
The 26th Western Photosynthesis Conference
Marconi Conference Center
Marshall, California, USA
January 5-8, 2017
www.westphotosynthesis.org


# 26TH WESTERN PHOTOSYNTHESIS CONFERENCE

## 5-8TH JANUARY 2017

## MARCONI CONFERENCE CENTER

## MARSHALL, CALIFORNIA, USA

**Event Locations (see map at the end of this booklet):**
Unless otherwise specified, all Talks will be held in Buck Hall. Coffee Breaks will be held at the entrance and patio of Buck Hall. The Poster presentations and Social Hours will be held in McCargo Hall and Porch. All meals will be held in the Redwood Building at the top of the Center.

## CONFERENCE ORGANIZERS:

**CHAIR:**

PROFESSOR BARRY D. BRUCE
DEPARTMENT OF BIOCHEMISTRY, CELLULAR, AND MOLECULAR BIOLOGY
UNIVERSITY OF TENNESSEE AT KNOXVILLE
KNOXVILLE, TENNESSEE USA
BBRUCE@UTK.EDU

**OUTGOING CO-CHAIR:**

PROFESSOR MARINUS PILON
DEPARTMENT OF BIOLOGY
COLORADO STATE UNIVERSITY
FT. COLLINS, COLORADO, USA
PILON@LAMAR.COLOSTATE.EDU

**INCOMING CO-CHAIR:**

PROFESSOR MATT POSEWITZ
DEPT. OF CHEMISTRY/GEOCHEMISTRY
COLORADO SCHOOL OF MINES
GOLDEN, COLORADO, USA
MPOSEWIT@MINES.EDU

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LOGO AND PHOTOS BY BARRY D. BRUCE

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# THE 26th WESTERN PHOTOSYNTHESIS CONFERENCE
## MARCONI CONFERENCE CENTER, MARSHALL, CA
## JANUARY 5th - 8th, 2017

The 26th Western Photosynthesis Conference
WPC/2017

***All talks will be held in Buck Hall and poster sessions are in the McCargo Hall and water will be available throughout the meeting. Coffee breaks will be held in the entrance to Buck Hall. The Young Investigators are denoted in the Program by an *graduate students and **post-docs.***

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## Thursday Jan 5, 2017

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| | | |
|---|---|---|
| **2:00-5:00 pm** | **Arrival and room check-in** | **Main Office** |
| **6:00-7:00 pm** | **Dinner** | **(TBD)** |
| **7:00-9:30 pm** | **Evening Talks; Buck Hall** | **Buck Hall** |
| **9:30-11:00 pm** | **Social Mixer/ Posters (All Presenters)** | **McCargo Hall** |

### Session I: Chair: Barry Bruce

7:00-7:05 pm **Welcome Address (Barry D. Bruce)**

7:05-7:35 pm **Prof. Ken Sauer (Invited Speaker), UC. Berkeley, LBL**
*THE PHYLOGENETICS OF PHOTOSYNTHETIC BACTERIA*

7:35-8:05 pm **Dr. Silvia Ramundo** (Invited Speaker) UC. San Francisco**
*EXPLORING MARS, A COLLECTION OF MUTANTS AFFECTING CHLOROPLAST-TO-NUCLEUS RETROGRADE SIGNALING*

8:05-8:25 pm **Prof. Richard Malkin (Invited Speaker), (UC. Berkeley)**
*REMEMBERING MY COLLEAGUE AND FRIEND, DAVID B. KNAFF*

8:25-8:45 pm **Dr. Non Chotewutmontri** (Post-doc), University of Oregon**
*GENOME-WIDE ANALYSIS OF TRANSLATIONAL DYNAMICS IN CHLOROPLASTS*

8:45-9:25 pm **Prof. Jean-David Rochaix (Keynote Speaker), University of Geneva**
*CONDITIONAL REPRESSION OF ESSENTIAL CHLOROPLAST GENES: NEW LINKS BETWEEN PROTEIN IMPORT AND PLASTID RNA METABOLISM?*

**9:25-9:30 pm** **Announcements (Barry D. Bruce)**

**9:30-11:00 pm** **Evening Social/Posters** **McCargo Hall**

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## Friday Jan 6, 2017

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| | | |
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| **7:00-8:30 am** | **Breakfast** | **Redwood Dining Hall** |
| **8:45-12:30 am** | **Morning Talks** | **Buck Hall** |

### Session II: Chair: Junko Yano

8:45-9:15 am **Prof. Yuval Mazor (Invited Speaker), Arizona State University**
*THE STRUCTURE OF PLANT PHOTOSYSTEM I, WHAT CAN WE SEE AT 2.6Å*

9:15-9:45 pm **Dr. Junko Yano (Invited Speaker), U.C. Berkeley/ LBL**
*FOLLOWING THE WATER OXIDATION REACTION OF PHOTOSYSTEM II WITH CRYSTALLOGRAPHY AND X-RAY SPECTROSCOPY AT XFELS*

9:45-10:15 am **Prof. Petra Fromme (Invited Speaker), Arizona State University**

*FEMTOSECOND CRYSTALLOGRAPHY OF PHOTOSYSTEM II BASED ON CONTINUOUS DIFFRACTION*

10:15-10:35 pm **Dr. Sarthak Mandal** (Post-doc), Arizona State University**
*ON THE MECHANISM OF TRIPLET STATE FORMATION AND ENERGY TRANSFER IN BACTERIAL REACTION CENTERS*

**10:35-10:55 am** **Coffee Break (20 min)** **(Buck Hall Entrance)**

## Session III: Chair: Devaki Bhaya

10:55-11:25 am **Dr. Julian Whitelegge (Invited Speaker), U.C. Los Angeles**
*HIGH-RESOLUTION MASS SPECTROMETRY OF LIPIDATED PROTEINS IN PHOTOSYNTHESIS*

11:25-11:55 am **Prof. Alex Worden (Invited Speaker), Monterey Bay Area Research Institute**
*ADVANCES IN UNDERSTANDING OF PHOTOSYNTHETIC MARINE PICOEUKARYOTES*

11:55-12:15 pm **Alexandra Fischer* (Graduate Student), UC. Berkeley**
*DEVELOPING TRANSIENT ABSORPTION SNAPSHOTS TO FOLLOW ZEAXANTHIN RADICAL CATION QUENCHING IN THYLAKOID MEMBRANES*

12:15-12:25 pm **Dr. Daniel Fishman, Department of Energy**
*OVERVIEW AND OPPORTUNITIES OF THE DOE EERE AND BETO PROGRAMS*

12:25-12:30 pm **Announcements (Barry D. Bruce)**

| | | |
|---|---|---|
| **12:30-1:30 pm** | **Lunch** | **Redwood Dining Hall** |
| **1:30-4:00 pm** | **Free time/an excursion** | **(Kayaking, Horseback riding, hike)** |
| **4:00 -5:30 pm** | **Poster Presentations (EVEN numbers)** | **McCargo Hall** |
| **5:30-7:00 pm** | **Dinner** | **Redwood Dining Hall** |
| **7:00-9:30 pm** | **Evening Talks** | **Buck Hall** |
| **9:30-11:00 pm** | **Social Mixer/Posters (ODD numbers)** | **McCargo Hall** |

## Session IV: Chairs: Graham Peers

7:00-7:30 pm **Prof. Beverley Green (Invited Speaker), University of British Columbia**
*EVOLUTION OF THE UNIQUE PHYCOBILIPROTEIN ANTENNA OF CRYPTOPHYTE ALGAE, ILLUMINATED BY 3D MOLECULAR STRUCTURE*

7:30-7:50 am **Dayna Peterson Forbrook* (Graduate Student), Arizona State University**
*NUCLEOTIDE DEPENDENT SUBUNIT EXCHANGE IN SPINACH SHORT-FORM RUBISCO ACTIVASE*

7:50-8:10 pm **Andrew Serban* (Graduate Student), Arizona State University**
*UNDERSTANDING THE ROLE OF NUCLEOTIDES AND CATIONS IN REGULATING SELF-ASSEMBLY OF RCA*

8:10-8:30 pm **Dr. Christof Klughammer, Walz Optoelectronics, Germany**
*OBSERVATION OF REDOX CHANGES OF FERREDOXIN, P700 AND PLASTOCYANIN IN SITU WITH THE DUAL-KLAS-NIR MEASURING SYSTEM*

8:30-8:50 pm **Niu Du* (Graduate Student), Scripps Institution of Oceanography/J.C.V. Institute**
*DEVELOPMENT OF A PH OSCILLATION METHOD FOR RAPID CARBON INFLUX/EFFLUX MEASUREMENTS*

8:50-9:25 pm **Prof. Graham Fleming (Historic Overview), U.C. Berkeley, LBL**
*TIME RESOLVING AND CALCULATING PHOTOSYNTHETIC ENERGY TRANSFER TIMESCALES AND MECHANICS- 1949 TO NOW*

9:25-9:30 pm **Announcements (Barry D. Bruce)**

**9:30-11:00 pm** **Evening Social/Posters** **McCargo Hall**

# Saturday Jan 7, 2017

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|---|---|---|
| **7:00 -8:30 am** | **Breakfast** | **Redwood Dining Hall** |
| **8:45-12:30 am** | **Morning Talks** | **Buck Hall** |

## Session V: Chairs: Arthur Grossman

8:45-9:15 **Prof. Andrew Allen (Invited Speaker), J Scripps Institution of Oceanography/J.C.V. Institute**
ECOLOGICAL GENOMICS OF MICRONUTRIENT LIMITATION IN MARINE DIATOMS

9:15-9:45 am **Prof. Devaki Bhaya (Invited Speaker), Carnegie Institution of Science**
*DIVERSITY IN PHOTOTROPHIC MICROBIAL COMMUNITIES*

