential electron can be transferred without loss and at high rates directly from PS I to the H<sub>2</sub>ase enzyme. The *PS I-molecular wire-H<sub>2</sub>ase* complex will be tethered to a gold electrode through a baseplate of cytochrome *c*<sub>6</sub>, which will additionally serve as a conduit of electrons from the gold to PS I. Cytochrome *c*<sub>6</sub> will be covalently bonded to the electrode through a self-assembling monolayer of functionalized alkane thiols. The device should be capable of transferring at least 1000 electrons per second from PS I to the H<sub>2</sub>ase to carry out the reaction:  $2\text{H}^+ + 2\text{e}^- + 2\text{h}\nu \rightarrow \text{H}_2$ . In the long run, it may be possible to eliminate the need for the H<sub>2</sub>ase enzyme by replacing it with Au or Pt nanoparticles. *Funded by the DOE H<sub>2</sub> Program.*

## Poster 22

### PSI/IsiA Supercomplex Formation in the *iscA*<sup>-</sup> Mutant of *Synechococcus* sp. PCC 7002

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An *iscA* deletion mutant (*iscA*<sup>-</sup>) in the strain *Synechococcus* sp. PCC 7002 has been shown to up-regulate *isiA* message levels when grown under both iron replete and iron deficient conditions. The presence of PS I-supercomplexes can be verified only in mutant cells grown under iron deficient conditions. Isolated PS I-supercomplexes from the mutant cells show characteristic spectroscopic properties of PS I-supercomplexes from wild type cells grown under iron stress. However, the mutant cells do not display the phenotypic chlorosis of wild type cells. Analysis of the PS I-supercomplexes verifies the presence of the stromal proteins PsaC, D, and E. EPR and time-resolved optical spectroscopies indicate that the F<sub>A</sub> and F<sub>B</sub> clusters are present, able to be reduced, and are capable of accepting electrons from F<sub>X</sub>. Steady state kinetics in the presence of NADP<sup>+</sup> and FNR indicate that these PS I-supercomplexes are capable of donating electrons to flavodoxin. In addition, multivariate statistical analysis of electron microscopy projections has found that single rings of IsiA form only around PS I trimers. Preliminary analysis shows that growth for prolonged periods of time under iron stress leads to a more homogeneous supercomplex population. *Funded by the DOE.*

## Poster 23

### THE IRON SULFUR CLUSTERS AND POLYPEPTIDES ASSOCIATED WITH THE PHOTOSYNTHETIC REACTION CENTER FROM *HELIOBACTERIUM MODESTICALDUM*

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Heliobacteria contain Type I reaction centers, a homodimeric core, and a minimal complement of low molecular mass polypeptides. Given their simplicity, the heliobacterial RC (HbRC) should be ideal for study of a prototypical homodimeric RC. There has recently been progress on understanding the nature of the secondary and tertiary electron acceptors. Firstly, the low molecular mass polypeptide that contains F<sub>A</sub> and F<sub>B</sub> has been identified. The change in the lifetime of the flash-induced kinetics from 75 ms to 15 ms on its removal shows that the former arises from the P798<sup>+</sup> [F<sub>A</sub>/F<sub>B</sub>]<sup>-</sup> recombination, and the latter from P798<sup>+</sup> F<sub>X</sub><sup>-</sup> recombination. Secondly, F<sub>X</sub> has been identified in HbRC cores by EPR and Mössbauer spectroscopy, and shown to be a [4Fe-4S]<sup>1+,2+</sup> cluster with a ground spin state of S = 3/2. Because all of the iron in HbRC cores is in the F<sub>X</sub> cluster, a ratio of ~22 Bchl g/P798 is calculated from chemical assays of non-heme iron and Bchl g. Thirdly, the N-terminal amino acid sequence of the F<sub>A</sub>/F<sub>B</sub>-containing polypeptide has led to the identification and cloning of its gene. The expressed protein can be rebound to isolated HbRC cores, thereby regaining both the 75 ms kinetic phase resulting from P798<sup>+</sup> [F<sub>A</sub>/F<sub>B</sub>]<sup>-</sup> recombination and the light-induced EPR resonances of F<sub>A</sub><sup>-</sup> and F<sub>B</sub><sup>-</sup>. The gene was named 'pshB' and the protein 'PshB' in keeping with the accepted nomenclature for Type I RCs. *Supported by the DOE.*

