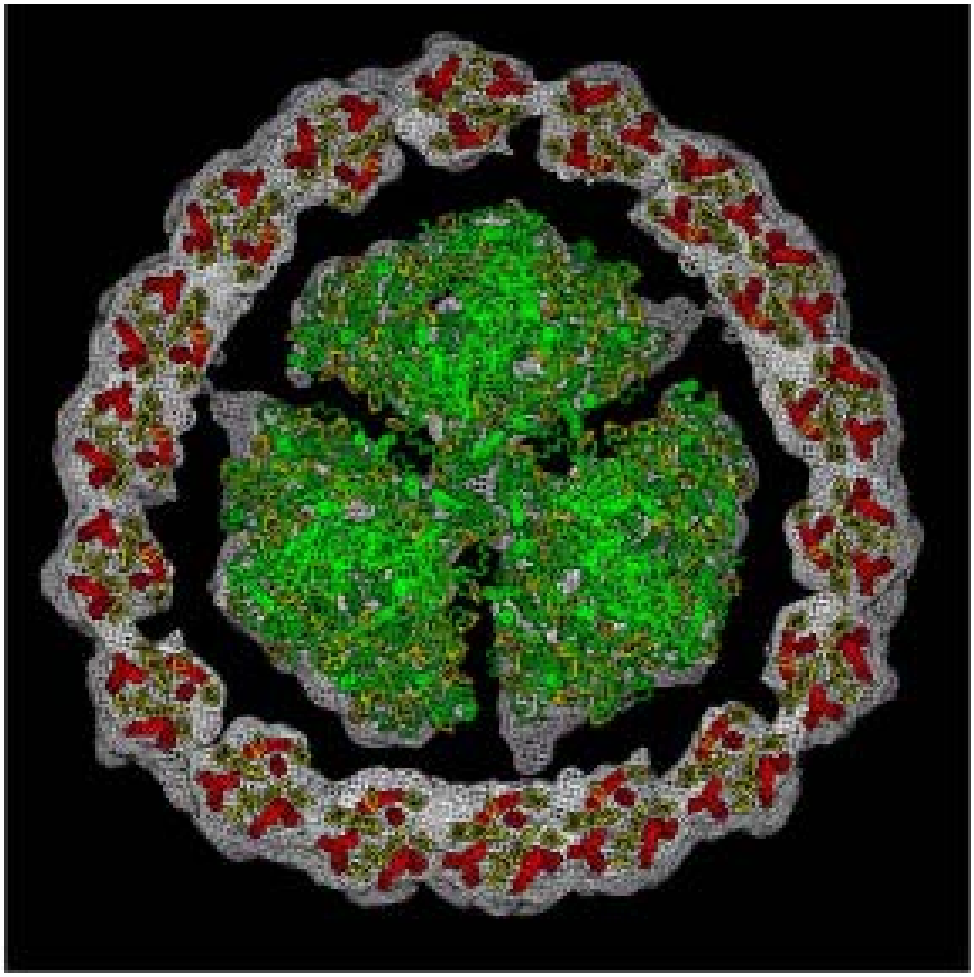
Twentieth Annual Eastern Regional Photosynthesis Conference



Swope Center
Marine Biological Laboratory
Woods Hole, MA
April 11—13, 2003

Twentieth Annual Eastern Regional Photosynthesis Conference

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Marine Biological Laboratory
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On the Cover: Electron micrograph image of the CP43'-PSI supercomplex overlaid with PSI trimer structure and CP43 helix organization derived from X-ray crystallography. Figure courtesy of Prof. James Barber.

A Note from the Chair: 20th Annual Eastern Regional Photosynthesis Conference

Welcome to Woods Hole for a weekend of photosynthesis and socializing. I would like to thank those who made helpful suggestions throughout the year leading up to this weekend at Woods Hole. In particular, Don Bryant, the chair of the 19th Annual Eastern Regional Photosynthesis Conference, has provided invaluable guidance. I would also like to thank our invited speakers, contributing speakers, poster presenters, and session chairs. I was really fortunate to have the assistance of Pam Oldham and the staff of Swope Center and the Marine Biology Laboratory to handle the local site issues. The web site for the conference was prepared by free-lance web designer Kristina Wood and Lorraine Walsh, a member of my department at the University of North Carolina at Greensboro, helped with the assembly of the program booklets. Finally, I am very grateful to our sponsor companies, Bruker Biospin, Dynamax, Elsevier, and DuPont, for their financial contributions. Special thanks goes to sponsoring wineries of Dry Ridge Valley and John Biggins who went to great lengths to organize a wine tasting event to follow his special lecture.

In order to promote the future ERPC's, the current web site at UNC-Greensboro (http://www.uncg.edu/che/ERPC2003/ERPC-20.html) will be maintained indefinitely. Information on next year's meeting, to be chaired by Art van der Est (Brock University), will be made available in the coming months. The conference program will be set up for downloading soon after the meeting; please let your colleagues know about this so that the science at this meeting can be accessed and appreciated even by those who were unable to attend.

Here is the current schedule for the ERPC over the next few years:

ERPC-21	**16-18 April, 2004 Art van der Est, Chair**
ERPC-22	**15-17 April, 2005 Tom Punnett, Chair**
ERPC-23	**21-23 April, 2006**
ERPC-24	**20-22 April, 2007**

The present chair and the future chairs are always interested in getting feedback on the organization of the ERPC and suggestions for future meetings.

Alice Haddy, Chair, ERPC-20
aehaddy@uncg.edu

Schedule of Events

The platform sessions and invited lectures will be held in Whitman Auditorium. All other events will be held in the Swope Center.

Friday, 11 April 2003

4:00-6:00 PM	Arrival and Registration
5:00 PM	Cocktails
6:15 PM	Dinner
7:30 PM	Session A: Invited Lecture – James Barber
9:00 PM	Mixer and Poster Viewing

Saturday, 12 April 2003

7:30 AM	Breakfast
8:30 AM	Session B: Contributed Papers
10:10 AM	Coffee Break
10:30 AM	Session C: Contributed Papers
12:30 PM	Lunch
1:30– 4:00 PM	Posters, Exhibits and Free Time
4:00 PM	Poster & Exhibit Session
5:00 PM	Cocktails, Poster Session Continues
6:00 PM	Dinner
7:00 PM	Session D: Invited Lecture - Robert Blankenship
8:00 PM	Special Lecture – John Biggins
9:00 PM	Mixer

Sunday, 13 April 2003

7:30 AM	Breakfast and preliminary checkout
8:30 AM	Session E: Contributed Papers
10:30 AM	Coffee Break and Final Checkout
11:00 AM	Session F: Invited Lecture – Gary Brudvig
11:50 AM	Closing Remarks
12:00 PM	Lunch
1:00 PM	Departure

Friday, 7:30 PM

7:30 PM Welcome

Session A, Invited Lecture 7:35 – 8:30 PM.
Chair: Alice Haddy

"Rings and Things"

James Barber
Wolfson Laboratories, Department of Biological Sciences,
Imperial College London

9:00 PM Mixer

Saturday, 8:30 AM

Session B, Contributed Papers 8:30 to 10:10 AM.
Chair: Fevzi Daldal

8:30-8:50 AM
1. The Effect of Incorporating Anthraquinone and 2, 3- Disubstituted Methyl Naphthoquinones into the A1 site of PSI: Transient EPR Studies. **Sarah Brown**[1], Yulia Pushkar[2], Dietmar Stehlik[2] and Art van der Est[1]. [1]Brock University. [2]Free University of Berlin.

8:50-9:10 AM
2. Molecular Dynamics simulations of monosubstituted 1,4-naphthoquinone electron acceptors in the A_1 binding site of Photosystem I. **Y.S. Bukhman**, H. Gordon and A. van der Est. Department of Chemistry, Brock University.

9:10-9:30 AM
3. Analysis of Structural Features that Dictate the Efficiency of Light Harversting in the PS I Reaction Center/Core Antenna Complex **Thomas G. Owens** and David G. Rand, Dept. of Plant Biology, Cornell University.

9:30-9:50 AM

4. Photosynthetic Excitons in PS I: An Evaluation of Key Exciton-Monomer Chlorophyll Interactions that Determine the Efficiency of Light Harvesting. **David G. Rand,** *Robert S. Knox and Thomas G. Owens. Dept. of Plant Biology, Cornell University. *Dept. of Physics and Astronomy, University of Rochester.

9:50-10:10 AM

5. Mutation Induced Modulation of Hydrogen Bonding To P700 Studied Using FTIR Difference Spectroscopy. **Gary Hastings**[A], Ruili Wang[A], Velautham Sivakumar[A]A, Yajing Li[B] and Kevin Redding[B]. [A]Department of Physics and Astronomy, Georgia State University. [B]Departments of Chemistry and Biological Sciences, The University of Alabama.

Coffee Break: 10:10 to 10:30 AM

Session C, Contributed Papers 10:30 – 12:30 AM.
Chair: Gary Hastings

10:30-10:50 AM

1. FTIR Difference Spectroscopy and Isotope Labeling For The Identification of The Carbonyl Modes of P700 in Photosystem I. **Ruili Wang**[A], Velautham Sivakumar[A], T. Wade Johnson [B]and Gary Hastings[A]. [A]Department of Physics and Astronomy, Georgia State University. [B]Department of Biochemistry and Molecular Biology, Pennsylvania State University.

10:50-11:10 AM

2. Methionine to Leucine Mutations of the A_0 Chlorophyll Ligand in the PsaA and PsaB Subunits of Photosystem I in *Synechocystis* sp. PCC 6803. **Rachel Cohen**[1], Wade Johnson[1] ,Wu Xu[2], Art van der Est[3], Dietmar Stehlik[4], Parag Chitnis[3] and John Golbeck[1]. [1]Department of Biochemistry and Molecular Biology, The Pennsylvania State University. [2]Department of Biochemistry, Molecular Biology, and Biophysics, Iowa State University. [3]Department of Chemistry, Brock University. [4]Institut fur Experimentalphysik, Freie Universitat Berlin**.**

11:10-11:30 AM

3. An Alternate Head Domain Position in the +2ALA Hinge Region Insertion Mutant Contributes to the Raised E_m of the [2Fe2S] Cluster. **Jason W. Cooley**[1], Arthur G. Roberts[2], Mike K. Bowman[3], David M. Kramer[2] and Fevzi Daldal[1]. [1]Department of Biology, Institute for Plant Sciences, University of Pennsylvania. [2]Institute of Biological Chemistry, Washington State University. [3]WR Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory.

11:30-11:50 AM

4. Photosynthesis Lends a Hand to Structural Genomics Efforts **P. D. Laible**, S. J. Hofman, H. M. Joshi, H. N. Scott, and D. K. Hanson. Biosciences Division, Argonne National Laboratory.

11:50-12:10 PM

5. Carotenoids and Carotenosomes of the Green Sulfur Bacterium *Chlorobium Tepidum* **Niels-Ulrik Frigaard** , Colleen E. Yunker and Donald A. Bryant. Department of Biochemistry and Molecular Biology, The Pennsylvania State University.

12:10-12:30 PM

6. Genetic approach for the elucidation of the BChl *c* biosynthesis pathway in the green sulfur bacterium *Chlorobium tepidum*. **A. Gomez Maqueo Chew**, N.-U. Frigaard and D. A. Bryant. The Pennsylvania State University, Department of Biochemistry and Molecular Biology.

12:30 PM **Lunch**

1:30-4:00 PM **Posters, Exhibits and Free Time**

4:00 PM **Poster & Exhibit Session**

5:00 PM **Cocktails, Poster Session Continues**

6:00 PM **Dinner**

7:00 PM

Session D, Invited and Special Lectures 7:00-8:30 PM.
Chair: Harry Frank

7:00-7:55 PM **Invited Lecture**

"Early Evolution of Photosynthesis and the Transition to an Aerobic World"

Robert Blankenship
Department of Chemistry and Biochemistry
Arizona State University

8:00-8:45 PM **Special Lecture**

"Field Photosynthesis in CA: *CO_2 to vines to wine*"

John Biggins
Dry Creek Ridge Winery
Sonoma County, CA

9:00 PM **Wine tasting session (*sponsored by Wineries in Dry Creek Valley, CA*) and Mixer**

Sunday, 8:30 AM

Session E, Contributed Papers 8:30-10:30 AM.
Chair: Marilyn Gunner

8:30-8:50 AM

1. Construction and Characterization of Genetically Modified *Synechocystis sp.* PCC6803 PhotosymstemII Core Complexes Containing Carotenoids with Shorter Pi-conjugation than Beta-carotene. **James A. Bautista[1]**, Cara A. Tracewell[2], Francis X. Cunningham, Jr.[3], Gary W. Brudvig[2] and Bruce A. Diner[1]. [1]Central Research and Development Department, E. I. Du Pont de Nemours and Company. [2]Department of Chemistry, Yale University. [3]Department of Cell Biology and Molecular Genetics, Microbiology Building, University of Maryland.