9:45-10:05 am **Dr. Patrick Shih** (Post-doc), Joint BioEnergy Institute**
*DATING THE ORIGINS OF CYANOBACTERIA AND OXYGENIC PHOTOSYNTHESIS*

10:05-10:25 am **Usha Lingappa* (Graduate Student), California Institute of Technology**
*MANGANESE OXIDATION BY EXTANT CYANOBACTERIA & THE EVOLUTION OF PHOTOSYSTEM II*

**10:25- 10:40 am** **Coffee Break (15 mins)** **(Buck Hall Entrance)**

## Session VI: Chair: Helmut Kirchhoff

10:40-11:10 am **Prof. Helmut Kirchhoff (Invited Speaker), Washington State University**
*STRUCTURAL BOUNDARY CONDITIONS FOR ENERGY CONVERSION IN THYLAKOID MEMBRANES*

11:10-11:30 am **Dr. Ricarda Hoehner (Post-doc), Washington State University**
*THE INTERPLAY OF POTASSIUM AND SODIUM IN THE KEA1KEA2 ARABIDOPSIS MUTANTS PROVIDES NEW INSIGHTS INTO CELLULAR ION HOMEOSTASIS AND ITS ROLE IN PHOTOSYNTHESIS*

11:30- 11:50 pm **Dr. Jose G. Garcia-Cerdan** (Post-doc), UC. Berkeley**
*CPSFL1, A CRAL-TRIO LIPID-BINDING DOMAIN PROTEIN ESSENTIAL FOR PHOTOAUTOTROPHIC GROWTH IN CHLAMYDOMONAS REINHARDTII, MODULATES CAROTENOID ACCUMULATION IN THE CHLOROPLAST*

11:50-12:20 pm **Prof. William Adams (Invited Speaker), University of Colorado at Boulder**
*ACCLIMATION OF ARABIDOPSIS THALIANA FOLIAR VASCULATURE, PHOTOSYNTHESIS, AND TRANSPIRATION DEPENDENT ON ENVIRONMENT*

12:20-12:40 pm **Dr. Shai Saroussi** (Post-doc), Carnegie Institution of Sciences**
*NOVEL PHOTOSYNTHETIC FUNCTIONS INVOLVED IN THE ACCLIMATION OF CHLAMYDOMONAS REINHARDTII TO NITROGEN DEPRIVATION*

| | | |
|---|---|---|
| **12:40 -1:30 pm** | **Lunch** | **Redwood Dining Hall** |
| **1:30 -4:00 pm** | **Free time or an excursion** | |
| **4:00 -5:30 pm** | **Posters/refreshments ODD posters** | **McCargo Hall** |
| **5:30 -7:00 pm** | **Conference Dinner** | **Redwood Dining Hall** |
| **7:00 -9:30 pm** | **Evening Talks** | **Buck Hall** |
| **9:30 -11:00 pm** | **Evening Social/Posters (EVEN)** | **McCargo Hall** |

## Session VII: Chair: Marinus Pilon

7:00-7:30 pm **Prof. Elaine Tobin (Historic Talk), UCLA**
*MY ROAD FROM PHOTOSYNTHETIC LIGHT HARVESTING TO CIRCADIAN RHYTHMS*

*7:30-8:00 PM* **Prof. Marinus Pilon (Invited Speaker), Colorado State University**
*COPPER DELIVERY FOR PHOTOSYNTHESIS IN PLANTS*

8:00-8:30 pm **Prof. Bill Cramer (Keynote Speaker), Purdue University**
*STRUCTURE-FUNCTION OF THE CYTOCHROME $B_6/F$ LIPOPROTEIN COMPLEX; INTERACTION WITH THE TRANS-MEMBRANE STT7 SER-THR KINASE THAT MEDIATES STATE TRANSITIONS*

8:30-8:50 pm **Dr. Raimund Fromme, Arizona State University**

*REACTION CENTERS IN PHOTOSYNTHESIS: HELIOBACTERIUM MODESTICALDUM HAS A UNIQUE STRUCTURE COMPARED TO THE KNOWN STRUCTURES OF REACTION CENTERS (PRC, PSII, &PSI)*

8:50-9:25 pm **Prof. Steve Theg (Invited Speaker and Inoue Memorial), UC. Davis**
*A MEMBRANE DEFECT MODEL FOR PROTEIN TRANSLOCATION ON THE TAT PATHWAY*

9:25-9:30 pm **Announcements (Barry D. Bruce)**

**9:30-11:00 pm** **Evening Social/Posters** **McCargo Hall**

## Sunday Jan 8, 2017

**77:00**

**-8:30 am** **Breakfast** **Redwood Dining Hall**
**8:30 -8:45 am** **Check-out** **Main Office**

### Session VIII: Chair: Matt Posewitz

8:45-9:15 am **Prof. John Peters (Invited Speaker), Montana State University**
*BALANCING ELECTRON FLOW AND ENERGY METABOLISM THROUGH FLAVIN BASED ELECTRON BIFURCATION*

9:15-9:45 am **Prof. Kevin Redding, Arizona State University**
*REWIRING PHOTOSYNTHESIS: FUSION OF PHOTOSYSTEM I TO HYDROGENASE*

9:45-10:05 am **Dr. Anne-Marie Carey** (Post-doc), Arizona State University**
*PHOTOCURRENT GENERATION BY PHOTOSYNTHETIC BACTERIAL REACTION CENTERS INTERFACED WITH A POROUS ANTIMONY-DOPED TIN OXIDE (ATO) ELECTRODE*

10:05-10:25 am **Jonathan Morris* (Graduate Student), UC. Berkeley**
*DISTINGUISHING CHARACTERISTICS OF THE REGULATION OF CAROTENOID DEPENDENT NON-PHOTOCHEMICAL, QUENCHING IN LIGHT HARVESTING PLANTS*

**10:25-10:40 am** **Coffee Break (15 min)** **(Buck Hall Entrance)**

### Session IX: Chair: Tom Moore

10:40 -11:10 am **Prof. Gary Moore (Invited Speaker), Arizona State University**
*MOLECULAR COATINGS FOR APPLICATIONS IN SURFACE CATALYSIS AND ARTIFICIAL PHOTOSYNTHESIS*

11:10 -11:40 am **Prof. Ana Moore, Arizona State University**
*PROTON COUPLED ELECTRON TRANSFER IN ARTIFICIAL PHOTOSYNTHETIC CONSTRUCTS*

11:40 –12:00 am **Dr. Fiona Davies , Colorado School of Mines**
*LARGE-SCALE CULTIVATION OF THE CYANOBACTERIUM SYNECHOCOCCUS SP. PCC 7002 AT THE AZCATI/ATP3 ALGAL TESTBED*

12:00-12:30 am **Prof. Matt Posewitz (Invited Speaker), Colorado School of Mines**
*PHOTOSYNTHETIC AND METABOLIC MODULATION IN CHLAMYDOMONAS, NANNOCHLOROPSIS AND SYNECHOCOCCUS*

12:30- 12:40 pm *Richard Malkin* and *Beverly Green Awards*, photos, and closing remarks

**12:40 pm** **Optional Box Lunch and Departure for Whale Watching (Bodega Bay)**

# Oral presenters and titles
# (listed alphabetical by last name)

ADAMS, WILLIAM

ACCLIMATION OF ARABIDOPSIS THALIANA FOLIAR VASCULATURE, PHOTOSYNTHESIS, AND TRANSPIRATION DEPENDENT ON ENVIRONMENT

ALLEN, ANDREW

ECOLOGICAL GENOMICS OF MICRONUTRIENT LIMITATION IN MARINE DIATOMS

BHAYA, DEVAKI

DIVERSITY IN PHOTOTROPHIC MICROBIAL COMMUNITIES

CAREY, ANNE-MARIE**

PHOTOCURRENT GENERATION BY PHOTOSYNTHETIC BACTERIAL REACTION CENTERS INTERFACED WITH A POROUS ANTIMONY-DOPED TIN OXIDE (ATO) ELECTRODE

CHOTEWUTMONTRI, PRAKITCHAI**

GENOME-WIDE ANALYSIS OF TRANSLATIONAL DYNAMICS IN CHLOROPLASTS

CRAMER, WILLIAM A.

STRUCTURE-FUNCTION OF THE CYTOCHROME B6F LIPOPROTEIN COMPLEX; INTERACTION WITH THE TRANSMEMBRANE STT7 SER-THR KINASE THAT MEDIATES STATE TRANSITIONS

DANIEL FISHMAN

OVERVIEW AND OPPORTUNITIES OF THE DOE EERE AND BETO PRROGRAMS

DAVIES, FIONA**

LARGE-SCALE CULTIVATION OF THE CYANOBACTERIUM SYNECHOCOCCUS SP. PCC 7002 AT THE AZCATI/ATP3 ALGAL TESTBED

DU, NIU*

DEVELOPMENT OF A PH OSCILLATION METHOD FOR RAPID CARBON INFLUX/EFFLUX MEASUREMENTS

FISCHER, ALEXANDRA*

DEVELOPING TRANSIENT ABSORPTION SNAPSHOTS TO FOLLOW ZEAXANTHIN RADICAL CATION QUENCHING IN THYLAKOID MEMBRANES

FLEMING, GRAHAM R.