## Poster 24

### Structural Studies of the Heliobacterial Reaction Center

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The structure and function of Type I homodimeric photosynthetic reaction centers, such as those found in *Chlorobium* and *Heliobacteria*, are poorly understood. Recently, we developed a procedure to purify the heliobacterial reaction center (HbRC) core from *Heliobacterium modesticaldum* using ion exchange chromatography. Four minor proteins were found to co-elute with the PshA homodimer, and efforts are currently under way to determine their amino acid sequences and their possible roles in photosynthesis. Efforts are also underway to crystallize the PshA homodimer in an attempt to solve its 3-dimensional structure. HPLC analysis has shown that menaquinone is present in the HbRC cores, but it can be removed by washing with diethyl ether. In agreement with previous studies, its removal has no effect on the charge recombination kinetics of P798<sup>+</sup>, the primary electron donor. The DEAE purification protocol has enabled us to identify the three [4Fe-4S] clusters that act as the intermediate and terminal electron acceptors. Two of the [4Fe-4S] clusters are located on a newly-discovered protein termed PshB. The gene for PshB is part of a dicistronic operon that contains a gene for a second ferredoxin-like protein of similar mass. We are cloning and overexpressing this gene to determine whether it can function in a manner similar to PshB. *Supported by the DOE Energy Biosciences Program.*

## Poster 25

### Calculation of the Vibrational Properties of Chlorophyll-*a*

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Chlorophylls are important pigments in photosynthetic processes, and they have been widely studied using vibrational spectroscopies. In spite of this, little work has been undertaken to quantitatively assess the vibrational structure of chlorophyll. With this in mind we have used density functional theory (DFT) to calculate the vibrational properties of chlorophyll-*a* (Chl-*a*) in the gas phase and in the presence of solvents. Gas phase calculations do not accurately simulate the vibrational properties of the carbonyl groups of either neutral or cationic Chl-*a*. That is, the calculated results do not agree with experimental observations.

Here we undertake calculations to investigate how different factors impact the carbonyl vibrational modes of Chl-*a*. Firstly, we use DFT in combination with the polarizable continuum model to investigate how different solvents impact the vibrational properties of Chl-*a*. Secondly, how additional peripheral groups modify the vibrational properties of Chl-*a* and Chl-*a*' are investigated. Thirdly, the effects of hydrogen-bonding to the carbonyl groups are investigated. Our calculations show that solvent effects, as described by the polarizable continuum model, cannot explain the differences between theory and experiment. Additional peripheral groups cannot explain the differences either. Hydrogen bonding to the carbonyl groups of Chl-*a* appear to be the only way to reconcile calculations with experimental spectra. We therefore propose that in all previously determined experimental IR spectra of Chl-*a* in solvent, hydrogen bonding effects predominate, possibly because water has never been completely removed in any of these previous experiments.

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

16	1999	Beck	Sension Merchant Dismukes	U. Michigan U.C.L.A Princeton
17	2000	Gogel	Diner Hu Bauer	DuPont U. Toledo Indiana U.
18	2001	Golbeck	Krauss Falkowski Klimov	Humboldt. U. Rutgers Russian Academy of Sciences
19	2002	Bryant	Beale Carpentier Golbeck	Brown Trois-Rivieres Penn State
20	2003	Haddy	Barber Blankenship Brudvig Biggins	Imperial College London Arizon State Univ. Yale Dry Creek Ridge Winery
21	2004	van der Est	Barber Cogdell Cramer Bruce	Imperial College London Univ. Glasgow Purdue Brock
22	2005	Punnett	Nelson Evans Bowes	Tel Aviv University Univ. College London Florida
23	2006	Polle	Sauer Bassi Dismukes	UC Berkeley University of Verona Princeton University
24	2007	Johnson Hastings	van der Est Fromme Tennekone Gunner	Brock Arizona State University Georgia State University City College of New York