8:50-9:10 AM

2. The Orientations of Core Antenna Chlorophylls in Photosystem II are Optimized to Maximize the Yield of Photosynthesis. [1]Sergej Vasil'ev, [2]Jian-Ren Shen, [2]Nobuo Kamiya and **[1]Doug Bruce.** [1]Department of Biological Sciences, Brock University. [2]RIKEN, Harima Institute/Spring-8.

9:10-9:30 AM

3. The X-ray Structure of Photosystem II Reveals a Novel Electron Transport Pathway Between P680, Cytochrome b_{559} and the Energy-quenching Cation, Chl_Z^+. Sergej Vasil'ev[1], Gary W. Brudvig [2] and Doug Bruce[1] [1]Department of Biological Sciences, Brock University. [2]Department of Chemistry, Yale University.

9:30-9:50 AM

4. Restoration of Photosystem I and II Activities during Re-hydration of Lichen *Hypogumnia Physoides* thalli. **G. Sridharan[1]**, N.G. Bukhov[1, 2], E.A. Egorova[1, 2] and R. Carpentier[1]. [1]Groupe de Recherche en Énergie et Information Biomoléculaires, Université du Québec à Trois-Rivières. [2]K.A. Timiriazev Institute of Plant Physiology Russian Academy of Sciences.

9:50-10:10 AM

5. Electrochemistry and Structure of Mn^{2+} in Bicarbonate Solutions: Towards Understanding the Bicarbonate Effect on the Donor Side of PSII. **J. Dasgupta[1]**, A.M. Tyryshkin[1], Y.N. Kozlov[2], A.A. Kazakova[2], S.V. Baranov[2], V.V. Klimov[2] and G.C. Dismukes[1]. [1]Department of Chemistry, Princeton University. [2]Institute of Basic Biological Problems, Russian Academy of Sciences.

10:10-10:30 AM

6. Replacing Ca^{2+} by VO^{2+}, Ga^{3+}, or Gd^{3+} in the PSII-WOC Increases the Affinity for Mn: Cooperativity in the Assembly Kinetics of the Mn_4 Cluster**. J. E. Bartlett[1]**, S. V. Baranov[1], G. M. Ananyev[2] and G. C. Dismukes[1]. [1]Department of Chemistry, Princeton University. [2] Institute of Marine and Coastal Sciences, Rutgers University.

10:30-11:00 AM Coffee Break, Final Checkout

Session F, Invited Lecture 11:00-11:50 AM.
Chair: Bruce Diner

"Water-Splitting Chemistry of Photosystem II: Intrusions into the Inner Sanctum of Photosynthesis"

Gary W. Brudvig
Department of Chemistry
Yale University

11:50 AM Closing Remarks

12:00 PM Lunch

1:00 PM Departure

Rings and Things

J. Barber, Wolfson Laboratories, Department of Biological Sciences, Imperial College London, South Kensington Campus, London SW72AZ, UK

Recent structural studies have revealed the similarity of the organisation of the six transmembrane helices of CP43, CP47 and the N-terminal domains of PsaA and PsaB. This similarity emphasises the common evolutionary origin of PSI and PSII. Interestingly, the chlorophyll *a/b* binding proteins of prochlorophytes also fall into this same category of six transmembrane helical proteins as does the IsiA chlorophyll *a* binding protein induced in cyanobacteria when they are depleted of iron. We showed that the six transmembrane helices of CP47 were arranged in a ring of three pairs and bound 14 chlorophylls (1) which has been confirmed by x-ray crystallography and extended to CP43 and PSI (2-4). Electron microscopy and single particle analyses have now shown that the IsiA protein forms an 18 subunit light harvesting antenna ring around PSI trimer in *Synechocystis* 6803 (5). A similar structure has also been detected in the marine prochlorophyte, *Prochlorococcus* low light strain SS120, except in this case it binds both chlorophyll a and chlorophyll b (6). Spectroscopic analyses indicate that the antenna ring in *Synechocystis* is functionally coupled and increases the light harvesting capacity of PSI by almost 100% (7). Electron cryomicroscopy and molecular modelling have identified peripheral chlorophylls of PSI which may aid this efficient energy transfer (8). The role of this newly discovered light harvesting system will be discussed in terms of its significance in the natural environment.

1 Rhee *et al* (1998) Nature 396: 283-286; 2. Zouni *et al* (2001) Nature 409: 739-743
3 Jordan *et al* (2001) Nature 411: 909-916; 4 Kamiya N, Shen JR (2003) Proc Natl Acad Sci USA 100: 98-102; 5 Bibby *et al* (2001) Nature 412: 743-745; 6 Bibby *et al* (2001) Nature 413: 590; 7. lMelkozernov *et al* (2003) Biochemistry in press; 8. Nield *et al* (2003) Biochemistry in press.

The Effect of Incorporating Anthraquinone and 2, 3- Disubstituted Methyl Naphthoquinones into the A1 site of PSI: Transient EPR Studies

Sarah Brown[1], Yulia Pushkar[2], Dietmar Stehlik[2] and Art van der Est[1]
[1]Brock University St Catharines Ontario L2S 3A1 Canada
[2]Free University of Berlin, Arnimallee 14, D-14195 Berlin Germany

The secondary electron acceptor in PSI is a vital part of the electron transfer chain. We are looking at the effect of incorporating structurally related non-native quinones, in order to determine what features of phylloquinone are responsible for its high binding affinity and high midpoint potential.
Previous experiments have shown that monosubstituted naphthoquinones with alkyl side chains of increasing length (up to n=6) are incorporated into the A1 site with the same orientation as phylloquinone in native PSI and with their alkyl side chains in the position normally occupied by the methyl group of phylloquinone, meta to the hydrogen bonded oxygen. For the longer alkyl substituents, this is an unexpected result since structurally they resemble the phytyl tail and not the methyl group. This suggests that that quinones bind preferentially with a substituent meta to the H-bonded oxygen which implies an important role for the methyl group in phylloquinone.
Here, we present results for disubstituted methyl napthoquinones in the A1 site. Transient EPR spectra show that these quinones have unusually large methyl hyperfine coupling which indicates that the methyl group and not the alkyl side chains reside meta to the hydrogen bonded oxygen.
The high midpoint potential of phylloquinone (PhQ) is another distinguishing feature. The rate of forward electron transfer from A1- to FX is not at the maximum on the Marcus curve, as demonstrated by the temperature dependence of the rate. 9, 10-Anthraquinone (AQ) has a reduction midpoint potential which is ~120mV more negative than PhQ in DMF solution. This implies that it has a larger ΔG value than phylloquinone and a determination of the rate of AQ- to FX electron transfer allows us to determine in which region of the Marcus curve the A1 => FX electron transfer lies. Transient EPR experiments on PSI incubated with AQ reveal that the rate of electron transfer from AQ- to FX is faster than from PhQ to FX in native PSI. In addition, temperature dependence experiments show that the activation energy is lower. From this we conclude that the rate of electron transfer lies in the normal region of the Marcus curve.

Molecular Dynamics simulations of monosubstituted 1,4-naphthoquinone electron acceptors in the A1 binding site of Photosystem I.

Y.S. Bukhman, H. Gordon and A. van der Est, Department of Chemistry, Brock University, St. Catharines, Ontario, Canada.

Electron transfer in Photosystem I (PS-I) is mediated by several small cofactors that act as electron acceptors. In the native system, the electron acceptor A_1 is a 2-methyl-3-phytyl-1,4-naphthoquinone, or phylloquinone (PhQ), which is bound to the protein on the stromal side of the membrane. In the absence of PhQ, PS-I is known to recruit plastoquinone (PQ), which functions in the A_1 site with reduced efficiency. Thus, under normal conditions, PhQ must compete with PQ from the PQ pool for the A_1 site. However, there are no obvious structural features of the PhQ and the A_1 binding site that account for the preferential binding of PhQ. The interaction between the binding site environment and the quinone may be examined through picosecond-scale Molecular Dynamics simulations. Here, we explore the behaviour of monosubstituted naphthoquinones (NQ) in the A_1 binding site. The length of the substituent alkyl chain is increased from 1 to 6 carbons, and the quinone molecule is manipulated within the binding site to place the alkyl chain at positions ortho and meta to the quinone's H-bonded oxygen atom, starting with the lowest energy conformers obtained via systematic conformation searches of 2-methyl-, 2-ethyl-, 2-butyl- and 2-hexyl-1,4-naphthoquinones. Our initial results suggest a high propensity for the alkyl side chain to reside in the space normally occupied by the phytyl tail of PhQ. This is contrary to EPR results, which suggest that the alkyl side chains are meta to the H-bonded carbonyl. Possible reasons for this difference will be discussed.

B3 Saturday, 9:10 AM

ANALYSIS OF STRUCTURAL FEATURES THAT DICTATE THE EFFICENCY OF LIGHT HARVESTING IN THE PS I REACTION CENTER/CORE ANTENNA COMPLEX

Thomas G. Owens and David G. Rand
Dept. of Plant Biology, Cornell University, Ithaca NY 14853-5908

The 2.5 Å x-ray crystallographic structure of the PS I reaction center/core antenna complex reveals a cluster of six chlorophylls in the central region of the complex that are involved in photochemistry and electron transfer, surrounded by a ring of 90 chlorophylls that make up the core antenna. Although this general separation between pigments involved in electron transfer from antenna pigments is also see in PS II and bacterial antenna systems, PS I is unique in that the quantum efficiency of energy transfer and photochemistry is essentially unity. The higher efficiency of energy transfer in PS I is a consequence of the spatial and spectral properties of the RC/core antenna complex, in particular those interactions at the interface between the electron transfer and antenna chlorophylls. An examination of the structural features (position and orientation) that determine the coupling between interacting pigments at this interface reveals some unexpected results. Interestingly, the majority of putative excitons that are present in the PS I complex are located at or near this interface and thus must play key roles in facilitating efficient energy transfer among these two groups of pigments.

B4 Saturday, 9:30 AM

PHOTOSYNTHETIC EXCITONS IN PS I: AN EVALUATION OF KEY EXCITON-MONOMER CHLOROPYLL INTERACTIONS THAT DETERMINE THE EFFICIENCY OF LIGHT HARVESTING

David G. Rand, *Robert S. Knox and Thomas G. Owens
Dept. of Plant Biology, Cornell University, Ithaca NY 14853-5908 and
***Dept. of Physics and Astronomy, University of Rochester, Rochester NY 14627-0171**

The relative rate of single energy transfer between the two states of an excitonic dimer (composed of two strongly interacting chlorophylls) and a monomeric chlorophyll, compared to the same rates calculated in the Förster (dipole-dipole, weak interactions) limit, depends explicitly on the spatial separation, relative orientations and spectral properties of the three pigments. The effects of the exciton interactions can be to either speed up or slow down the energy transfer rates, depending primarily on the relative orientations of the pigments. We apply our understanding of these interactions in simulation models to the reaction center/core antenna complex of PS I. In particular, we examine those key exciton-monomer interactions whose energy transfer rates are critical to the efficiency of light harvesting in PS I. From an evolutionary perspective, we determine if the positions and orientations of the chlorophylls participating in these key exciton-monomer interactions lead to an increase or decrease in energy transfer rates compared to the corresponding rates calculated in the Förster limit. Further, we examine how the spectral properties of the exciton and monomer chlorophylls influence energy transfer rates as a means of predicting spectral assignments for some of these key pigments in the complex.

B5 Saturday, 9:50 AM

Mutation Induced Modulation of Hydrogen Bonding To P700 Studied Using FTIR Difference Spectroscopy

Gary Hastings[A], Ruili Wang[A], Velautham Sivakumar[A], Yajing Li[B] and Kevin Redding[B]

[A]Department of Physics and Astronomy, Georgia State University, Atlanta, GA 30303
[B]Departments of Chemistry and Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487

Site directed mutagenesis in combination with Fourier-transform infrared difference spectroscopy has been used to study how hydrogen bonding modulates the electronic and physical organization of P700, the primary electron donor in photosystem I. Wild type PS I particles from *Chlamydomonas reinhardtii*, and a mutant in which ThrA739 is changed to alanine [TA(A739) mutant] were studied. ThrA739 is thought to provide a hydrogen bond to the chlorophyll-a' molecule of P700 (the two chlorophylls of P700 ($P700^+$) will be called P_A and P_B (P_A^+ and P_B^+)). The mutation considerably alters the ($P700^+$-P700) FTIR difference spectra. Employing Fourier deconvolution procedures to enhance the resolution of the difference spectra, we were able to describe all of the mutation induced changes in the difference spectra in terms of difference band assignments that were proposed recently (Hastings, G., Ramesh, V. M., Wang, R., Sivakumar, V. and Webber, A. (2001) *Biochemistry*, 40, 12943-12949).