TIME RESOLVING AND CALCULATING PHOTOSYNTHETIC ENERGY TRANSFER TIMESCALES AND MECHANICS 1949 TO NOW

FROMME, PETRA

FEMTOSECOND CRYSTALLOGRAPHY OF PHOTOSYSTEM II BASED ON CONTINUOUS DIFFRACTION

FROMME, RAIMUND

REACTION CENTERS IN PHOTOSYNTHESIS. HELIOBACTERIUM MODESTICALDUM HAS A UNIQUE STRUCTURE COMPARED TO THE KNOWN STRUCTURES OF BACTERIAL REACTION CENTERS AS THE PHOTOSYTEMS I AND II

GARCIA-CERDAN, JOSE G.**

CPSFL1, A CRAL_TRIO LIPID-BINDING DOMAIN PROTEIN ESSENTIAL FOR PHOTOAUTOTROPHIC GROWTH IN CHLAMYDOMONAS REINHARDTII, MODULATES CAROTENOID ACCUMULATION IN THE CHLOROPLAST

GREEN, BEVERLEY

EVOLUTION OF THE UNIQUE PHYCOBILIPROTEIN ANTENNA OF CRYPTOPHYTE ALGAE, ILLUMINATED BY 3D MOLECULAR STRUCTURES

HOEHNER, RICARDA**

THE INTERPLAY OF POTASSIUM AND SODIUM IN THE KEA1KEA2 ARABIDOPSIS MUTANTS PROVIDES NEW INSIGHTS INTO CELLULAR ION HOMEOSTASIS AND ITS ROLE IN PHOTOSYNTHESIS

KIRCHHOF, HELMUT

STRUCTURAL BOUNDARY CONDITIONS FOR ENERGY CONVERSION IN THYLAKOID MEMBRANES

KLUGHAMMER, CHRISTOF

OBSERVATION OF REDOX CHANGES OF FERREDOXIN, P700 AND PLASTOCYANIN IN SITU WITH THE DUAL-KLAS-NIR MEASURING SYSTEM

LINGAPPA, USHA*

MANGANESE OXIDATION BY EXTANT CYANOBACTERIA AND THE EVOLUTION OF PHOTOSYSTEM II

MALKIN, RICHARD

REMEMBERING MY COLLEAGUE AND FRIEND, DAVID B. KNAFF

MANDAL, SARTHAK**

ON THE MECHANISM OF TRIPLET STATE FORMATION AND ENERGY TRANSFER IN BACTERIAL REACTION CENTERS

MAZOR, YUVAL

THE STRUCTURE OF PLANT PHOTOSYSTEM I, WHAT CAN WE SEE AT 2.6Å

MOORE, ANA

PROTON COUPLED ELECTRON TRANSFER IN ARTIFICIAL PHOTOSYNTHETIC CONSTRUCTS

MOORE, GARY

MOLECULAR COATINGS FOR APPLICATIONS IN SURFACE CATALYSIS AND ARTIFICIAL PHOTOSYNTHESIS

MORRIS, JONATHAN*

DISTINGUISHING CHARACTERISTICS OF THE REGULATION OF CAROTENOID DEPENDENT NON-PHOTOCHEMICAL QUENCHING IN LIGHT HARVESTING PLANTS

PETERS, JOHN

BALANCING ELECTRON FLOW AND ENERGY METABOLISM THROUGH FLAVIN BASED ELECTRON BIFURCATION

PETERSON, DAYNA*

NUCLEOTIDE DEPENDENT SUBUNIT EXCHANGE IN SOB RUBISCO ACTIVASE

PILON, MARINUS

COPPER DELIVERY FOR PHOTOSYNTHESIS IN PLANTS

POSEWITZ′ MATTHEW C.

PHOTOSYNTHETIC AND METABOLIC MODULATION IN CHLAMYDOMONAS, NANNOCHLOROPSIS AND SYN-ECHOCOCCUS

RAMUNDO, SILVIA**

EXPLORING MARS, A COLLECTION OF MUTANTS AFFECTING CHLOROPLAST-TO-NUCLEUS RETROGRADE SIGNALING

REDDING, KEVIN

REWIRING PHOTOSYNTHESIS: FUSION OF PHOTOSYSTEM I TO HYDROGENASE

ROCHAIX, JEAN-DAVID

ONDITIONAL REPRESSION OF ESSENTIAL CHOLORPLAST GENES: NEW LINKS BETWEEN PROTEIN IMPORT AND PLASTID RNA METABOLISM?

SAROUSSI, SHAI**

NOVEL PHOTOSYNTHETIC FUNCTIONS INVOLVED IN THE ACCLIMATION OF CHLAMYDOMONAS REINHARDTII TO NITROGEN DEPRIVATION

SAUER, KENNETH

THE PHYLOGENETICS OF PHOTOSYNTHETIC BACTERIA

SERBAN, ANDREW*

UNDERSTANDING THE ROLE OF NUCLEOTIDES AND CATIONS IN REGULATING SELF-ASSEMBLY OF RCA

SHIH, PATRICK**

DATING THE ORIGINS OF CYANOBACTERIA AND OXYGENIC PHOTOSYNTHESIS

THEG, STEVEN

A MEMBRANE DEFECT MODEL FOR PROTEIN TRANSLOCATION ON THE TAT PATHWAY

TOBIN, ELAINE

MY ROAD FROM PHOTOSYNTHETIC LIGHT HARVESTING TO CIRCADIAN RHYTHMS

YANO, JUNKO

OLLOWING THE WATER OXIDATION REACTION OF PHOTOSYSTEM II WITH CRYSTALLOGRAPHY AND X-RAY SPECTROSCOPY AT XFELS

WHITELEGGE, JULIAN P.

HIGH-RESOLUTION MASS SPECTROMETRY OF LIPIDATED PROTEINS IN PHOTOSYNTHESIS.

WORDEN, ALEXANDRA Z.

ADVANCES IN UNDERSTANDING OF PHOTOSYNTHETIC MARINE PICOEUKARYOTES

# Talk ABSTRACTS (in order of talks)

## SESSION I:

### THE PHYLOGENETICS OF PHOTOSYNTHETIC BACTERIA

**KENNETH SAUER**

EMERITUS PROFESSOR OF CHEMISTRY, UNIVERSITY OF CALIFORNIA-BERKELEY, AND SENIOR AFFILIATE, LAWRENCE BERKELEY NATIONAL LABORATORY

During the past decade expansion in the genomic database for photosynthetic bacteria has been enormous. The phylum of cyanobacteria that are responsible for the appearance and growth of dioxygen in Earth's atmosphere contains tens of thousands of taxa. My study, which began about three years ago, involves a comparison of sequences of proteins involved in forming the reaction centers of Photosystem I (17 proteins) and Photosystem II (33 proteins). The majority of the chosen taxa, accessible from the Joint Genome Institute databank, span a representative assembly of 54 taxa. These taxa match the set used in the comparable analysis recently published by Patrick Shih and colleagues (2016); however, the set of genes that they selected using diversity-driven genome sequencing did not include any that coded for reaction center proteins. Nevertheless, each set of genes from the same 54 taxa can be assembled into a pattern or tree that can be compared for the two orthogonal sets of proteins. The agreement is remarkably close between the arrangements predicted by each of these approaches. I have grouped the 54 taxa into 5 major clades and further organized each of these into higher-order subclades. The pattern for the reaction-center protein genes is totally consistent with the maximum-likelihood pattern shown in Fig. 1 of Shih, *et al.* My approach provides a basis for tree organization that informs about both near and distant taxa, and about the pattern and timeline of genetic divergence during cyanobacterial evolution.


### EXPLORING MARS, A COLLECTION OF MUTANTS AFFECTING CHLOROPLAST-TO-NUCLEUS RETROGRADE SIGNALING

**RAMUNDO, SILVIA**[1]; PERLAZA, KARINA[1]; LAM, MABLE[1]; JONIKAS, MARTIN[2]; ROCHAIX, JEAN-DAVID[3]; & WALTER, PETER[1];

[1]DEPT OF BIOCHEMISTRY AND BIOPHYSICS, UCSF, SAN FRANCISCO, CA, USA; [2]DEPT OF PLANT BIOLOGY, CARNEGIE INSTITUTION FOR SCIENCE, PALO ALTO, CA, USA; [3]DEPT OF MOLECULAR BIOLOGY AND PLANT BIOLOGY, UNIVERSITY OF GENEVA, GENEVA, CH

Chloroplasts are plant cell organelles specialized in harvesting light energy via photosynthesis. Although chloroplasts have their own genome, the large majority of chloroplast proteins are transcribed from nuclear genes and imported post-translationally. Thus, the nucleus controls the majority of chloroplast processes and must rely on chloroplast signals that monitor the status of the organelle. Recently, we discovered a chloroplast-to-nucleus signaling pathway in Chlamydomonas reinhardtii, a unicellular green algae, which allows to sense and respond to perturbations in chloroplast protein homeostasis (1, 2). In this work, the ClpP protease, which degrades damaged chloroplast proteins, was depleted. The cellular changes that ensued, including protein aggregation, induction of chaperones, proteases, and proteins involved in lipid trafficking and membrane homeostasis, emerged as telltale signs of an unfolded protein response (UPR). While the UPR of the endoplasmic reticulum (ER) has been extensively studied and exclusive sensors, transmitters, and transcriptional activation responses have been identified and characterized, the identities of the components in this chloroplast-UPR (cpUPR) remain unknown. To identify factors that mediate this signal transduction, we carried out a genetic screen that identified mutants that are either i) defective in inducing the cpUPR (silencing mutants) or ii) constitutively in a cpUPR induced-state (activating mutants). We will report on MARS1, an uncharacterized protein which potentially acts as signal transmitter of this pathway. 1. Ramundo S. et al. The Plant Cell (2014); 2. Ramundo S. and Rochaix JD, Autophagy (2014)

### REMEMBERING MY COLLEAGUE AND FRIEND, DAVID B. KNAFF

**RICHARD MALKIN**

EMERITUS PROFESSOR OF PLANT & MICROBIAL BIOLOGY, UNIVERSITY OF CALIFORNIA-BERKELEY

After completing a postdoc at the University of California, Berkeley, David spent the rest of his career as a professor in the department of chemistry and biochemistry at Texas Tech University. During 50 years of plant biochemistry re-

search, he authored more than 220-refereed publications, mentored hundreds of students, and collaborated with scientists worldwide. He was editor-in-chief of the journal Photosynthesis Research and a long-term participant of the Western Photosynthesis Conference. Unfortunately, this year David B. Knaff died on Jan. 27 in Lubbock, Texas, he was 74.

## GENOME-WIDE ANALYSIS OF TRANSLATIONAL DYNAMICS IN CHLOROPLASTS

**PRAKITCHAI CHOTEWUTMONTRI** & ALICE BARKAN

INSTITUTE OF MOLECULAR BIOLOGY, UNIVERSITY OF OREGON, EUGENE, OR 97403 USA.

The translation of chloroplast mRNAs has long been known to be regulated by developmental, environmental and physiological cues. However, progress in recognizing examples of translational regulation, identifying translational regulators, and dissecting mechanisms of translational modulation has been limited by the assays that have been available to monitor ribosome behavior in vivo. We are using genome-wide ribosome profiling methods that provide a quantitative and high resolution readout of ribosome positions in vivo to (i) identify nucleus-encoded proteins that are required for the translation of specific chloroplast mRNAs; (ii) analyze the impact of various light regimes on chloroplast ribosome behavior; and (iii) describe the translational dynamics of chloroplast mRNAs during the differentiation of photosynthetic leaf cells. I will discuss ribosome profiling data that provide a comprehensive description of chloroplast gene expression at four stages of chloroplast differentiation as displayed along the maize seedling leaf blade, and in mature bundle sheath and mesophyll cells. The developmental dynamics of chloroplast protein output fall into several patterns. Programmed changes in mRNA abundance make a strong contribution to the developmental shifts in protein output, but output is further adjusted by changes in translational efficiency. RNAs with prioritized translation early in development are largely involved in chloroplast gene expression, whereas those with prioritized translation in photosynthetic tissues are generally involved in photosynthesis. Differential gene expression in bundle sheath and mesophyll chloroplasts results primarily from differences in mRNA abundance, but differences in translational efficiency amplify mRNA-level effects in some instances. In most cases, rates of protein output approximate steady-state protein stoichiometries, implying a limited role for proteolysis in eliminating unassembled or damaged proteins under non-stress conditions.

## CONDITIONAL REPRESSION OF ESSENTIAL CHOLORPLAST GENES: NEW LINKS BETWEEN PROTEIN IMPORT AND PLASTID RNA METABOLISM?

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Most chloroplast genomes contain between 100 and 200 genes with different roles in photosynthesis, plastid gene expression and metabolism. In addition, several essential genes with unknown function have been identified. To better understand the role of these essential genes, we have developed a repressible chloroplast gene expression based on the nuclear Nac2 gene driven by the vitamin-sensitive MetE promoter and Thi4 riboswitch in the green unicellular alga *Chlamydomonas reinhardtii*. Nac2 encodes a protein targeted to the chloroplast which specifically acts on the *psbD* 5'UTR and is required for the accumulation of *psbD* mRNA. This system can be used for repressing any chloroplast protein gene provided its coding sequence is fused to the *psbD* 5'UTR. Using this strategy we have been able to conditionally deplete *Chlamydomonas* cells of RpoA, the ✔ subunit of the chloroplast RNA polymerase[1], Rps12, a plastid ribosomal protein[1], ClpP1, the catalytic subunit of the ATP-dependent ClpP protease[2], and Orf1995 and ORF2971. In all these five cases conditional repression leads to an arrest of cell growth. These studies have revealed novel aspects of chloroplast gene expression and signaling, in particular a regulatory negative feedback circuitry, a chloroplast-triggered autophagic response as well as a plastid protein unfolded response. Although the sequences of ORF1995 and ORF2971 are poorly conserved, they are related to Ycf1 and Ycf2 in plants, respectively. Depletion of ORF1995 profoundly affects plastid RNA metabolism and reveals that it mainly interacts with several components of the TIC and TOC protein import machinery in agreement with recent results obtained in land plants demonstrating that Ycf1 is part of the TIC translocon[3]. The results reveal an unexpected link between the chloroplast protein trans-

location machinery and RNA metabolism although it is not yet clear whether this link is direct or indirect.

**References:** [1]Ramundo S et al. (2013) Plant Cell 25, 167-186; [2]Ramundo S et al. (2014) Plant Cell 26, 2201-2222; [3]Kikuchi S et al. (2013) Science 339, 571-574.

## Session II

### THE STRUCTURE OF PLANT PHOTOSYSTEM I, WHAT CAN WE SEE AT 2.6Å

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Photosystem I (PSI) is one of two large reaction centers responsible for converting light photons into the chemical energy needed to sustain life. In the thylakoid membranes of plants, PSI is found together with its integral light-harvesting antenna, LHCI, in a membrane supercomplex containing hundreds of light harvesting pigments. I will discuss the structure of the plant PSI-LHCI complex which is now solved to a resolution of 2.6Å. The new structure contains the complete structure of the PsaK subunit, new details showing the binding mode of chlorophyll B molecules in the LHCI antenna. As well as multiple lipids, which appear to be important for the PSI-LHCI interface.

### FOLLOWING THE WATER OXIDATION REACTION OF PHOTOSYSTEM II WITH CRYSTALLOGRAPHY AND X-RAY SPECTROSCOPY AT XFELS

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The development of XFELs has opened up opportunities for studying the dynamics of biological systems beyond what is possible at synchrotron radiation (SR) sources. Intense XFEL pulses enable us to apply both x-ray diffraction and spectroscopic techniques to dilute systems or small protein crystals. By taking advantage of ultra-bright femtosecond x-ray pulses, one can also collect the data under functional conditions of temperature and pressure, in a time-resolved manner, after initiating reactions, and follow chemical dynamics during catalytic reactions and electron transfer. Such an approach is partic- ularly beneficial for biological materials and aqueous solution samples that are susceptible to x-ray radiation damage. We have developed spectroscopy and diffraction techniques necessary to fully utilize the capability of the XFEL x-rays for a wide-variety of metalloenzymes, like Photosystem II, and to study their chemistry under functional conditions (room temperature, ambient pressure). One of such methods is simultaneous data collection for x-ray crystallography and x-ray spectroscopy, to look at overall structural changes of proteins and chemical changes at metal catalytic sites. The other method is soft x-ray absorption spectroscopy of metalloenzymes by developing a spectrometer capable of studying dilute biological systems under ambient conditions. We have used the above techniques to study the water oxidation reaction of Photosystem II multi-subunit protein complex, in which the Mn4CaO5 cluster catalyzes the reaction. The current status of this research and the mechanistic understanding of the water oxidation reaction based on the X-ray techniques is presented.

### FEMTOSECOND CRYSTALLOGRAPHY OF PHOTOSYSTEM II BASED ON CONTINUOUS DIFFRACTION

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Time-resolved serial femtosecond crystallography (TR-SFX) has opened a new era in the study of dynamic processes in biology. The majority of time resolved studies have so far relied on techniques where small crystals were delivered to the XFEL femtosecond X-ray beam by use of liquid or viscous jets where the reaction has been initiated by light excitation of the crystals on the fly. TR-SFX sing liquid jets has been successfully applied for study of the photodynamics of PYP[1,2], myoglobin[3], and reactions in photosynthesis[4-6]. Of special interest is the water splitting reaction in Photosystem II, which produces all the oxygen in the atmosphere. PSII catalyzes water to $O_2$, in oxygenic photosynthesis, which provides all higher life on earth with oxygen and energy. The reaction involves in a complex reaction, where Oxygen Evolving Complex (OEC) cycles through 5 states from $S_0$ to $S_4$. During this cycle, four electrons are extracted from the water via OEC in four light-driven charge separation events. PSII is the only system in nature capable of forming molecular oxygen from water and sunlight. The catalytic center of the oxygen evolving complex (OEC) consists of a $Mn_4O_XCa$ metal center that couples the -electron chemistry of water oxidation to the one-electron photochemistry of the reaction center by sequentially storing oxidization states in this series of the S-state**.** We have recently discovered and developed a way to break the nexus between Bragg peak resolution and spatial resolution for Photosystem II using a new approach where structure determination is based on nano/microcrystals that allow for a limited displacement of molecules in the crystals based on continuous diffraction [8]. In this paradigm shifting research we discovered that random and uncorrelated displacements of the PSII dimer by a root mean square of 0.8 Å diminishes the formation of Bragg peaks at 4.5 Å resolution and beyond but leads to the observation of continuous diffraction. The presence of continuous diffraction beyond Bragg peak resolution is due to random (uncorrelated) displacements of rigid structural units (here: the PSII dimer), which causes a disruption of the constructive interference that forms the Bragg peaks. Concomitant to the fall-off of Bragg peaks there is an increase in the incoherent sum of continuous diffraction from the PSII dimers. The continuous diffraction from the sum of the single molecules in the crystal extended to the edge of the detector (at 3.5 Å), enabling structural refinement to 3.5Å resolution [8] and have recently be extended to 2.5 Å resolution. Preliminary data will be also presented on XES spectroscopy data, that were evaluated using the manifold method with single photon spectra collected on the crystals "on the fly". **References:** [1]Tenboer J, et al . (2014). Science 346: 1242-1246; [2]Pande K, et al.. Science 352: 725-729; [3]Barends TR, et al Science 350: 445-450; [3]Kupitz C, Basu S, et al. Nature 513: 261-265; [5]Kern J, et al. Nat Commun 5: 4371; [6]Ayyer K, et al. Nature 530: 202-206.