C1 Saturday, 10:30 AM

FTIR Difference Spectroscopy and Isotope Labeling For The Identification of The Carbonyl Modes of P700 in Photosystem I

Ruili Wang[A], Velautham Sivakumar[A], T. Wade Johnson[B]and Gary Hastings[A]

[A]Department of Physics and Astronomy, Georgia State University, Atlanta, GA 30303.
[B]Department of Biochemistry and Molecular Biology, Pennsylvania State University, PA 16802

In order to identify the carbonyl modes of P700, the primary electron donor in photosystem I, light-induced ($P700^+$-P700) Fourier-transform infrared difference spectra have been obtained using PS I particles from *Synechocystis* sp. PCC 6803 that are unlabeled, uniformly ^2H labeled and uniformly ^{15}N labeled at room temperature. FTIR difference spectra obtained from ^{15}N labeled PS I particles indicates that infrared bands in the region 1750-1650 cm_{-1} are not related to nitrogen atoms. Upon uniform ^2H labeling of PS I, however, the ($P700^+$-P700) FTIR difference spectra are considerably altered. All difference bands associated with ester/keto carbonyl modes of the chlorophylls of P700 and $P700^+$ downshift ~4-5/1-3 cm-1 upon ^2H labeling, respectively. We show that a negative difference band at 1698 cm^{-1} is associated with keto carbonyl modes of both chlorophylls of P700. We also show that a negative difference band at ~1639 cm^{-1} is unlikely to be associated with keto carbonyl modes of the chlorophylls of P700.

Methionine to Luecine Mutations of the A0 Chlorophyll Ligand in the PsaA and PsaB Subunits of Photosystem I in Synechocystis sp. PCC 6803

Rachel Cohen[1], Wade Johnson[1] ,Wu Xu[2], Art van der Est[3], Dietmar Stehlik[4], Parag Chitnis[3], John Golbeck[1]

[1] Department of biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, 16802. [2] Department of Biochemistry, Molecular Biology, and Biophysics, Iowa State University, Ames, IA, 50010. [3] Department of Chemistry, Brock University, Ontario, Canada L2S 3A1. [4] Institut fur Experimentalphysik, Freie Universitat Berlin, Arnemalle 14, 14195, Berlin Germany.

The PsaA and PsaB proteins that form the heterodimeric core of Photosystem I (PSI) are related by a pseudo-C_2 symmetry axis. The electron transfer cofactors, beginning with P_{700}, and continuing with the primary and secondary electron acceptors, A_0 and A_1, are located on redundant branches, which converge at the F_X iron-sulfur cluster. This bifurcating electron transfer chain begs the question of whether the electron utilizes one branch (a unidirectional scheme) or both branches (a bidirectional scheme) in electron transfer. To address this question, we constructed mutants in which the axial methionine ligand on A_0 was replaced with non-ligating leucine on both the PsaA (*am*l) and PsaB (*bml*) branches. EPR spectroscopy shows that when the samples are frozen under constant illumination, the amount of F_A and F_B reduced is about the same in the wild-type and the *aml* and *bml* mutants. The amplitude of the g = 2.0028 resonance derived from $P700^+$ by illumination at 77 K is also about the same in the wild-type and the *aml* and *bml* mutants. In contrast, optical kinetic spectroscopy at room temperature shows that the amplitude of long-lived *(i.e* > 1 ms) $P700^+$ in the *aml* mutant is only 60% that of the *bml* mutant and the wild-type. Temperature dependent analysis Faster kinetic phases (*i.e.* 10 μs and 500 μs) are found in the *aml* mutant, but the amplitudes are flash-energy dependent, indicating a possible origin in as antenna chlorophyll triplet states. The fast phases are nearly absent in the wild-type, and the $_{bml}$ mutant. HPLC analysis done on the *aml* and *bml* mutants confirm the presence of two phylloquinone in the A_1 site. These results show that electron transfer is altered in the *aml* mutant, but not in the *bml* mutant, suggesting that at room temperature the PsaA side is active and the PsaB side is relatively inactive in cyanobacterial PS I.

C3 Saturday, 11:10 AM

AN ALTERNATE HEAD DOMAIN POSITION IN THE +2ALA HINGE REGION INSERTION MUTANT CONTRIBUTES TO THE RAISED E_m OF THE [2FE2S] CLUSTER.

Jason W. Cooley[1], Arthur G. Roberts[2], Mike K. Bowman[3], David M. Kramer[2] and Fevzi Daldal[1]
[1]Department of Biology, Institute for Plant Sciences, University of Pennsylvania, Philadelphia, PA 19104-6018 (jcooley@sas.upenn.edu; Ph: 215-898-5385)
[2]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340
[3]WR Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352-0999

Mutant strains of *Rhodobacter capsulatus* that have alanine insertions in the hinge region of the iron sulfur containing subunit of the bc_1 complex have raised [2Fe2S] cluster E_m's in the absence of Q_o site inhibitors, implying that the environment of the metal center is altered. The change in the E_m of the [2Fe2S] cluster in such mutants has been proposed to be due to an increased binding of the Fe-S protein to the Q_o site inhabitant ubiquinone. To probe the validity of this attractive proposal we have monitored this redox-sensitive interaction by using electron paramagnetic resonance (EPR) spectroscopy, and found the interaction similar to that seen with the native enzyme. Moreover, in +2Ala chromatophore membranes that are depleted of ubiquinone the [2Fe2S] cluster still exhibited an E_m about 55 mV more positive than similarly treated wild type membranes, implying that additional factors might also be involved. To address whether or not the location of the [2Fe2S] cluster in the bc_1 complex affects this observed E_m value we determined the relative orientation of the [2Fe2S] cluster in the +2Ala mutant by analyzing layered membrane samples using EPR spectroscopy. We found that the orientation of the [2Fe2S] cluster of the bc_1 complex in the +2Ala not treated with inhibitors is different than that found in the native enzyme. Yet it shares a similar orientation with the native enzyme when both are in the presence of stigmatellin. Furthermore, ubiquinone-depleted, layered chromatophore membranes from the +2Ala mutant have a predominant orientation of the [2Fe2S] cluster similar to that seen with the untreated +2Ala membranes and dissimilar to that seen with the wild type membranes lacking ubiquinone. These findings suggest that the environment derived from the newly observed orientation, independent of a stabilized or destabilized [2Fe2S]-ubiquinone complex, also contributes to the raised [2Fe2S] E_m in this mutant.

C4 Saturday, 11:30 AM

PHOTOSYNTHESIS LENDS A HAND TO STRUCTURAL GENOMICS EFFORTS

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Membrane proteins present unparalleled challenges for structural and functional genomics programs. We present a system which seeks to overcome some of the current membrane protein hurdles by exploiting the unique physiology of photosynthetic bacteria B specifically *Rhodobacter* B for the heterologous expression of membrane proteins. Why *Rhodobacter*? Unlike the situation when membrane proteins are overexpressed in *E. coli*, inclusion body formation can be minimized in *Rhodobacter* by concomitant synthesis of foreign protein and new membrane. The coordinate protein and membrane synthesis of *Rhodobacter* occurs naturally when cultures are depleted of oxygen in preparation for photosynthetic growth B both the transmembrane protein complexes of the photosynthetic apparatus and large quantities of a new, intracytoplasmic membrane (ICM) that houses them are induced simultaneously. The ICM differs from the cell membrane in its morphological and physical properties, and, most importantly, in its kinetics of biogenesis. In our engineered system, heterologous expression and ICM localization of target membrane proteins is accomplished by placing their genes under the control of oxygen-inducible promoter(s) of genes encoding proteins for the photosynthetic apparatus. Reporter proteins have been used to identify optimal expression vectors and *Rhodobacter* hosts. Using standardized and semi-automated protocols, natively-folded, ICM-encapsulated membrane proteins can be purified rapidly by detergent extraction of the ICM and subsequent affinity chromatography. This *Rhodobacter* expression system represents an advance towards the development of a strategy for obtaining structures for this important class of proteins at a more rapid pace.

C5 Saturday 11:50 AM

CAROTENOIDS AND CAROTENOSOMES OF THE GREEN SULFUR BACTERIUM *CHLOROBIUM TEPIDUM*

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Chlorobium tepidum is an obligate anaerobic green sulfur bacterium. About 8% of the pigmentation in cells is carotenoids, mainly chlorobactene. Several genes functioning in the carotenoid biosynthetic pathway were identified by gene inactivation and shown to encode: phytoene synthase (*crtB*), phytoene desaturase (*crtP*), ζ-carotene desaturase (*crtQ*), γ-carotene desaturase (*crtU*), and two tentative *cis-trans* isomerases (*crtH* paralogs). Only the carotenoid-free *crtB* mutant showed a severe growth deficiency. Interestingly, the pathway leading to lycopene is identical to that in plants and cyanobacteria and different from that in most other bacteria. The chlorosome antenna organelle in *C. tepidum* contains BChl *c*, carotenoids, and BChl *a* in a molecular ratio of about 80:8:1. Vestigial chlorosomes, denoted carotenosomes, was isolated from a *bchK* mutant of *C. tepidum* which completely lacks BChl *c*. These carotenosomes contained carotenoids, BChl *a*, CsmA and CsmD in ratios comparable to those in wild type chlorosomes, but all other chlorosome proteins normally found in wild type chlorosomes were found only in trace amounts or not detected. The BChl *a* species has an absorption peak at 798 nm and corresponds to an oligomeric CsmA-BChl *a* complex which most likely forms the baseplate structure of both chlorosomes and carotenosomes. Minor protein components identified in carotenosomes may suggest that a transporter complex of EmrA, EmrB, and TolC homologs may function in chlorosome biogenesis.

C6 Saturday, 12:10 AM

Genetic approach for the elucidation of the BChl *c* biosynthesis pathway in the green sulfur bacterium *Chlorobium tepidum*

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The green sulfur bacterium *Chl. tepidum* can grow at very low light intensities due to its large light-harvesting antenna, the chlorosome. Chlorosomes are structures that contain between 150,000 and 200,000 bacteriochlorophyll *c* (BChl *c*) molecules that direct the excitation energy to the reaction center. In addition to BChl c_F, which makes up about 90% of the pigment content in the organism, *Chl. tepidum* also produces BChl a_P and Chl a_{PD}. Based on the well studied pathway for BChl *a* in purple bacteria and on the gene duplications found in the *Chl. tepidum* genome, we predicted the function of several genes involved in the biosynthesis of BChl *c*. In order to verify the function of these genes we constructed mutants with the target gene inactivated and used several analytical techniques, such as HPLC and MS to analyze the phenotype of the mutant BChl *c.* We have successfully identified three genes, *bchH, bchS* and *bchT* that encode the large subunit of the Mg-chelatase, the genes responsible for the methylation of the $C8^2$ and $C12^1$ carbons, *bchQ* and *bchR*, one of the genes responsible for the hydration of the $C3^1$ carbon, *bchV*, and two genes *bchP* and *bchO*, that are involved in the reduction of the isoprenoid tails of BChl *a* and Chl *a.* The physiological characterization of these *Chl. tepidum* mutant strains is underway.

D1 Saturday, 7:00 PM Invited Lecture

Early Evolution of Photosynthesis and the Transition to an Aerobic World

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Understanding the origin and evolution of photosynthesis has proven to be a remarkably challenging task. It is now clear that a single, simple branching diagram cannot describe the evolutionary path of photosynthesis. A number of lines of evidence force us to this conclusion, including whole genome analyses of photosynthetic organisms, which revealed that large scale horizontal gene transfer has taken place amongst these groups, including the genes that code for the photosynthetic apparatus. The photosynthetic apparatus has been assembled largely by gene recruitment from other metabolic pathways. We have continued these studies, to focus in more detail on the transition between anoxygenic and oxygenic photosynthesis. Additional work has indicated that there is an ancient relationship between photosynthesis and nitrogen fixation.

References

J. R. Raymond, O. Zhaxybayeva, S. Gerdes, J. P. Gogarten, and R.E. Blankenship (2002) Whole genome analysis of photosynthetic prokaryotes, *Science*, **298**, 1616-1620.