## ON THE MECHANISM OF TRIPLET STATE FORMATION AND ENERGY TRANSFER IN BACTERIAL REACTION CENTERS

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In purple bacterial reaction centers (RCs), triplet energy transfer from the primary donor P, a bacteriochlorophyll dimer to the carotenoid (Car) is the key step to protect RCs from damage under excess light illumination. The dynamics of P+HA– charge separation, recombination and spin conversion strongly influence the generation of triplet P and its energy transfer to Car via the cofactor BB. The mechanistic pathways of triplet excited state formation and triplet energy transfer in RCs remain a matter of debate largely due to the lack of sufficient spectral and kinetic information. Here, the rates and pathways of P+HA– recombination and triplet energy transfer are investigated in the wild-type (WT) and mutant Rhodobacter sphaeroides RCs using transient absorption spectroscopy over a wide spectral and temporal region in the temperature range from 77 K to 298 K. Two major aspects are addressed in this work. First, an investigation of WT, Car-less R26 and M182HL mutant RCs is performed to provide a detailed picture regarding the generation and dissipation of triplet energy in RCs and unravel the critical role played by BB in the energy transfer. The M182HL RC, where BB is replaced by a bioteriopheophytin (Φ) exhibits slower rate of triplet energy transfer due to the change of the electronic coupling and an increased triplet energy of Φ than BB. The second aspect of this work is to address the influence of the oxidation potential of the primary donor in electron and energy transfer of RCs. For this purpose we have used a highly oxidizing mutant RCs (M160LH + L131LH + M197FH) which has a P/P+ mid-point potential of 765 mV. This RC shows a lower yield of P+HA– and a faster charge recombination resulting in a lower yield of triplet P and slower rate of energy transfer.

# SESSION III

## HIGH-RESOLUTION MASS SPECTROMETRY OF LIPIDATED PROTEINS IN PHOTOSYNTHESIS.

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Post-translational modifications with hydrophobic moieties such as fatty acids and lipids are rarely detected using traditional bottom-up proteomics. Protocols for analysis of intact membrane proteins allow straightforward detection of these modifications where they typically lead to extended retention in reverse-phase chromatography separations. Bringing the power of high-resolution Fourier-transform mass spectrometry to bear on lipidated proteins has illuminated a number of challenges that require special consideration. Top-down tandem mass spectrometry is rarely sufficient to fully localize the modification site requiring strategies such as 'middle down' or MS3 whereby the intact protein is sparsely cleaved to yield larger fragments for greatly improved coverage after dissociation. During analysis of a cyanobacterial membrane preparation a small 13.5 kDa protein was found eluting at high organic concentration and it was hypothesized that the protein was a lipidated version of Psb27. The monoisotopic mass of 13533 Da could be accounted for by removal of a 21 amino acid signal sequence and modification of the N-terminal Cys residue with a modification of 842.77 Da. In a subsequent top-down experiment the N-terminus was further characterized by high-resolution MS2 and MS3 experiments to better characterize the modification. Prosight software initially failed to assign any b-ions. Manual interpretation revealed b1-b4 ions consistent with assignment of N-palmitoylation and S-diacylglycerol with a pair of 18:1 fatty acids. MS3 of the b1-ion provided further confirmation of the pattern of fatty acylation.

## ADVANCES IN UNDERSTANDING OF PHOTOSYNTHETIC MARINE PICOEUKARYOTES

**ALEXANDRA Z. WORDEN**

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Picoeukaryotes represent the smallest free living eukaryotic cells known and have important ecological roles in marine environments. Many of these eukaryotic microbes are photosynthetic, such as the green alga *Micromonas*, and are therefore amendable to lab experimentation. Using *Micromonas* cultures grown in high-tech bioreactors we have been learning about unique aspects of their cellular biology and physiology under nutrient limitation. At the same time, new deep-branching lineages are being discovered that appear to be widespread in marine ecosystems, but remain uncultured. Here, we will discuss some of the latest advances in knowledge of the biology and evolution of these unicellular eukaryotes, as well as controls of their growth and distributions in the world oceans.

## DEVELOPING TRANSIENT ABSORPTION SNAPSHOTS TO FOLLOW ZEAXANTHIN RADICAL CATION QUENCHING IN THYLAKOID MEMBRANES

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In order to respond to rapid changes in light intensity, plants have developed a series of mechanisms known as non-chemical quenching (npq). The fastest set of these

mechanisms is known as qE, which turns on within seconds to minutes of high light exposure. Previous work has proposed a possible qE mechanism involving charge transfer and the formation of a zeaxanthin radical cation. This cation has been observed selectively using transient absorption spectroscopy (TA) in high light exposed plant thylakoids. In order to tie this mechanism to a qE timescale, we are developing a technique called transient absorption snapshots. This involves following the appearance of the zeaxanthin radical cation signal as the thylakoids adapt to bright light. Together with fluorescence lifetime snapshots, which track the fluorescence lifetime as a sample adapts to high light, TA snapshots will provide insight into the timescale of this charge-transfer mechanism.

### OVERVIEW AND OPPORTUNITIES OF THE DOE EERE AND BETO PRROGRAMS

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## SESSION IV

### EVOLUTION OF THE UNIQUE PHYCOBILIPROTEIN ANTENNA OF CRYPTOPHYTE ALGAE, ILLUMINATED BY 3D MOLECULAR STRUCTURES

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Cryptophyte algae acquired their plastids from a red alga by secondary endosymbiosis along with many nuclear-encoded genes for plastid proteins, including the membrane-intrinsic LHC family. Unlike the other algal groups with secondary plastids, the cryptophytes retained a relict of the other red algal antenna, the phycobilisome, in the form of the phycoerythrin b subunit, acquired a novel subunit of unknown origin, and evolved a completely novel antenna system unique to them. Genomic, transcriptomic and proteomic data show that a number of evolutionary mechanisms were involved in the transition between these two bilin-based antenna systems. Gene loss: all nuclear and plastid phycobilisome genes except one; Gene gain: a small nuclear-encoded protein ("new" a subunit) with no homologs in any other organism. Result: a tetrameric (a1b.a2b) phycobiliprotein unique to cryptophytes, where the a subunit interactions play the major role in determining its 3D structure. Relocation: acquisition of tripartite targeting sequences by the a subunits result in the transport of assembled tetramers into the thylakoid lumen. Gene duplication/divergence gave families of a subunit genes with a surprisiing amount of diversity. Recently-determined three-dimensional structures show that much of the sequence diversity could be accomodated on surface loops and free N-termini. However, in the genus Hemiselmis, a single amino acid insertion in the a subunit sequences cause a large conformational change of the tetramer and loss of electronic coupling, with no reported physiological consequences and little effect on the conformation of the basic ab dimer. What was the driver for the replacement of the phycobilisome with this new type of antenna? Our current hypothesis is: "It's the Economy!" Nitrogen is the limiting nutrient in most marine environments, and the new antenna is much more economical in terms of nitrogen requirement than the large multiproteiin phycobiliosome structure. (Supported by DARPA-QuBE, NSERC, ARC, US-DOE, Moore Foundation)

### NUCLEOTIDE DEPENDENT SUBUNIT EXCHANGE IN SPINACH SHORT-FORM RUBISCO ACTIVASE

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the most abundant enzyme on Earth and represents the primary entry point of CO2 into the biosphere. Rubisco's extremely slow carboxylation rate of

ribulose-1,5-bisphosphate and its propensity toward inhibition make it a notoriously inefficient enzyme, however, Rubisco activase (Rca) is essential in maintaining Rubsico activity by catalyzing the rapid release of trapped inhibitors to reactivate Rubisco for CO2 fixation. Rca uses ATP hydrolysis as the driving force behind a conformational motion that restores activity to inhibited Rubisco active sites. In this work, spinach-β (Soβ) Rca was shown to differ greatly from tobacco Rca in ATPase activity in the presence of manganese and magnesium. Notably, Soβ subunits equilibrate between oligomers on a much slower time scale than cotton-β Rca. Soβ Rca subunit exchange rates may be relevant to the structural adjustments generating Rubisco-binding competent conformations of Rca. A fluorescence based Rca subunit exchange assay was developed to examine subunit exchange kinetics. Soβ Rca was successfully labeled with green and red fluorophores and utilized in a Förster resonance energy transfer (FRET) assay to monitor subunit exchange kinetics. A striking nucleotide effect was observed that correlated with thermal stability, indicating oligomeric conformational rigidity is based on nucleotide state. While ATP-analogs slow exchange to near zero, ATP speeds up exchange, and the fastest rates are with ADP. The high thermal stability of Soβ Rca under certain conditions suggests subunit-subunit interactions in assemblies that are sufficiently tight to prevent exchange. In combination with assembly assays, FRET assays and reactivation studies will provide critical information about the structure/function relationship of Rca in the presence of different nucleotides. This information will provide

valuable insight into understanding the Rubisco reactivation mechanism.

## UNDERSTANDING THE ROLE OF NUCLEOTIDES AND CATIONS IN REGULATING SELF-ASSEMBLY OF RCA

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Higher plant Rubisco activase (Rca) is a stromal ATPase necessary for Rubisco reactivation. Its functional state is thought to consist of ring-like hexameric species, similar to other members of the AAA+ protein superfamily. Despite observing hexameric assemblies under particular conditions, the assembly mechanism and the role played by nucleotides and cations are only partially understood. Progress towards elucidating the self-association pathway has been slow, primarily due to Rca's polydispersity in solution which renders traditional biochemical approaches difficult to interpret. Therefore, we chose to use Fluorescence Correlation Spectroscopy (FCS) to investigate the relationship between quaternary structure and function. Consistent with our previously published FCS work on cotton β Rca, tobacco β Rca appears to assemble in a step-wise fashion. However, tobacco Rca appears to assemble more rapidly, with equilibrium binding constants in the sub-micromolar range, forming substantial amounts of hexamers at much lower concentrations (3 μM for tobacco, 23 μM for cotton). Also consistent with previous work, tobacco β Rca assembly changes in response to nucleotide type and Mg2+ concentrations, suggesting a possible mechanism for regulating Rca activity. When compared with activity data, the proposed self-assembly model suggests that the hexamer has the highest ATPase activity. We have also generated the subunit-subunit interface mutant R294V in tobacco β Rca, which appears to also assemble in a step-wise fashion, despite appearing to be largely disordered based on Thermofluor assays. We find that contrary to tobacco, the spinach β-isoform appears to be in equilibrium with much larger oligomers, making FCS data difficult to interpret. Therefore spinach β Rca may require the addition of the α-isoform to properly assemble. This feature would suggest that assembly may be regulated by expressed isoform composition in addition to nucleotides and cations. Understanding how these variables are able to modulate self-assembly of Rca is critical for the interpretation of biochemical and biophysical experiments, as well as gaining an understanding of the physiological regulation of Rubisco carboxylation activity by means of dynamic Rca assembly-disassembly processes.

## OBSERVATION OF REDOX CHANGES OF FERREDOXIN, P700 AND PLASTOCYANIN IN SITU WITH THE DUAL-KLAS-NIR MEASURING SYSTEM

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Properties and possibilities of the new DUAL-KLAS-NIR measuring system are demonstrated. The successor of the DUAL-PAM system allows assessment of chlorophyll fluorescence and differential transmission changes in the near-infrared (NIR) spectral region. With the new system two pulse-modulated fluorescence signals with green and blue excitation and four pulse-modulated dual-wavelength difference signals (780-820 nm, 820-870 nm, 870-965 nm and 840-965 nm) are recorded. By deconvolution of the four NIR-signals simultaneous observation of redox changes of ferredoxin (Fd), P700 and plastocyanine (PC) in intact leaves is possible. The major opto-electronical components as well as the principles of data acquisition and signal deconvolution are outlined. Deconvolution via multilinear regression is based on “differential model spectra” (DMS) of Fd, P700 and PC that are derived from selective changes of these three components under appropriate physiological conditions. Whereas information on maximal changes of Fd is obtained upon illumination after dark-acclimation, maximal P700 and PC changes can be readily induced by saturating light pulses (SP) in the presence of far-red (FR) background light. Via on-line deconvolution and by using the information of maximal changes, the new measuring system enables the observation of redox states of Fd, P700 and PC in situ. A time resolution of 1 ms in continuous recording mode is achieved. It can be extended down to 30µs by cyclic single channel averaging. Under steady state light conditions quantitative information on the three redox states can be obtained by dark-pulses (Fd) and saturation pulses (P700

and PC). The possibility of determination of the relative and absolute ratios PC/P700 and Fd/P700 in situ are discussed.

## DEVELOPMENT OF A PH OSCILLATION METHOD FOR RAPID CARBON INFLUX/EFFLUX MEASUREMENTS

**DU, NIU**[1]; DICKSON, ANDREW[2]; ALLEN, ANDREW[1]; & MITCHELL, BRIAN (GREG)[1]

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Improving photosynthetic efficiency has been the new and hot topic in algal research and industrial applications. However, the measurement of carbon influx/efflux, which is a critical parameter for photosynthetic efficiency determination, has not been very well developed for laboratory phytoplankton culture condition. The most commonly used radioactive labeling technique was proposed in the 1950s for the study of primary productivity in natural environment. The method is well suited for ecology study for its high sensitivity, yet falls in short for physiological study because it is destructive and measures carbon fixation arbitrarily between net and gross rates. Other more recently developed approaches remain insufficient for the laboratory study that requires high accuracy and high sampling rates, etc. In this work, a pH oscillation method for rapid carbon uptake assay was developed and tested, starting from an original method for measuring carbonic anhydrase activity in cells, which lets the pH in the culture medium drift from pH 5 to 11. By using advances in understanding of carbonate chemistry for precise calculations of carbonate speciation and concentration changes, this method was improved to be far more sensitive than published methods. The assay requires only a small pH change (< 0.1 pH units) minimizing changes in carbonate speciation, and is optimized for measurement of chlorophyll concentration at 1 – 5 mg/L. Preliminary results using an automated prototype system have proven the feasibility of the method for precisely determining instantaneous carbon uptake in laboratory phytoplankton cultures. Case studies were as well performed to demonstrate the use of this approach for solving scientific questions most efficiently and effectively.

## TIME RESOLVING AND CALCULATING PHOTOSYNTHETIC ENERGY TRANSFER TIMESCALES AND MECHANICS 1949 TO NOW

**FLEMING, GRAHAM R.**

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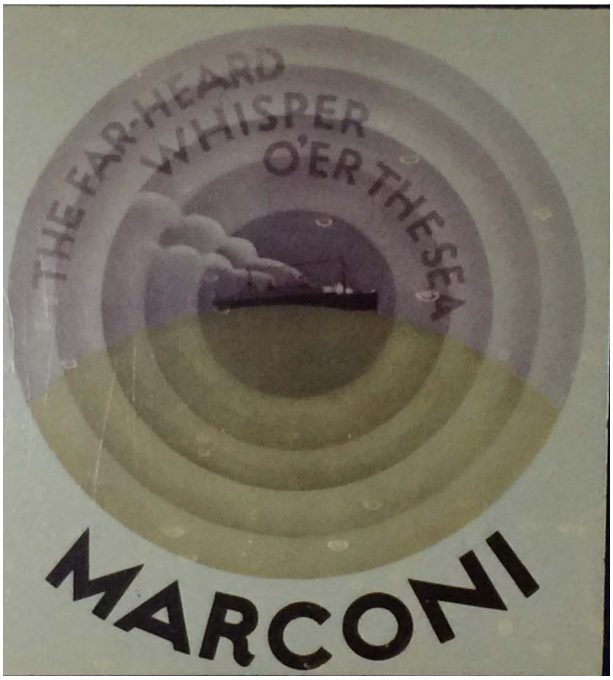
Once the concept of a dilute reaction center connected to a large (~ 300Chl/RC) antenna system became established, the concepts of number of energy transfer “hops” for

trapping at the RC and the timescale per hop became apparent. The number of suggested hops ranged from 10 to 10,000 but L. Duysens made realistic estimates (~750 hops) in 1964 and by 1966 suggested the timescale per hop of 10-13 s. Also in 1966 G.W. Robinson talked of exciton "spreading" in 50 fs. At the time there was considerable skepticism that such timescales could ever be observed directly. In this talk I will sketch the progress in making the measurements and in quantitatively describing the dynamics in photosynthetic light harvesting proteins. I will then briefly show how the fundamental microscopic knowledge gained from these studies can be built into a realistic model of the thylakoid membrane that exhibits both open and closed reaction centers and non-photochemical quenching.




## SESSION V

### ECOLOGICAL GENOMICS OF MICRONUTRIENT LIMITATION IN MARINE DIATOMS

**ANDREW ALLEN**

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Diatoms and other eukaryotic are important contributors to global primary production, the biological pump, and to the paleo record. In order to identify genes and cellular processes that are significant for the ecological and evolutionary success of diatoms and other phytoplankton we have conducted a variety of functional and ecological genomics studies. Recently we have obtained new insights from laboratory studies regarding cellular mechanisms that govern iron and nitrogen assimilation as well as diel partitioning of metabolism. For example, observations regarding major coordinated shifts in transcript control of primary and intermediate metabolism over light:dark cycles are contributing to a new view of the significance of distinctive diatom pathways, such as mitochondrial glycolysis and the ornithine-urea cycle. Also recent field work has suggested that Southern Ocean diatom communities are controlled by multiple layers of interactive micronutrient colimitation. Primary producers appeared to be simultaneously limited by iron and cobalamin availability, and data suggest that availability of cobalamin is controlled by gammaproteobacterial response to those phytoplankton. Therefore, iron limitation, widely documented in the ocean, may be better understood as micronutrient colimitation that is mediated by phytoplankton-bacterial interactions. Results from these studies will be discussed.

### DIVERSITY IN PHOTOTROPHIC MICROBIAL COMMUNITIES

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Cyanobacteria survive in diverse and extreme ecosystems, and provide us an opportunity to understand the molecular mechanisms that allow them to adapt to and thrive in changing environments. Our focus has been on microbial communities that form layered biofilms or microbial mats in hot-springs of Yellowstone National Park. Previous research showed that 16S rRNA profiles in these communities can be correlated with environmental gradients of temperature and light. The comparative genomic, metagenomic and transcriptomic analysis of genomes of two thermophilic Synechococcus isolates that dominate at different temperatures has given us key insights into the genetic and metabolic diversity of these phototrophic populations. In describing some of our findings, I will focus on how they have provided the inspiration for our current research in area of synthetic biology and host-phage interactions. Recently we have used deep amplicon sequencing to understand how the unexpectedly high degree of genomic diversity is established and maintained and how this micro-diversity might impact photosynthetic activity. The genetic diversity in these populations appears also allow us to explore the importance of niche adaptation strategies and the structure of microbial mat communities. I will also describe our attempts to understand the role of cyanophages and their co-evolution with cyanobacterial hosts in these environments.