J. Raymond, O. Zhaxybayeva, J. P. Gogarten, and R.E. Blankenship (2003) Evolution of photosynthetic prokaryotes: a maximum likelihood mapping approach. *Phil. Trans of the Royal Soc. B,* **358**, 223-230.

J. Raymond, J. Siefert,, C. Staples, R. E. Blankenship (2003) The Natural History of Nitrogen Fixation, submitted

D2 Saturday, 8:00 PM Special Lecture

Field Photosynthesis in CA: *CO_2 to* vines *to wine*

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Modern viticulture practices will be reviewed with emphasis on the management of leaf canopy to optimize photosynthetic capacity and fruit quality and yield. Related aspects include a consideration of the regulation of anthocyanin/phenol levels, fruit acid composition, pH and fruit ripening enzymes by phytochrome. The conversion of fruit to wine will be briefly discussed with special attention given to the biochemistry of events occurring during the aging of wine.

Construction and Characterization of genetically modified *Synechocystis sp.* PCC6803 Photosystem II Core Complexes Containing Carotenoids with shorter pi-conjugation than beta-carotene

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Cytochrome b559 and the Chl_Z accessory chlorophylls are located at the periphery of the Photosystem II (PSII) reaction center and function as alternate electron donors to $P680^+$ under conditions where oxidation of the manganese cluster by $P680^+$ is blocked. It has been postulated that beta-carotene (two beta-rings, 11 conjugated double bonds), of which there are two per reaction center, enables this long distance electron transfer from the periphery to the center by acting as an electron wire, forming an intermediate cation radical. To test this function of carotenoids in PSII, we manipulated the carotenoid biosynthetic pathway of *Synechocystis* PCC 6803 to produce carotenoids with shorter conjugated pi-electron systems and to examine the consequences of their expression on the function of PSII. We expect that the shortened conjugated system should result in a higher reduction potential for carotenoid oxidation and a shorter distance over which such a carotenoid might act as an electron wire bridging two redox components. We used three different strategies to modify carotenoid expression in *Synechocystis*. Separately, we 1) replaced the gene encoding phytoene desaturase (Pd) by a kanamycin resistance cassette; 2) replaced the zeta-carotene desaturase (Zd), also by a kanamycin resistance cassette; and 3) replaced the Zd gene with the Pd gene of *Rhodobacter capsulatus* coupled to a spectinomycin resistance cassette. All three strains were allowed to fully segregate using antibiotic selection. The first two strategies accumulated phytoene (3 conjugated double bonds) and zeta-carotene (7 conjugated double bonds), respectively, and no beta-carotene. These strains required glucose and dim light for growth and contained functional Photosystem I (PSI) but no functional PSII. The third strategy was intended to block the formation of lycopene (11 conjugated double bonds), which in the wild type is converted to beta-carotene by the *Synechocystis* carotenoid beta-cyclase, but would allow for the formation of neurosporene (9 conjugated double bonds), which can be converted to beta-zeacarotene (one beta-ring, 9 conjugated double bonds). We have found that this strain successfully assembles PSII and tolerates brighter light conditions than the previous two strains. Our preliminary pigment analysis using HPLC coupled to a photodiode array detector revealed that this strain produces no beta-carotene but accumulates neurosporene, spheroidene, beta-zeacarotene, and their corresponding hydroxy derivatives. Experiments aimed at examining low temperature oxidation of Chl_Z and carotenoid in carotenoid-modified and wild type PSII core complexes are underway.

E2 Sunday, 8:50 AM

The orientations of core antenna chlorophylls in photosystem II are optimized to maximize the yield of photosynthesis.

[1]Sergei Vasil'ev, [2]Jian-Ren Shen, [2]Nobuo Kamiya and [1]Doug Bruce

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In photosystem II the probability that energy absorbed by core antenna chlorophyll is transferred to the reaction center exceeds 98%. Although efficiency of transfer is ensured by proximity, fine-tuning the orientations of antenna chlorophyll can fractionally modify it. This level of refinement has been assumed to be superfluous as so many subsequent processes limit the overall rate of photosynthesis. Nevertheless, could natural selection act on the most efficient step of energy conversion? Our Monte Carlo simulations sampled the orientation space of key antenna chlorophyll in a kinetic model based on the X-ray structures of photosystem II. Our results revealed that even though the orientation of these key chlorophyll could only affect the quantum efficiency of PSII by approximately 1% they were indeed optimized to maximize photosynthesis.

E3 Sunday, 9:10 AM

The X-ray structure of Photosystem II reveals a novel electron transport pathway between P680, Cytochrome b_{559} and the energy-quenching cation, Chl_Z^+.

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When water oxidization by photosystem II (PSII) is impaired, an oxidized chlorophyll (Chl_Z^+) is formed that quenches excitation and may prevent photodamage. Both the identification of this Chl^+ and the mechanism of its oxidation and reduction are controversial. Using the available X-ray structures of PSII we calculated the efficiency of two proposed quenchers, Chl_Z^+(D1) and Chl_Z^+(D2). Of these two, only Chl_Z^+(D1) can quench to the degree observed experimentally. We also identify a chain of closely-spaced pigments in the structure from *Thermosynechococcus vulcanus* that we propose to form a novel electron transport pathway between Chl_Z(D1), β-carotene, $P680^+$ and Cytochrome b_{559}.

E4 Sunday, 9:30 AM

RESTORATION OF PHOTOSYSTEM I AND II ACTIVITIES DURING RE-HYDRATION OF LICHEN *HYPOGUMNIA PHYSOIDES* THALLI

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Photochemical efficiencies of photosystem I (PSI) and photosystem II (PSII) were evaluated in lichen, *Hypogumnia physoides* thalli during the transition from dry to hydrated state. PSII reaction centers are photochemically inactive in dry thalli as indicated by the complete loss of chlorophyll fluorescence rise, whereas the primary electron donor of PSI, P700 is oxidized irreversibly under continuous light. Upon application of a multiple and, particularly, single-turnover pulses, P700 oxidation is partially reversed in dry lichen, owing to the recombination between $P700^+$ and reduced acceptor F_X of PSI. During re-hydration of dried thalli, the light-induced redox reactions in PSII and PSI was restored gradually, which was much faster for PSI. When partially or fully hydrated thalli were irradiated with strong white light, the $P700^+$ reduction was composed of two slow components. This finding suggests that PSII is unable to reduce all PSI centers. Oppositely, no slow component was found in the reoxidation kinetics of Q_A^- in such thalli after irradiation with white light, this is attributable to the inability of PSII to reduce the plastoquinone pool to significant levels. Importantly, the half-times of fluorescence decay and of the fast component of $P700^+$ reduction observed after irradiation with white light were similar. It is concluded that slow alternative electron transport routes may contribute to the energetics of photosynthesis to a larger extent in *Hypogumnia physoides* compared to higher plants.

E5 Sunday, 9:50 AM

Electrochemistry and Structure of Mn^{2+} in Bicarbonate Solutions: Towards Understanding the Bicarbonate Effect on the Donor Side of PSII

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The complexation equilibria between Mn^{2+} and bicarbonate (ie., speciation) is crucial to its transport properties and ability to participate in assembly of the Mn_4 core of the Water Oxidizing Complex (WOC). Mn^{2+} together with bicarbonate serves as a more efficient electron donor than Mn^{2+} alone to apo-WOC-PSII. Mn-bicarbonate complexes are also speculated to have played a seminal role in the evolution of the WOC-PSII from an ancestral anoxygenic bacterial precursor. Herein, electrochemistry (EC) and EPR methods are used to study the formation of Mn^{2+} complexes in bicarbonate solutions and their oxidation potentials. EC reveals that bicarbonate titrations form two complexes having low oxidation potentials with E_0= 0.67V and 0.52V. These species are correlated to complexes with Mn^{2+}-to-ligand stoichiometries of 2:1 (Mn^{2+} cluster) and 1:2 (mono-Mn^{2+}) respectively. Room temperature EPR shows that the equilibrium speciation is dominated by mono-Mn^{2+} complexes (1:1 and/or 1:2) with no or less than 5% of the clusters present (within expt. error). Low temperature dual-mode EPR of frozen solutions using water/CH_3OH (25%) to ensure glass formation detected exclusively mono-Mn^{2+} species. Pulsed ENDOR, ESEEM and 2D-HYSCORE detected the complete (rhombic) hyperfine tensor of ^{13}C, establishing that $H^{13}CO_3^-$ is a direct ligand to Mn^{2+}. ^1H ENDOR shows that 2-3 water ligands are lost upon HCO_3^- binding. The dominant species responsible for the EC oxidation at E_0= 0.52V is thus predicted to be $Mn^{2+}(HCO_3^-)_2(OH_2)_{3\text{-}4}$.

REPLACING Ca^{2+} BY VO^{2+}, Ga^{3+}, OR Gd^{3+} IN THE PSII-WOC INCREASES THE AFFINITY FOR Mn: COOPERATIVITY IN THE ASSEMBLY KINETICS OF THE Mn_4 CLUSTER

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The functional role of the Ca^{2+} cofactor in water splitting has not been conclusively established, and only recently has a direct measure of its interaction energy with the high-affinity Mn site in the PSII Water Oxidizing Complex been established by EPR (Tyryshkin et al. poster). Here we show that the positive cooperativity in Mn and Ca binding affinities at these sites can be measured by the net rate of formation of the first photooxidized Mn^{3+} during the initial assembly step, eventually leading to the reconstitution of the inorganic core of the apo-WOC from the free cofactors (photoactivation). The lag phase in the rate of recovery of O_2 production from apo-WOC was measured and found to accelerate in the presence of Ga^{3+} (1.5x), Gd^{3+} (2x), or VO^{2+} (2x), which bind competitively and reversibly to the Ca^{2+} site. The data indicate that occupation of the Ca site by Ca^{2+} (or VO^{2+}, Gd^{3+}, Ga^{3+}) increases the affinity for Mn^{2+} at the high affinity Mn site and suppresses its decay after photooxidation to Mn^{3+}. Importantly, none of the inorganic mutants at the Ca^{2+} site support O_2 production in the reconstituted enzyme (other than Sr^{2+}), indicating that Ca^{2+} expels them in later steps which yield functional enzyme. A structural model that may account for this interaction and is also supported by EPR studies (see Tyryshkin et al.) suggests that Ca^{2+} binds to a site in PSII which shares a bridging ligand to the high affinity Mn^{3+} following photooxidation and proton ionization: $[Mn^{2+}OH_2Ca^{2+}] \leftrightarrow [Mn^{3+}OHCa^{2+}]$. The resulting state has a higher affinity for Mn^{2+} and thus cooperative assembly of a functional Mn_4 core occurs.

Water-Splitting Chemistry of Photosystem II: Intrusions into the Inner Sanctum of Photosynthesis

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The active site for water oxidation in photosystem II (PSII) consists of a tetranuclear Mn cluster, a redox-active tyrosine called Y_Z, Ca^{2+} and Cl^-. Based on a consideration of biophysical and inorganic chemistry, we proposed that the O-O bond-forming step in PSII involves nucleophilic attack by a calcium-bound water molecule on an electron-deficient Mn(V)=O species.[1-2] In order to test this proposal, we determined the affinity of a series of metal ions for the Ca^{2+}-binding site.[3] The pK_a of the aqua-ion is identified as the factor that determines the functional competence of the metal ion, consistent with a role of Ca^{2+} as a Lewis acid that binds a substrate water and tunes its reactivity. To examine the chemistry of a Mn(V)=O species, we synthesized and structurally characterized the complex $[(H_2O)(terpy)Mn(O)_2Mn(terpy)(OH_2)](NO_3)_3$, terpy = 2,2':6,2''-terpyridine. This complex catalyzes the conversion of oxone or hypochlorite to O_2.[4] Kinetic and isotope labeling studies are consistent with a mechanism involving a Mn(V)=O intermediate. Supported by the NIH (GM32715).

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Poster 1

OPTICAL SPECTROSCOPIC STUDIES OF GEOMETRIC ISOMERS OF SPHEROIDENE

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Spheroidene is a naturally-occurring carotenoid that adopts two geometric isomeric forms, all-*trans* and 15,15'-*cis*, in the pigment-protein complexes of photosynthetic bacteria from *Rb. sphaeroides*. It has been postulated that the biological functions of light-harvesting and photoprotection performed by spheroidene depend on the configuration of the molecule. The present work seeks to examine this hypothesis by isolating various geometric isomers of spheroidene and studying their spectral and photochemical properties. In this study, high performance liquid chromatography (HPLC) was used to isolate several geometric isomers of naturally-occurring and synthetic 11-locked-*cis*-spheroidene, 13-locked-*cis*-spheroidene and 15,15'-locked-*cis*-spheroidene. The HPLC elution profiles showed a number of peaks, the absorption spectra of some of which displayed strong "cis-bands" in the region near 350 nm in addition to the prominent absorption bands in the visible region. Resonance Raman spectroscopy has been used to deduce the configurations of the molecules. Semi-empirical molecular orbital computations have been used to correlate the structures of the molecules with their spectral properties.