### DATING THE ORIGINS OF CYANOBACTERIA AND OXYGENIC PHOTOSYNTHESIS

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The rise of oxygen ~2.3 billion years ago is the most fundamental geological change in Earth's history and was ultimately precipitated by a biological innovation – the evolution of oxygenic photosynthesis. Cyanobacteria were responsible for inventing this metabolism; however, the evolutionary timing of Cyanobacteria and oxygenic photosynthesis is widely debated. I have developed improved molecular clock approaches that incorporate information from gene duplication and endosymbiosis events to more accurately date ancient evolutionary events. Moreover, the recent discoveries of close relatives to oxygenic Cyanobacteria, named Melainabacteria, have filled a large gap in our understanding of the evolution of the Cyanobacterial phylum. I have utilized this increased phylogenetic coverage in combination with our developed molecular clock methodologies to investigate the rise of oxygen and Cyanobacteria. Our results demonstrate that crown group Cyanobacteria postdate the rise of oxygen circa 2.0 billion years ago. Furthermore, we observed that the divergence between Cyanobacteria and Melainabacteria – which marks an upper age limit for the evolution of oxygenic photosynthesis – occurred just prior to the rise of oxygen circa 2.5 Ga. These results provide insight into the evolution of oxygenic photosynthesis by unifying disparate yet complementary lines of evidence from biology, paleontology, and geology.

### MANGANESE OXIDATION BY EXTANT CYANOBACTERIA AND THE EVOLUTION OF PHOTOSYSTEM II

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Oxygenic photosynthesis is arguably the most important bioenergetic innovation in Earth history. Manganese plays a special role in this history because of its role in the water-oxidizing complex (WOC) of photosystem II (PSII). One hypothesis that is supported by evidence in both the sedimentary rock record and comparative biology is that Mn(II)-oxidizing phototrophy may have been a direct evolutionary precursor to oxygenic photosynthesis. If this was the case, it is possible that extant Cyanobacteria are still be capable of phototrophic Mn(II) oxidation. Here we present data with Synechocystis sp. PCC 6803 wild-type cells, PSII D1 mutants, and isolated membranes that show Mn(II) oxidation occurring by a light-dependent, PSII-dependent mechanism. This effect demonstrates at least some phylogenetic breadth (examined in Synechocystis, Nostoc, and Gloeobacter). To begin evaluating the ecological scope of this process, we also investigated Mn(II) oxidation in thick marine cyanobacterial mats (dominated by Scytonema) in a tidal lagoon on Little Ambergris Cay (Turks and Caicos, B.W.I.). Taken together, these data suggest that Cyanobacteria may play an important role in Mn cycling in modern environments. Ongoing work is focused on understanding the physiological value and molecular mechanics of this process, and its evolutionary implications for the origin of the WOC.

## SESSION VI

### STRUCTURAL BOUNDARY CONDITIONS FOR ENERGY CONVERSION IN THYLAKOID MEMBRANES

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In plants, the energy transforming machinery is harbored in the intricate thylakoid membrane system that is divided in strictly stacked and unstacked regions. Previous and recent electron microscopic data reveals exact numbers for critical membrane properties like the width of the thylakoid lumen, the diameter of stacked grana, or the separation distance between adjacent membranes in grana (stroma partition). What is the physiological meaning of

these numbers? The talk will present evidence that the geometric parameters of stacked grana were tuned by evolution to allow control of energy conversion and its regulation. This capability to fine-tune photosynthetic functions is based on the principal that small alterations of membrane properties can cause big changes, i.e. geometrical membrane parameters are at the threshold between two states.

## THE INTERPLAY OF POTASSIUM AND SODIUM IN THE KEA1KEA2 ARABIDOPSIS MUTANTS PROVIDES NEW INSIGHTS INTO CELLULAR ION HOMEOSTASIS AND ITS ROLE IN PHOTOSYNTHESIS

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Cellular and chloroplast ion homeostasis are crucial for biological processes including photosynthesis. In nature this fine-tuned system is challenged by abiotic stress such as soil salinity. Increased sodium levels in soils have become a major agricultural issue, affecting photosynthetic performance and drastically diminishing crop yields [1]. Toxic Na ions are taken up by plants and compete with cellular potassium (K) an essential macronutrient for plant function. To cope with the consequences of soil salinity, a better understanding of cellular ion flux and ion flux in chloroplasts in particular is key. Our lab identified Arabidopsis mutants deficient in the two K+/H+ exchange antiporters, KEA1 and KEA2 [2]. Mutant plants display a heterogeneous phenotype with compromised photosynthesis primarily in pale young leaves. Older leaves turn green and exhibit robust photosynthetic performance [2]. Using Total Reflection X-Ray Fluorescence measurements we showed increased K accumulation in individual leaves harvested from two independent kea1kea2 loss-of-function mutant lines compared to corresponding wild-type tissue [3]. We noticed that the K accumulation is pronounced in the young, pale green mutant leaves [3]. kea1kea2 mutants grown in the presence of 75 mM NaCl show a phenotype rescue behavior resulting in restored chlorophyll content and recovered photosynthesis performance [2]. Rescued kea1kea2 plants show a drop in K levels back to wild-type values particularly in young leaves of kea1kea2. In addition, we identified two suppressor mutant alleles that genetically rescue the kea1kea2 phenotype. In the case of kea1kea2 suppressor mutants we noticed a decrease in cellular K contents compared to the original kea1kea2 mutant. We speculate that defective sensing of proper K level and a subcellular misdistribution of K surplus are responsible for the chloroplast malfunction and poor photosynthesis in kea1kea2 plants. The adjustment and rebalancing of subcellular K pools can be achieved by moderate salt stress treatments or by inducing suppressor mutations. [1] Schroeder et al. 2013, Nature, 497, 60-66; [2] Kunz et al., 2014 PNAS, 111 (20), 7480–7485; [3] Höhner et al., 2016, SAB, 125, 159-167

## CPSFL1, A CRAL_TRIO LIPID-BINDING DOMAIN PROTEIN ESSENTIAL FOR PHOTOAUTOTROPHIC GROWTH IN CHLAMYDOMONAS REINHARDTII, MODULATES CAROTENOID ACCUMULATION IN THE CHLOROPLAST

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Chlamydomonas reinhardtii is an excellent model organism to study regulation of photosynthesis in eukaryotes. From a large collection of non-photosynthetic (acetate-requiring) DNA insertional mutants, we have isolated the extremely light-sensitive mutant cpsfl1. Exposing heterotrophic dark-grown cultures of the cpsfl1 mutant to light leads to severe photoinhibition and photobleaching. Photoinhibited cpsfl1 cells exhibit abnormal repair process of photosystem II (PSII) in the dark. The cpsfl1 mutant displays a light-green color under dark growth conditions due to a 40% reduction

of total carotenoids, whereas chlorophylls are reduced by 10% of WT levels. Complementation with a wild-type copy of the CPSFL1 gene rescues the mutation. CPSFL1 encodes a 31.5 kDa chloroplast protein that contains a CRAL_TRIO lipid-binding domain. A yellow-in-the-dark (chlL) and cpsfl1 double mutant (chlL cpsfl1), which lacks chlorophyll when grown in the dark, exhibits a drastic 80% reduction of carotenoids compared to chlL. The low carotenoid content observed in cpsfl1 and chlL cpsfl1 is caused by an impairment in phytoene synthesis. In lipid binding studies, recombinant CPSFL1 protein exhibits strong phosphatidic acid (PA) binding in vitro. Moreover, heterologous expression of recombinant CPSFL1 protein in E. coli cells that constitutively overexpress carotenoids, results in purified CPSFL1 protein bound to minor amounts of carotenoids. These results suggest a role of CPSFL1 in transiently binding carotenoids and interacting with membranes containing PA. We suggest that the CPSFL1 protein modulates carotenoid accumulation by controlling routing and/or transport of carotenoids within the chloroplast.

## ACCLIMATION OF ARABIDOPSIS THALIANA FOLIAR VASCULATURE, PHOTOSYNTHESIS, AND TRANSPIRATION DEPENDENT ON ENVIRONMENT

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Temperature during the evolution of three Arabidopsis thaliana ecotypes from Sweden, Poland, & Italy determines to what extent photosynthesis and the capacity for foliar sugar export acclimate to growth light intensity and temperature while precipitation during their evolution determines the extent of acclimatory adjustment of foliar vein density, water-transport capacity of the xylem, and transpiration rate in response to growth temperature. All ecotypes exhibited upregulation of photosynthesis in response to high versus low light or cool versus hot temperature, with the extent of upregulation increasing with increasing habitat latitude (greatest in Swedish ecotype). The upregulation of photosynthesis to high growth light intensity was supported by thicker leaves (more mesophyll palisade cells), higher vein density, and augmented phloem and xylem. However, in contrast to photosynthetic capacity, transpiration rate in high-light acclimated leaves increased with decreasing habitat latitude (highest in the Italian ecotype). Moreover, upregulation of photosynthetic capacity in response to cool growth temperature occurs under a condition in which evaporative demand is diminished compared to growth at hot temperature. Thus, while photosynthetic capacity, leaf thickness, and phloem features increased with increasing habitat latitude (decreasing habitat temperature) in response to growth at low temperature (greatest increase in Swedish ecotype), transpiration rate, vein density, and xylem features increased with decreasing habitat precipitation in response to growth at high temperature (greatest increase in Polish ecotype). These findings are consistent with habitat precipitation levels being greatest at the Italian site, intermediate at the Swedish site, and lowest at the Polish site. Whereas acclimation of photosynthetic capacity was positively correlated with foliar sugar-export capacity under all growth conditions, transpiration rate (and water-transport capacity of the xylem) could thus be adjusted separately from photosynthetic capacity.