Poster 2

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

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The main-form (MFPCP) and the high-salt (HSPCP) peridinin-chlorophyll *a*-proteins from the dinoflagellate, *Amphidinium carterae*, were investigated using absorption, fluorescence, and fluorescence excitation spectroscopy. Pigment analysis demonstrated that the MFPCP contains eight peridinins and two chlorophyll (Chl) *a*, whereas the HSPCP has six peridinins and two Chl *a*. The absorption spectra of the complexes were taken at 10K and analyzed by summing the individual 10K spectra of Chl *a* and peridinin taken in 2-MTHF. To fit the MFPCP spectrum, two Chl *a* spectra with an intensity ratio of 2:1 at 434 nm and 440 nm and two blue-shifted peridinin molecules were required. Fitting the HSPCP spectrum required two Chl *a* of the same intensity at 435 nm and 440 nm and only one peridinin molecule blue-shifted relative to the others. The absorption spectral profiles of the complexes in the Q region were fit using Gaussian functions. Fluorescence spectra from both complexes display several features that become evident only at the low temperature. Fluorescence excitation spectroscopy has been used to determine the energy transfer efficiencies between peridinin and chlorophyll in both complexes.

Poster 3

OPTICAL SPECTROSCOPY OF LINEAR POLYENES

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Three polyenes, decatetraene, dodecapentaene, and tetradecahexaene, were synthesized from hexadiene using living polymerization techniques, and the all-trans isomers were obtained using HPLC. The spectroscopic properties of the molecules were studied using steady-state absorption, fluorescence, and fluorescence excitation spectroscopy. All three molecules display vibrationally resolved $S_0 \rightarrow S_2$ ($1^1A_g \rightarrow 1^1B_u$) absorption and $S_1 \rightarrow S_0$ ($2^1A_g \rightarrow 1^1A_g$) fluorescence. Performing the spectroscopy at 77 K facilitated the accurate determination of the spectral origins from the spectral lineshapes. The origins of the fluorescence spectra were assigned by Gaussian deconvolution. The $S_2 - S_1$ energy gaps increase with increasing lengths of π-electron conjugation. The data provide a systematic series of molecules for understanding the structural features controlling the spectroscopic properties of π-electron conjugated systems.

Poster 4

SOLVING A DIPOLE STRENGTH RIDDLE: THEORY, AND UPDATED D VALUES IN FOUR CHLOROPHYLLS (CHL-A, CHL-B, BCHL-A, BCHL-C)

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As structural and kinetic information become more plentiful and accurate, chlorophyll-proteins are being subjected to extensive theoretical analysis (see, e. g., Novoderezhkin et al. [1]). Dipole strengths (D) for chlorophyll Qy transitions are essential to many such calculations but the literature provides a large number of quantitative values that are very difficult to sort out. Most of the problem is connected with the dependence of D on index of refraction n. In addition to the effective field problems we discussed recently at ERPC-19 and PS-2001 (Brisbane), a significant error by a factor n2 is found to exist in very prominent sources, sometimes implicitly [2] and sometimes explicitly [3]. We believe we have located the source of this error. We present the results of this detective work [4] and an update on dipole strengths in the chlorophylls [5].

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Poster 5

When a lumenal protein interferes with stromal side affairs. Energy transfer in the phycobilin and chlorophyll antenna in a mutant of Synechocystis 6803 lacking PsbU.

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PsbU is a lumenal protein associated with the oxygen evolving apparatus in red algae and cyanobacteria. Mutant cells lacking PsbU are characterized by slightly decreased rates of oxygen evolution and decreased levels of variable fluorescence. We are using a combination of low temperature fluorescence and absorbance spectroscopy to characterize energy transfer in *Synechocystis* PCC 6803 *psb*U$^-$ cells. 77K emission spectra for excitation of the phycobilisome at 600nm show an increase at 680nm in psbU$^-$ cells as compared to wild-type. The difference observed in the 77K emission spectra with 600nm excitation is not observed for Chl excitation at 435nm. These results suggest that there is increased fluorescence from the phycobilisomes in *psb*U$^-$ cells and that the decreased variable fluorescence is likely due to an increased contribution of bilin fluorescence at F_o. The increased phycobilisome fluorescence suggests that the interaction between PSII and the phycobilisome is altered in the *psb*U$^-$ cells. This hypothesis is counterintuitive as psbU is a luminal side structural protein and the phycobilisome interacts with PSII on the stromal side of the thylakoid membrane. Time-correlated single photon counting will be used to determine if the decay time for the phycobilisome is increased in the *psb*U$^-$ cells and for the basis of a computer model that will simulate the interactions between the pigments involved. To determine the relative affinity of the phycobilisome for PSII in the psbU$^-$ cells and wild-type a detergent binding assay will be used.

Poster 6

FAST REPETITION RATE ANALYSIS OF VARIABLE BACTERIOCHLOROPHYLL *A* FLUORESCENCE IN *RHODOBACTER SPHAEROIDES* CULTERED UNDER LOW AERA-TION IN A CHEMOSTAT

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Photosynthetic unit (PSU) development was re-examined in steady-state grown *Rb. sphaeroides* by IR-fast repetition rate fluorometry, a highly sensitive technique for the measurement of functional absorption cross-sections (Σ), inter-unit connectivity and rates of reaction center turnover and cyclic electron flow. Steady-state aerobic cells that were bleached of bacterio-chlorophyll *a* (BChl) by growth in a chemostat with limiting malate, were switched to 3% O_2 to induce PSU formation and BChl synthesis. Cell numbers remained constant over 24 h, while BChl levels rose to a steady state with a $t_{1/2}$ of ~9 h, and thin sections showed that the cytoplas-mic membrane began to invaginate and form intracytoplasmic membranes (ICM) after 2 h. The core light-harvesting 1 (LH1) complex was assembled first, followed by the peripheral LH2 complex which predominated after 6 h. Rises in Σ at 795 nm mirrored the LH2 rise, while Σ measured at 470 nm followed the LH1 increase. Accelerated reaction center (RC) turnover rates (1-2 ms) were observed in the aerobic cells and over the first 3 h at low aeration. This represents the first observation of a functional RC in aerobic cells, reminiscent of the RC activity of aerobic anoxygenic photoheterotrophic bacteria, which also have low BChl contents. RC turnover achieved a steady-state rate of ~6 ms with $t_{1/2}$ of ~5 h, the same $t_{1/2}$ seen for LH1. These studies establish that PSUs are assembled in a sequential manner with the functionally essential LH1-RC cores appearing first, followed by the accessory LH2 complex.

Poster 7

NEAR-INFRARED TRANSIENT ABSORPTION ANALYSIS OF THE EXCITED STATE DYNAMICS OF SPIRILLOXANTHIN IN SOLUTION AND IN THE LH1 COMPLEX OF *RHODOSPIRILLUM RUBRUM*

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The spectroscopic properties of spirilloxanthin dissolved in *n*-hexane and bound to the corelight-harvesting (LH1) complex *of Rhodospirillum (Rs.) rubrum* were studied by near-IR ultrafast transient absorption spectroscopy. Global analysis of the kinetic traces measured after excitation of spirilloxanthin to the S_2 ($1\ B_u^+$) state enabled us to estimate the species-associated difference-spectra that correspond to the excited state absorption from S_2 and S_1 ($2A_g^-$). Analysis of the absorption originating from the S_2 state, has provided further insight into the nature of the spirilloxanthin excited states, while by analyzing the profile of the S_1-S_2transition, we place the energy of the S_1 state of *all-trans* spirilloxanthin at 11500 cm^{-1}, both in solution and in the LH1 complex. This low value virtually excludes excitation energy transfer from the S_1 state to bacteriochlorophyll, thus explaining the observed low energy transfer efficiency from spirillo-xanthin to bacteriochlorophyll in the LH1 complex of *Rs. rubrum*. Our results indicate that the S* state, recently found in spirilloxanthin, both in solution and in the LH1 complex, where it gives rise to ultrafast carotenoid triplet formation via singlet-fission [Gradinaru, C.C. *et al*. (2001) Proc. Natl. Acad. Sci. USA 98, 2364], does not show any detectable spectral features in the near-IR region. In addition, no evidence was found in the *Rs. rubrum* LH1 complex for the ultrafast formation of a radical cation of the type recently observed with spheroidene in the LH2 complex of *Rhodobacter sphaeroides* [Polívka *et al*. (2002) J. Phys. Chem. B 106, 11016].

Poster 8

IRON REGULATION OF MARINE PHYTOPLANKTON PHOTOSYNTHESIS: THEORY AND OBSERVATIONS

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Iron supply plays an important regulatory role in phytoplankton primary production in certain ocean regions and laboratory work has shown that iron regulates photosynthetic physiology by altering photosynthetic electron transport. However, iron-specific physiological parameters of photosynthesis have not been quantitatively related to observed phytoplankton photosynthetic responses under iron-replete and iron-limited conditions, in part because there is no analytical theory that explicitly links the parameters and response. Here we present such an analytical theory that employs iron-dependent parameters of photosynthetic performance to independently describe the photosynthesis vs. irradiance (P^b vs E) response in terms of light absorption and photosynthetic electron transport. This model reproduces with considerable fidelity the P^b vs E responses observed during two *in situ* iron addition experiments: one in the equatorial Pacific Ocean and one in the Southern Ocean. These results suggest that the carbon uptake responses of ambient phytoplankton communities to iron addition can be predicted if the mechanistic parameters of photosynthesis are quantified.

Poster 9

PHOTOSYNTHETIC EXCITONS IN PS I: THE DEPENDENCE OF SINGLET ENERGY TRANSFER RATES ON PIGMENT ORIENTATION AND SPECTRAL CHARACTERISTICS

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As the interaction energy between two or more chlorophylls approaches one-half the width of the chlorophyll absorption band, the individual pigments can no longer be described as distinct entities. In this limit of strong intermolecular interactions, electronic excitation of N strongly interacting pigments is described in terms of a system of N exciton states where the states are delocalized to varying degrees over the N pigment sites and depend on the properties of the individual pigments that make up the exciton. Using an approach first outlined by Sumi [1], we show how the presence of excitons can be easily incorporated into energy transfer calculations using a *mixed site-state* approach (where the sites are the weakly interacting pigments and the states describe the exciton properties). We examine the effects of excitons on the rate of energy transfer between an excitonic dimer (two strongly interacting chlorophylls) and a more weakly interacting monomeric chlorophyll by comparing the energy transfer rate in the strong coupling (exciton) limit to that calculated in the weak coupling (dipole-dipole or Förster) limit. Our analysis quantifies the dependence of the transfer rates in the strong and weak coupling limits on the orientation of the transition dipoles and spectral properties of the chlorophylls. This analysis is applied to key interactions in the PS I reaction center/core antenna complex.

[1] H. Sumi (1999), J. Phys. Chem. B 103, 252-260.

Poster 10

Forward Electron Transfer in Photosystem I from Stromal Subunit Deletion Mutants of *Synechococcus* PCC 7002.

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The electron transfer from phylloquinone (A_1) to the iron-sulfur cluster F_X in Photosystem I (PS I), is biphasic and the two phases are thought to represent either electron transfer in the two branches of cofactors or in two different local environments. We have investigated the A_1^- to F_X electron transfer kinetics in subunit deletion mutants of *Synechococcus* PCC7002 using time-resolved EPR. Deletion of PsaE and/or PsaF is found to have no significant effect on forward electron transfer from A_1 to F_X. However, when Triton X-100 is used to solubilize PS I from the mutants a marked change in the polarization patterns is observed consistent with a change in the ratio of the two kinetic phases of electron transfer to F_X. Because the PsaE and PsaF subunits are located on the stromal side of the membrane close to the phylloquinone in the PsaA branch (QK_A), but far from P700, it is likely that the differences in kinetics are due to changes in the environment of QK_A and not the partitioning of the electrons between the two branches. Previous photo-accumulation studies of the triton preparations of the deletion mutants showed that the phylloquinone is much more easily protonated suggesting that the detergent opens a water channel to the phylloquinone. Consistent with this interpretation the spin polarization patterns of $P_{700}^+A_1^-$ indicate that the lifetime of electron transfer from A_0 to A_1 may also be slowed in these samples. This implies that alteration of the environment of the quinone in the PsaA branch of cofactors can result in fast electron transfer to F_X and supports the notion that the two kinetic phases may be associated with different protein conformations.

Poster 11

QUINONE REDUCTION VIA SECONDARY B-BRANCH ELECTRON TRANSFER IN MUTANT BACTERIAL REACTION CENTERS

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Symmetry-related branches of electron transfer cofactors B initiating with a primary electron donor (P) and terminating in quinone acceptors (Q) B are common features of photosynthetic reaction centers (RC). Experimental observations show activity of only one of them B the A branch B in wild-type bacterial RCs. In a mutant RC, we now demonstrate that electron transfer can occur along the entire, normally inactive B-branch pathway to reduce the terminal acceptor Q_B on the time scale of nanoseconds. The transmembrane charge-separated state $P^+Q_B^B$ is created in this manner in a *Rhodobacter capsulatus* RC containing the F(L181)Y-Y(M208)F-L(M212)H-W(M250)V mutations (YFHV). The W(M250)V mutation quantitatively blocks binding of Q_A, thereby eliminating Q_B reduction via the normal A-branch pathway. Full occupancy of the Q_B site by the native UQ_{10} is ensured (without the necessity of reconstitution by exogenous quinone) by purification of RCs with the mild detergent, Deriphat 160-C. The time constants for the formation and decay of states $P^+H_B^B$ and $P^+Q_B^B$ in the YFHV mutant RC (as purified or upon addition of competitive inhibitors of Q_B binding) are compared with the values for similar states $P^+H_A^B$, $P^+Q_A^B$, and $P^+Q_B^B$ formed in the wild-type RC by A-side activity alone. We find that $P^+Q_B^B$ states formed via the two branches are distinct, and that $P^+Q_B^B$ formed by the B side does not decay via the normal (indirect) pathway that utilizes the A-side cofactors when present. These differences may report on structural and energetic factors that further distinguish the functional asymmetry of the two cofactor branches.

Poster 12

Chemical Rescue of Site Modified Ligands to the [4Fe-4S] cluster F_B with N-methyl-N'-(3-thiopropane)-4,4'-bipyridinium

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PsaC is a 9.3 kDa subunit of Photosystem I which contains two [4Fe-4S] clusters F_A and F_B. It has been shown previously that both [4Fe-4S] clusters can be quantitatively inserted into recombinant protein by incubation with 2-mercaptoethanol, iron, and sulfide under anaerobic conditions. In PsaC each [4Fe-4S] cluster is ligated by 4 cysteine residues arranged into two consensus iron sulfur binding sites CxxCxxCxxxCP, where C is cysteine, P is proline and x are any other amino acids. Mutating cysteine 14, a second cysteine that ligates the cluster F_B, to a glycine results in the formation of a [4Fe-4S] with altered EPR properties in the F_B site of PsaC. Previous work from our laboratory has shown that the role of cysteine 14 is taken up by an external thiolate (the 'rescue' ligand) that is used during the [4Fe-4S] cluster reconstitution procedure. Taking advantage of this, we propose to attach a viologen molecule in place of the second cysteine ligand to F_B. If successful, we should be able to measure the rate of electron transfer to the tethered viologen molecule using time-resolved optical spectroscopy. We have synthesized N-methyl-N'-(3-thiopropane)-4,4'-bipyridinium dichloride and attached it as a rescue ligand to C14G-PsaC. The EPR spectrum of chemically-reduced C14G-PsaC shows signals from F_A (low spin), F_B (high spin) and the viologen radical. A change in the microwave power saturation behavior of the EPR signal of the viologen shows that it is bound to F_B. The next step is to rebind C14G-PsaC with bound viologen to P700-F_X cores. These studies will focus defining the limits of distance (R) and free energy (ΔG) that can be tolerated while maintaining a high (>90%) quantum yield of electron transfer from F_B to the tethered viologen acceptor.

Poster 13

LIGHT-INDUCED CHANGES IN THE COMPOSITION OF CHLOROPHYLL-PROTEIN COMPLEXES AND PHOTOCHEMIACAL ACTIVITY IN PHOTOSYSTEM I SUBMEMBRANE PARTICLES

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The effects of irradiation of photosystem I (PSI) submembrane particles using intense white light (2000 µE m^{-2} s^{-1}) at chilling temperatures (4°C) were studied. PSI-dependent oxygen uptake activity was stable during the first 3 h of photoinhibitory illumination in the presence of superoxide dismutase (SOD). Without added SOD, oxygen uptake almost doubled during this period presumably due to denaturation of native membrane bound SOD or its release from the PSI membranes. The total chlorophyll (Chl) content and the magnitude of light-induced absorbance changes at 830 nm (ΔA_{830}) were also barely affected during the first 3-3.5 h of photoinhibitory treatment. However, further exposure to strong light markedly accelerated Chl breakdown followed by the decline of oxygen uptake rates and ΔA_{830}. This corresponded with the disappearance of the bands attributed to PsaA/B polypeptides on electrophoretic gels. Despite the unchanged maximum magnitude of ΔA_{830} during the first 3-3.5 h of photoinhibitory treatment, the light-response curves of P700 oxidation were gradually altered demonstrating a several fold increase in the ability of weak actinic light to oxidize P700. The major Chl *a*-protein (CP1) band gradually disappeared during the first 4 h of light exposure with a corresponding increase in the Chl content of a band with lower electrophoretic mobility ascribed to the formation of oligomers containing CP1, light-harvesting complex I (LHCI)-680, and LHCI-730. This aggregation of Chl-protein complexes likely due to photoinhibitory-induced cross-linking favouring light-harvesting, is proposed to explain the enhanced capacity of weak light to oxidize P700 in photoinhibited PSI submembrane fractions compared to untreated ones. However, co-solutes (glycinebetaine and sucrose) could minimize the alteration of photochemical activity and chlorophyll-protein complexes against photodamage of PSI submembranes particles.

Poster 14

THE STRUCTURE STUDY OF LIGHT HARVESTING COMPLEX I BY SOLID STATE NMR

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Solid state NMR is a powerful tool in studying structure and mechanism of membrane proteins, which are not soluble in water and difficult to crystallize. A complete assignment of protein sequence is a prerequisite for its structure and dynamics study. In our study, the multidimensional solid state NMR methods were applied on a u-^{13}C, ^{15}N labeled intact Light Harvesting complex I (LH1) of Rodobacter sphaeroides. A set of two dimensional and three dimensional homo- and hetero- nuclei correlation spectra were acquired on a Chemagnetics Infinity 600MHz spectrometer. Partial assignment of the amino acid sequence of LH1 α and β subunit was achieved by analyzing the inter- and intra- residue spectra. Our goal is to fully assign the amino acid sequence and to extract distance restraints from dipolar coupling.

Poster 15

THE STUDY OF E. COLI ATP SYNTHASE *C* SUBUNIT BY SOLID STATE NMR SPECTOSCROPY

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Solid state NMR is a very promising method for studying membrane proteins as microcrystals, precipitates, embedded in the raw membrane, or in bicelle-protein suspensions. Recent advances facilitated the assignment of solid protein samples. In this work, we attempt to characterize the structure dynamics and mechanism of the *c* subunit of E. coli ATP synthase. The *c* subunit is part of the membrane bound F_0 domain which functions mainly as a proton channel and/or pump in proton translocation. Numerous studies revealed that the underlying mechanism of this molecule is the rotation of its specific subunits; however, the participation of the c subunit in this motion is not clarified. Solution NMR data by Girvin et al. of solubilized and dissociated *c* subunits provide evidence for the conformational change following deprotonation of the Asp61 residue. Our goal is to explore this hypothesis using solid state NMR data on the intact F_0. ^{13}C and ^{15}N enriched ATP synthase was over-expressed and purified from E. coli, and homo- and heteronuclear spectra were acquired for assignment purposes. Partial or complete assignment of the *c* subunit will allow to btain further structural information regarding the torsional angles, tertiary contact distances, hydrogen bonds, and proton transfer

Poster 16

THE DITHIOL:DISULFIDE OXIDOREDUCTASES DsbA AND DsbB OF *RHODOBACTER CAPSULATUS* ARE NOT DIRECTLY INVOLVED IN CYT *C* BIOGENESIS BUT THEIR INACTIVATION RESTORES THE CYT *C* BIOGENESIS DEFECT OF CcdA-NULL MUTANTS

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The cytoplasmic membrane protein CcdA and its homologues in other species like the DsbD of *Escherichia coli* are thought to supply the reducing equivalents required for the biogenesis of *c*-type cytochromes that occurs in the periplasm of Gram-negative bacteria. CcdA-null mutants of the facultative phototroph *Rhodobacter capsulatus* are unable to grow under photosynthetic (Ps^-) conditions, and do not produce any active cytochrome (cyt) *c* oxidase ($Nadi^-$) due to a pleiotropic cyt *c* deficiency. However, under Ps or respiratory (Res) growth conditions these mutants revert frequently to yield $Ps^+/Nadi^+$ colonies that produce *c*-type cyts despite the absence of CcdA. Characterization of the revertants revealed that reversion into Ps^+ growth phenotype was mediated by a frame-shift mutation in ORF3149 encoding the dithiol:disulfide oxidoreductase DsbA. Indeed, a *dsbA ccdA* double mutant was shown to be $Ps^+/Nadi^+$, establishing that in *R. capsulatus* inactivation of *dsbA* suppresses the *c*-type cyts deficiency due the absence of *ccdA*. Next, the ability of the wild type *dsbA* allele to suppress the Ps^+ growth phenotype of the *dsbA ccdA* double mutant was exploited to isolate *dsbA*-independent revertants of *ccdA*. Sequence analysis revealed that the latter revertants carried mutations in *dsbB.* As with *dsbA*, a *dsbB ccdA* double mutant was also $Ps^+/Nadi^+$ and produced *c*-type cyts. Therefore, the absence of either DsbA or DsbB restores *c*-type cyts biogenesis in the absence of CcdA. Finally, it was also found that the DsbA-null or DsbB-null single mutants of *R. capsulatus* are Ps^+ and produce *c*-type cyts, but are impaired for Res growth. This finding demonstrates that in *R. capsulatus,* unlike in *E. coli,* the dithiol:disulfide oxidoreductases DsbA and DsbB are not essential for cyt *c* biogenesis even though they are important for respiratory growth.

Poster 17

THE *PsbS* GENE REGULATES DUAL ASPECTS OF PSII FUNCTION IN LEAVES

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The reversible radical pair model of PSII permits description of light utilization in terms of rate constants for H^+-dependent increases in thermal deactivation of singlet chlorophyll in the antenna system (k_N) and photochemistry in open (i.e. Q_A non-reduced) reaction centers (k_{P0}). We applied simultaneous measurements of steady state fluorescence and gas exchange (O_2 evolution or CO_2 uptake) to leaves from nine lines of *Arabidopsis thaliana* that exhibited either enhanced expression of the normal *psbS* gene or possessed distinct single-site mutations in this gene. The single-copy *psbS* gene encodes the 22-kilodalton pigment-binding S protein of the PSII antenna system and is essential for expression of nonphotochemical quenching (i.e. k_N) in higher plants. Reversible changes in the *in vivo* rate constants were measured during brief cycles of exposure to low (LL, 100 μmol $m^{-2}s^{-1}$) and high quantum flux rates (HL, 1000 μmol $m^{-2}s^{-1}$) under an atmosphere of 360 μl CO_2 l^{-1} and 1% O_2 at 23° C. Across all lines (including wild type) a significant (up to 40%) increase in k_{P0} in HL compared to LL was observed that was strongly and positively associated with the capacity to increase k_N in HL. Discrepancies between estimates of whole chain electron transport based on CO_2 exchange *versus* fluorescence in HL in contrasting *PsbS* genotypes were correlated with changes in k_{P0}. Changes in k_{P0} could explain prior observations of a slightly concave dependence of the quantum yield of whole chain electron transport *versus* the quantum yield of PSII photochemistry based on fluorescence *in vivo*. This concavity had been attributed solely to allocation of electrons to alternate acceptors not detectable by conventional gas exchange methods. Changes in k_{P0} may instead reflect energy-dependent alterations in patterns of quantum equilibration among PSII antenna chromophores.

Poster 18

A SOLUBLE FORM OF CYTOCHROME C_y AS AN ELECTRON CARRIER IN *Rhodobacter capsulatus.*

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The facultative photosynthetic bacterium *Rhodobacter capsulatus* has two *c*-type cytochromes (cyt), the soluble cyt c_2 and membrane-attached cyt c_y, that act as electron carriers during photosynthetic (Ps) and respiratory (Res) growth of this species. In this study, a soluble form of cyt c_y (cyt S-c_y) was constructed by fusing genetically the signal sequence of cyt c_2 to the cyt *c* domain of cyt c_y. The chimeric cyt S-c_y thus obtained was unable to support Ps growth of a *R. capsulatus* strain that lacks both the cyt c_2 and cyt c_y, presumably due to its low amount. However, this mutant yielded frequently two distinct classes of Ps^+ revertants. The first group of revertants included cyt S-c_yR3 and cyt S-c_yR5 that carried an additional mutation (H53Y and K39R, respectively) in the cyt *c* domain of cyt c_y. The cellular levels of mutant forms of cyt c_y in these revertants were slightly higher than the parent strain, in agreement with their improved Ps growth. On the other hand, the second group of revertants like FJ2-R4 contained a chromosomal mutation that is unrelated to cyt S-c_y. This mutation further increased cellular levels of cyt S-c_y and its derivatives, and conferred more vigorous Ps growth. Reduction-oxidation titration of membrane supernatants revealed that the redox midpoint potential (E_m) of cyt S-c_yR5 was around +362 mV, similar to that of the membrane-bound native cyt c_y (+365 mV). However, light-activated time-resolved spectroscopic measurements indicated that the electron transfer kinetic from the cyt bc_1 complex to the RC mediated by cyt S-c_yR5 was different than that seen with the membrane-bound cyt c_y, as it exhibited both a fast and a slow phase. The implications of these findings in terms of the structure and function of soluble and membrane-bound cyts and their role in Ps and Res growth conditions will be discussed.

Poster 19

THE REVERSIBILITY OF DIFFERENT OXIDATION STATES IN THE MANGANESE II,II DIMER Mn_2bpmp

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To model structural and functional parts of the water oxidizing complex in Photosystem II, a dimeric Mn_2bpmp (bpmp=2,6-bis[[N,N-di(2-pyridylmethyl)amino]methyl]-4-methylphenol) complex was synthesised. Flash photolysis with $Ru^{II}(bpy)_3$, in the prescence of an electron accpetor, results in the stepwise extraction of three electrons by the Ru^{III} $(bpy)_3$ from the $Mn_2^{II,II}$-dimer, which then reaches the $Mn_2^{III,IV}$ oxidation state.The reversibility of oxidation in the $Mn_2^{II,II}$-dimer Mn_2bpmp and the possibility of making a catalytic cycle between its different oxidation states was investigated with electron paramagnetic resonance spectroscopy (EPR). The Mn_2bpmp was oxidised with the help of the photosensitizer $Ru^{II}(bpy)_3$, the electron acceptor penta-amminechlorocobalt(III)chloride (Co^{III}) and reduced by the exogenous reductant *p*-Benzohydroquinone (HQ). Phosphate buffer inhibits photoinduced oxidation of the Mn_2 dimer by replacement of the acetate bridges by the strong phosphate ligand. When using MES photoinduced oxidation is achieved. The $Mn^{II,II}$ state was detected in the dark using EPR. Flash photolysis results in generation of the $Mn^{III,IV}$ state, and at the same time the reduced electron accpetor, Co^{II}, is observed. After exposure to many flashes the uncoupled Mn_2^{IV} state is detected. Addition of HQ achieves the semiquinone radical signal and $Mn^{II,II}$ is recovered. Further flash photolysis increases the yield of the $Mn^{II,II}$ state. A high amount of $Mn^{II,II}$ state is recovered after HQ addition in excess. Flash photolysis using Duroquinone instead of Co^{III} showed that it is a good electron acceptor. Our results indicate that the oxidation of the Mn_2bpmp is reversible and a catalytic cycle between the different oxidation states is possible.

Poster 20

SYNTHETIC MANGANESE COMPLEXES RELEVANT TO PHOTOSYSTEM II. A REDOX-TRIGGERED SHAPE-SHIFTING TETRANUCLEAR CLUSTER

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A tetranuclear manganese complex resides at the active site of the Photosystem II (PSII) water oxidase. While a crystal structure of PSII has been reported, the structure of the manganese cluster is poorly defined. Synthetic compounds of relevance to the enzyme active site complex are being sought. With model complexes, we wish to test various hypotheses regarding the structure and function of the S_n states. In this presentation, we examine the interesting behavior of $[(Mn_2O_2)_2(tphpn)_2]^{4+}$ upon redox changes. In the solid state complex, the Mn atoms are arranged 4+ rectangle, while in the 5+ complex there is a dramatic rearrangement to a tetrahedral shape. Reduction of $[(Mn_2O_2)_2(tphpn)_2]^{4+}$ by one electron provides a species whose EPR spectrum closely resembles that of the PSII S_0 state (see Figure above). Spectral and magnetic properties of the reduced form (3+) strongly suggest a structural transformation has occurred, as was the case for the oxidized form.

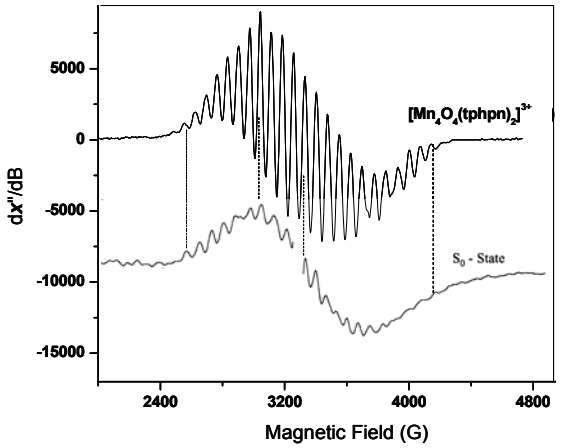

Figure. EPR spectra of $[(Mn_2O_2)_2(tphpn)_2]^{3+}$ (top) and Photosystem II S_0 state (bottom).

Poster 21

TWO REDOX-ACTIVE CAROTENOID MOLECULES IN PHOTOSYSTEM II

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Photosystem II (PS II) is a membrane-bound protein complex that is responsible for catalyzing the oxidation of H_2O to O_2 in oxygenic photosynthesis. Photosystem II (PS II) contains secondary electron transfer paths involving cytochrome b_{559} (Cyt b_{559}), chlorophyll (Chl) and □-carotene (Car) that are active under conditions when oxygen evolution is blocked such as in inhibited samples or at low temperature. These pathways allow for cyclic electron transfer in the reaction center, which dissipates excess energy and prevents photodamage. There are two β-carotene molecules bound to the D1 and D2 polypeptides of PS II. Intermediates of the secondary electron transfer pathways of Photosystem II from *Synechocystis*, *Synechococcus* and spinach have been investigated using low temperature near-IR spectroscopy and electron paramagnetic resonance (EPR) spectroscopy. Low temperature illumination generates Chl and Car radical cations. We present evidence for two spectroscopically distinct redox-active carotenoids in the *Synechocystis* photosystem II reaction center. The Car^+ near-IR absorption peak varies in wavelength and width as a function of illumination temperature. Also, the rate of decay of the Car^+ peak varies as a function of wavelength. Factor analysis indicates there are two spectral forms of Car^+ (Car_A^+ has an absorbance maximum of 982 nm and Car_B^+ has an absorbance max of 1010 nm) that decay at different rates. In *Synechocystis* PS II we observe a shift of the Car^+ peak to shorter wavelength when oxidized Tyrosine D ($Y_D^\bullet$) is present in the sample that is explained by an electrostatic interaction between $Y_D^\bullet$ and a nearby □-carotene that disfavors oxidation of Car_B. These results are consistent with two secondary electron transfer pathways in the reaction center.

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Poster 22

PULSED HIGH-FREQUENCY EPR STUDY ON THE LOCATION OF CAROTENOID AND CHLOROPHYLL CATION RADICALS IN PHOTOSYSTEM II

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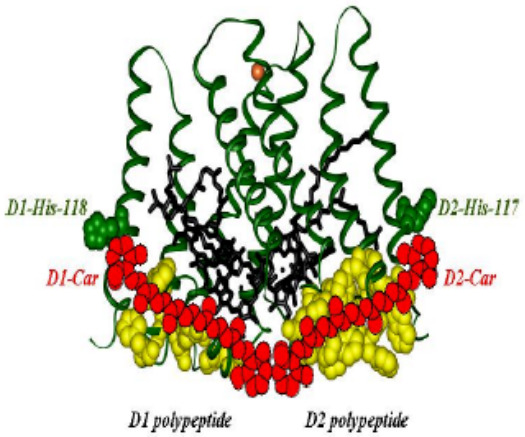

When the primary electron-donation pathway from the water-oxidation complex in photosystem II (PS II) is inhibited, chlorophyll (Chl_Z and Chl_D), β-carotene (Car) and cytochrome b_{559} are alternate electron donors in a photoprotection mechanism. Previous studies have demonstrated that high-frequency EPR spectroscopy (at 130 GHz), together with deuteration of PS II, yields resolved Car^+ and Chl^+ EPR signals (Lakshmi et al. (2000) *J. Phys. Chem. B* 104, 10445). The present study describes the use of pulsed high-frequency EPR spectroscopy to measure the location of the carotenoid and chlorophyll radicals relative to other paramagnetic cofactors in PS II. Based on the distance estimates obtained in this study and by analogy to carotenoidbinding sites in other pigment-protein complexes, possible binding sites are discussed for the Car cofactors of PS II. This work is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under contract W-31-109-Eng-38 (ANL) and DE-F-G02-01ER15281 (Yale).

Poster 23

Cooperative Assembly of the Inorganic Cofactors during Photo-reconstitution of the Water Oxidizing Complex in PSII: O_2 Reconstitution Kinetics and Dual-mode EPR Study

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Intermediates formed during assembly of the inorganic core of the water splitting enzyme $[Mn_4O_xCa_1Cl_y]_{WOC}$ from the free cofactors have been characterized structurally by dual-mode EPR spectroscopy (Mn^{2+} & Mn^{3+}) and by the kinetics of restoration of O_2 production (photoacti-vation) over broad concentration ranges: Mn^{2+} (10-250 □M), Ca^{2+} (8-100 mM) and bicarbonate (0-4 mM). Maintaining an optimal ratio of Mn^{2+}:Ca^{2+} = 1:400 is necessary to achieve high photoactivation in these ranges. Addition of bicarbonate improves the O_2 yield (by 50%) and accelerates the rate of photooxidatioin of the intial Mn^{2+} (4 fold). These effects are due to formation of a ternary complex $[Mn^{2+}(HCO_3)]$ within PSII ([ ] = apo-WOC). This highly active precursor is easier to oxidize than $[Mn^{2+}(OH_2)]$, and forms a more stable photooxidized intermediate, $[Mn^{3+}(HCO_3)]$. Dual-mode EPR of both Mn^{2+} and Mn^{3+} identifies three light-induced Mn^{3+} intermediates by their distinct g_{eff}, ^{55}Mn hyperfine and ZFS coupling. These are attributed to $[Mn^{3+}(OH)]$, $[Mn^{3+}(HCO_3)]$ and $[Mn^{3+}(OH)Ca^{2+}]$. A fourth intermediate, $[Mn^{3+}(OH_2)]$, is EPR-inactive. Ligand field theory is applied to identify the coordination symmetry and electronic state configuration ($^5B_{1g}$). The coordination environment of $[Mn^{3+}(OH)]$ shows a pronounced pH-dependence which is distinct from the effect of bicarbonate binding. This pH-dependence is eliminated upon binding of Ca^{2+}, thus revealing that Ca^{2+} binds nearby and locks the coordination state of Mn^{3+} into a single form.

Poster 24

Chloride requirement for oxygen evolution in chloride depleted PSII preparations

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Chloride (Cl^-) is a cofactor in the oxygen evolving complex (OEC) of photosystem II (PSII), but study of its dependence is hampered by the difficulty in removing Cl^- from PSII. Steady state enzyme kinetics has been used to measure the rate of oxygen evolution in PSII samples lacking only the 17 kDa or both 17 and 23 kDa extrinsic proteins in order to determine factors affecting Cl^- dependence of restoration of oxygen evolution activity. Removal of the 17 kDa protein was achieved by washing PSII BBY-type samples in medium containing 25 mM MES/NaOH and 300 mM sucrose, pH 6.0, (Cl^--free) several times. After the washing procedure PSII retained 30-40% of the initial oxygen evolution activity which was restored up to 90% by re-addition of Cl^- ions only. Removal of the 17 and 23 kDa proteins was achieved by mild alkaline treatment at pH 7.5 in the presence of Na_2SO_4 at 4°C, a treatment that has been used for Cl^- depletion by other groups. We found that the method removed chloride as well as the extrinsic polypeptides with $t_{1/2}$=45-55 s. Our results show that the Cl^- requirement for PSII lacking the 17 kDa protein was modulated by medium pH, with Michaelis constants estimated as: $K_M(Cl^-) \leq 30$ μM at pH 5.6; $K_M(Cl^-) = 50\text{-}70$ μM at pH 6.0; $K_M(Cl^-) = 350\text{-}400$ μM at pH 7.2. Removal of the 23 kDa protein along with the 17 kDa protein led to a decrease in affinity for Cl^-. Restoration of oxygen evolution activity was possible in the presence of Ca^{2+} ions. Addition of Ca^{2+} to PSII lacking the 17 and 23 kDa extrinsic proteins increased Cl^- requirement, with $K_M(Cl^-) = 0.8 - 1$ mM vs. 70 μM as measured for PSII that retained the 23 kDa extrinsic protein at pH 6.0. In the absence of the 17 kDa extrinsic protein, we found an increase in the g=4.1 S_2 state signal intensity in PSII samples lacking Cl^-, compared to samples to which Cl^- was re-added. Fluoride ions inhibited oxygen evolution activity by competing with Cl^-. At pH 6, preliminary analysis of the inhibitory effects of fluoride revealed two inhibition constants of $K_i \approx 800$ μM for competitive and $K_i' \approx 17$ mM for uncompetitive inhibition.

Poster 25

PHOTOSYSTEM II HETEROGENEITY: PICOSECOND CHLOROPHYLL FLUORESCENCE DECAY KINETICS REVEAL THAT PSII∃ CENTERS MAINTAIN SHORTER FLUORESCENT LIFETIMES THAN PSII∀ CENTERS. A PHOTOPROTECTATIVE MECHANISM OF DAMAGED PSII MAY OCCUR DURING ITS REPAIR.

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Thylakoid membrane fractions were prepared from specific regions of thylakoid membranes of spinach (*Spinacia oleracea*). These fractions, which include grana (B3), stroma (T3), grana core (BS), margins (Ma) and purified stroma (Y100) were prepared using a non-detergent method including a mild sonication and aqueous two-phase partitioning. The significance of PSII∀ and PSII∃ centres have been described extensively in the literature. Previous work has characterized two types of PSII centres which are proposed to exist in different regions of the thylakoid membrane. ∀-centres are suggested to aggregate in stacked regions of grana whereas ∃-centres are located in unstacked regions of stroma lamellae.

We have measured low temperature absorption spectra, absorbance cross-sections, fluorescence induction kinetics, and low temperature fluorescence emission spectra that characterize the thylakoid membrane subfractions. The results from those experiments clearly illustrate the widely held notions of PSII/PSI and PSII∀/PSII∃ spatial separation. Most notably this study suggests that chlorophyll fluorescence decay lifetimes of PSII∃ centres are shorter than those of PSII∀ centres at F_0 and F_M. This is contradictory to early work which predicted shorter fluorescent lifetimes for PSII∀ centers from whole thylakoids. We believe that the short lifetimes of PSII∃ may reflect a photoprotective mechanism, aiding PSII∃ in the stroma as it is repaired following photodamage.

Poster 26

Loss of functional, PSII reaction centers associated with the incorporation of a chlorophyll into the inactive branch pheophytin-binding site

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All photosynthetic reaction centers have two parallel and symmetry-related electron transfers across the membrane. In photosystem II (PS II) only one of the pathways (active branch) is used for electron transfer. Since both pathways have nearly identical cofactor distance and orientation relationships as well as midpoint potentials, the issue is why the electron is transferred up one and not both pathways. To address this issue we have used site directed mutagenesis to provide potential Mg ligands (histidine) to replace the pheophytin (Pheo) cofactors with chlorophylls (Chl). We mutated residue D1-L210 to histidine to provide a Mg ligand for the inactive branch Pheo ($Pheo_{inactive}$). Analyses of the pigment composition of PS II reaction centers (PS II RCs) indicated that the histidine substitution resulted in the incorporation of a Chl in place of Pheo, as predicted. This was further supported by the absorption spectra of wild type (WT) and D1-L210H RCs where the D1-L210H mutant had a ~40-50% reduction in the Pheo Qx absorption compared to WT. In addition, the Pheo Qx band was red shifted to 543.5-544 nm, which demonstrated that the remaining Pheo was $Pheo_{active}$. Comparison of the Chl CD spectra of WT and D1-L210H RCs recorded at 4°C revealed that a positive band peaking at 682 nm and negative lobe centered at 669 nm in WT were reversed in the mutant. This observation demonstrated the excitonic interaction of pigments was changed in the mutant, particularly energy coupling to P680. The result from Chl a fluorescence emission spectrum at 77 K showed that D1-L210H RCs displayed a fluorescence emission maximum at 682 nm that was identical to that of WT, but the narrower shape of spectrum in the mutant reflected marked changes in energy transfer in these genetically modified RCs. On the other hand, the transient absorption measurements suggested that the lifetime component for charge separation was slightly slower in this mutant than in WT (5.2 ps versus 3.6 ps). Unexpectedly, the D2L210H mutants were unable to evolve oxygen, lacked variable Chl fluorescence and could not reduce Q_A. These results are discussed in terms of a photochemical model for charge separation in PS II.

Poster 27

The Effect of Drought on the Tropical Rain Forest of Biosphere 2 Center: Remote Sensing by using a New Developed Laser-based Fluorometer

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Estimating ecosystem photosynthesis is a fundamental and practical research problem in carbon cycling. We developed the Fast Repetition Rate (FRR) fluorometer for remote sensing of Chl at a distance (5 – 50 m) by using a modulated laser source with the optical power >1 W, and the wavelength of 660 nm. A high sensitive avalanche photodiode module with a bandwidth 20 MHz is used as a fluorescence detector. It permitted us to measure fluorescence parameters of plants at harsh environmental conditions even at full sun light. The instrument is equipped by a precise positioning system coupled with CCD camera that allows us for any targets in the working tropical rainforest (TRF) mesocosm to be selected. The instrument was installed inside TRF at Biosphere 2 Center (Arizona) in September 2003. Remote network connection allows us access and control the instrument via the Internet. Drought stress on plants in TRF mesocosm has two time scales. First, during of a day drought stress was negligible in the morning but increased to the end of the day (30% changes). Second, during several weeks, longer-term trend occurred, including quantum efficiency F_v/F_m - going down; t_1 - delayed; σ_{PSII} - going up; estimated electron transfer rate (ETR) - going down. The time-constant for Q_A^- reoxidation (t_1) is the most reliable parameter that reflects drought stress. Our results show that during drought experiment there was a strong correlation between Net Ecosystem CO_2 Exchange (NEE) and estimated ETR. We suppose that water is an activator of electron transfer between Q_A and Q_B.

Poster 28

ON THE AXIAL HISTIDINE LIGANDS OF P700

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Light-induced Fourier-transform infrared (FTIR) difference spectroscopy has been used to study the photo-oxidation of P700 in photosystem I (PS I) particles from *Chlamydomonas reinhardtii*. Recently it was suggested that a difference band observed near 1656(+)/1637(-) cm^{-1} in ($P700^+$-P700) FTIR difference spectra is partly due to a vibrational mode of HisB656, that provides an axial ligand to the chlorophyll-a of P700 (Hastings, G, Ramesh, V., Wang, R., Sivakumar, V. and Webber, A. (2001) *Biochemistry* 40, 12943-12949). To test this idea we have obtained ($P700^+$-P700) FTIR difference spectra using PS I particles from a site-directed mutant of *C. reinhardtii* in which HisB656, is changed to asparagine. The ($P700^+$-P700) FTIR difference spectrum for the HN(B656) mutant lacks a 1659(+)/1637(-) cm^{-1} difference band, that is present in the wild type spectrum. The most obvious interpretation of this observation is that the difference band is associated with a vibrational mode of HisB656, that is not present in the mutant spectrum. The ($P700^+$-P700) FTIR difference spectrum for the HN(B656) mutant also displays a difference band at 1636(-)/1653(+) cm^{-1} that we assign to a vibrational mode of HisA676, which provides an axial ligand to the chlorophyll-*a'* of P700. The HN(B656) mutant spectrum also displays a difference band at 1679(-)/~1690(+) that we assign to an asparagine carbonyl mode. Other than the changes described above the spectra for wild type and the HN(B656) mutant are very similar, indicating that asparagine is a good substitute for HisB656, and can ligate the chlorophyll-a of P700, possibly mediated via a water molecule.

Poster 29

Photo-Oxidation of P740, the Primary Electron Donor in Photosystem I From *Acarychloris marina.*

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FTIR difference spectroscopy in combination with deuterium exchange experiments have been used to study the photo-oxidation of P740, the primary electron donor in photosystem I from *Acarychloris marina*. Comparison of ($P740^+$-P740) and ($P700^+$-P700) FTIR difference spectra show that P700 and P740 share many structural similarities. However, there are several distinct differences also: 1/ Only the ($P740^+$-P740) spectrum is altered upon proton exchange. The P740 binding pocket is therefore more accessible than the P700 binding pocket. 2/ Broad, "dimer" absorption bands are observed for both $P700^+$ and $P740^+$. These bands differ significantly in sub-structure, however, suggesting differences in the electronic organization of $P700^+$ and $P740^+$. 3/ Bands are observed at 2727 and 2715(+) cm^{-1} in ($P740^+$-P740) spectra, but are absent in ($P700^+$-P700) spectra. These bands are due to formyl C-H modes of chlorophyll-*d*. Therefore, P740 consists of two chlorophyll-*d* molecules. Deuterium induced modification of ($P740^+$-P740) FTIR spectra indicate that only the highest frequency 13^3 ester carbonyl mode of P740 down-shifts indicating that this ester mode is weakly H-bonded. In contrast, the highest frequency ester carbonyl mode of P700 is free from H-bonding. Deuterium induced changes in ($P740^+$-P740) FTIR spectra could also indicate that one of the Chl-*d* 3^1 carbonyls of P740 is hydrogen bonded.

Poster 30

Reconstitution of Q_B function in bacterial RCs with non-native quinones

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While many quinones will replace ubiquinone at the Q_A site in *Rb. Sphaeroides*, few quinones have been found to function at Q_B. The determinants of Q_B binding and function are therefore unknown. A project has been started to increase the driving force from Q_A to Q_B by synthesizing a low potential napthoquinone to act as Q_A. The synthesis of 2-dimethyl,3-methyl-napthoquinone will be described. In addition, preliminary studies were made to check the activity of various quinones at the Q_B site when ubiquinone-10 is Q_A. The appearance of a slow phase of the charge recombination reaction is taken to indicate the presence of Q_B. Benzo- and napthoquinones, methyls, hydroxyl, chloros, tails, aminos, and methylaminos were tested. As expected most of the added, non-native quinones show little slow phase of the back reaction attributed to Q_B. There is some Q_B activity with the methyl substituted benzoquinone, duroquinone as well as with vitamin K_1 with K_ds comparable to that found for UQ_0. At high concentration many of the quinones begin to inactivate the RCs. Funds from USDA.

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2	1985	Brudvig	Blankenship Youvan	Amherst Cold Spring Harbor
3	1986	Frank	Mauzerall McCarty Prince	Rockefeller Cornell Exxon
4	1987	Ownes	Beale Bryant Carpentier	Brown Penn State Trois-Rivieres
5	1988	Bruce	Gantt Holzwarth Marrs	U. Maryland Muelheim DuPont
6	1989	Redlinger	Gest Horton Ort	Indiana U. Rob. Hill Inst., Sheffield U.I.U.C
7	1990	Diner	Brudvig Daldal Warncke	Yale U. Penn U. Penn
8	1991	Niederman	Biggins Knox Lam	Brown Rochester Rutgers
9	1992	Peterson	Berry, Joe Drake Gibbs, Martin	Carnegie Inst. Wash. Smithsonian, Edgewater, MD Brandeis
10	1993	Biggins	Blankenship Hind Lorimer	Arizona Brookhaven Natl. Lab DuPont

ERPC		Chair	Speaker	
11	1994	DePaula	Niederman Therien	Rutgers U. Penn
12	1995	Gunner	Armstrong Berry, James O. Owens	Boston College SUNY-Buffalo Cornell
13	1996	McDermott	DePaula Greenbaum Lecomte/Falzone	Haverford 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 Univ.-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 (Special)	Imperial College London Arizona State U. Yale Dry Creek Ridge Winery, Sonoma CA