## NOVEL PHOTOSYNTHETIC FUNCTIONS INVOLVED IN THE ACCLIMATION OF CHLAMYDOMONAS REINHARDTII TO NITROGEN DEPRIVATION

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Nutrient availability is a major factor that impacts the growth and productivity of photosynthetic organisms. Accordingly, to optimize crop yields, substantial efforts are focused on supplementation of agricultural soils with key nutrients. Yet in nature, the growth of photosynthetic bacteria, algae and plants are frequently impacted by nutrient availability; these limitations elicit suites of acclimation responses that involve various aspects of cellular metabolism. A component of these responses is a dramatic modification of photosynthetic activity, which helps accommodate the energetic/metabolic constraints imposed by the environment and limits photo-damage. During nitrogen (N) deprivation, the capacity to de-novo synthesize amino acids is reduced and therefore imbalance between N and carbon feeds back on the Calvin-Benson-Bassham Cycle to retard carbon fixation. Consequently, the

major sink for electrons and ATP, generated by photochemical reactions, is a markedly declined. In analyses of the unicellular microalgae Chlamydomonas reinhardtii, we found that type-II NADPH dehydrogenase (NDA2) plays a key role in modulating photosynthesis during N deprivation. It drives NDA2-dependent cyclic electron flow, chlororespiration and thermal dissipation excess excitation energy from PSII. Therefore, NDA2 has an essential photoprotective function associated with the dissipation of energy that cannot be coupled to CO2 fixation or cell maintenance. Our ability to evaluate photosynthetic acclimation responses during N deprivation provides us with the opportunity to identify novel factors associated with the flexible nature of photosynthesis. Indeed, by applying specific ecophysiological screening methods to a library of Chlamydomonas strains defective in green-lineage conserved genes (encoding proteins designated the 'GreenCut'), we identified a novel component associated with photoprotection during acclimation of the photosynthetic apparatus to N deprivation. Both the screening procedure and this acclimation-associated mutant will be discussed.

## SESSION VII

### MY ROAD FROM PHOTOSYNTHETIC LIGHT HARVESTING TO CIRCADIAN RHYTHMS

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I arrived at U.C.L.A. as an affirmative action appointment as an Assistant Professor in 1975. There was no lab for me to occupy and my set up money was almost non-existent. However, I was supported by three plant biology faculty members who helped me get my research started—Karl Hamner, Sam Wildman and Philip Thornber. Karl Hamner was at the end of his career studying circadian rhythms and he let me use his remaining grant money to hire a technician before my own grant was funded. Once he retired, I was given his lab space, which I suspect led me to my own studies of circadian rhythms. Sam Wildman was studying ribulose bis-phosphate carboxylase/oxygenase (Rubisco) and had purified it and ultimately crystallized it. Philip Thornber had discovered that chlorophylls were bound by proteins (a radical idea at the time) and was able to separate chlorophyll protein complexes on non-denaturing "green gels". Over time, Philip expanded his studies of chlorophyll/protein complexes to purple bacteria and ultimately achieved his goal of solubilizing all of the cholorophyll-protein complexes from thylakoid membranes of higher plants without the generation of any free pigments. I had demonstrated during my post-doctoral work at Brandeis that light acting on phytochrome could cause changes in gene expression. It was already clear that light was necessary for chloroplast development and thus it seemed likely that major chloroplast proteins were affected. Thanks to samples of the Rubisco small subunit and of light-harvesting chlorophyll proteins I was able to raise antibodies to these proteins and use them to demonstrate that indeed light caused a dramatic increase in their mRNAs as measured by in vitro translation of polyA RNA, a new technique at the time. Indeed, progress in techniques continued to make progress in understanding the events involved in forming functional photosynthetic chloroplasts possible. In 1974, recombinant DNA technology was just getting underway, with Paul Berg of Stanford a leader in developing the techniques. He was also concerned about potential biohazards of cloning and was part of a National Academy committee to study the bio-safety ramifications of the technology and which suggested that until such time as safety was established, scientists should stop experiments with recombinant DNA. Of course, it quickly became clear that while scientists would make every effort to operate safely, there would not be a long time before cloning became widely used. My research at U.C.L.A. did use cloning and I studied the phytochrome regulation of transcription of one of the chlorophyll a/b-binding proteins. Over the ensuing years I was able to collaborate with Philip, using molecular techniques to study the biogenesis and assembly of several chlorophyll-protein complexes. Our collaboration became even closer when we married and collaborated on raising and educating our four children from previous marriages. Looking back on the progress of work on photosynthesis, I am impressed by the roles played by technical developments that allowed an ever more detailed analysis of the process of making the photosynthetic apparatus and understanding its structure.

### COPPER DELIVERY FOR PHOTOSYNTHESIS IN PLANTS

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The use of Cu-microRNAs to regulate Cu enzymes is conserved in multicellular photosynthetic eukaryotes. It was proposed that the Cu-microRNAs serve to economize the available cellular Cu for use in essential Cu proteins such as plastocyanin (PC), allowing plants to maintain photosynthesis during Cu deficiency. Testing the function of the Cu-microRNAs is challenging because several different Cu-microRNA families exist with multiple loci for most. To assess the importance of the Cu-microRNA-mediated regulation for plant productivity under Cu deprivation, we designed a novel target mimicry construct for all 4 Cu-miRNAs (miRNA 397, 398, 408 and 1444) and transformed both poplar and Arabidopsis. Analyses of target mimic-expressing plants showed that the Cu-miRNAs can indeed be manipulated and that mimicry affects both the target transcript stability and protein accumulation. Molecular and physiological characterization of the mimicry lines in comparison with wild-type plants provided support for a refined copper economy model. How is the prioritization of Cu delivery to PC achieved? PC mRNA is not a target of a microRNA. In order for Cu to reach PC in the thylakoid lumen, cytosolic Cu is first transported over the inner chloroplast envelope and subsequently over the thylakoid membrane. Cu transport is mediated by two P1B-type ATPases, PAA1/HMA6 (inner chloroplast envelope) and PAA2/HMA8 (thylakoid membrane). Because PAA1 or PAA2 might be involved in the regulation of sub-cellular Cu distribution we investigated if these proteins are them-selves regulated by Cu. We found that PAA2 protein is most stable at low Cu concentrations and its abundance decreases significantly with Cu addition to plant growth media. This regulation occurs post-translationally, via turnover mediated by the CLP protease system. Most likely, PAA2 becomes a CLP substrate when the transporter binds Cu. (Support: Agriculture and Food Research Initiative competitive grant 2012-67-13-19416 and by the US NSF MCB 1244142).

## STRUCTURE-FUNCTION OF THE CYTOCHROME B6F LIPOPROTEIN COMPLEX; INTERACTION WITH THE TRANS-MEMBRANE STT7 SER-THR KINASE THAT MEDIATES STATE TRANSITIONS

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The structure of the cytochrome b6f complex, which lies at the cross-roads of the photosynthetic electron transport chain of oxygenic photosynthesis, has been solved to a resolution of 2.5 Å[1] (PDB 4OGQ). Stt7, a 754 amino acid residue serine/threonine kinase found in *Chlamydomonas reinhardtii,* which mediates state transitions involving the distribution of chlorophyll antenna proteins between the two photosystems, is believed to have a trans-membrane topology and to be activated on the electrochemically positive (p) side of the membrane by redox action of the cytochrome b6f complex[2,3]. Plastoquinol oxidation by the b6f complex on the p-side activates the kinase domain of Stt7 on the trans (n) side, leading to phosphorylation of the light-harvesting chlorophyll proteins and their consequent redistribution ("state transition") between the two photosystems[4,5]. Its interaction with the b6f complex and its structural organization is unclear. In the present study, the Stt7 kinase has been cloned, expressed, and purified in a heterologous host, *E. coli*[6]. The kinase is active *in vitro* in the presence of reductant and is purified as a tetramer, determined by analytical ultracentrifugation, electron microscopy, and electrospray ionization mass spectrometry, with a molecular weight of 332 kDa, consisting of an 83.41 kDa monomer.[6] Far-UV circular dichroism spectra show Stt7 to be 58 % α-helical and, through increased thermal stability of Stt7 secondary structure in the presence of the b6f complex, document a physical interaction with b6f[6]. The activity of wild-type Stt7 and its Cys-Ser double mutant at positions 68 and 73 in the presence of a reductant imply that the disulfide form of these two cysteines is inhibitory[6]. It is hypothesized that kinase activation in vivo could result from direct interaction between Stt7 and the b6f complex, or long-range reduction of Stt7 by superoxide,[6] generated in the b6f complex via the plasto-semiquinone generated by the p-side 1-electron oxidation of plastoquinol[7]. **References:** (1) Structure (2014), 22, 1008–1015; (2) Science (2003), 299, 1572-1575; (3) PLoS Biology (2009) 7, e1000045; (4) PNAS (1997), 94, 1585-1590; (5) EMBO J. (1999), 18, 2961-2969; (6) J. Biol. Chem, 291, 21740-21750 (2016); (7) Biochemistry (2013), 52, 8975-8983. **Research Support:** NIH GM-038323 (WAC), P30 CA23168, P30 DK063491 (JPW).

## REACTION CENTERS IN PHOTOSYNTHESIS: *HELIOBACTERIUM MODESTICALDUM* HAS A UNIQUE STRUCTURE COMPARED TO THE KNOWN STRUCTURES OF BACTERIAL REACTION CENTERS AS THE PHOTOSYTEMS I AND II

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In the focus of photosynthetic reactions is the process of converting the light energy into chemical energy this process is maintained by protein pigment complexes which are called reaction centers(RC). The importance of these

RC's can be seen as fundamental for any photosynthetic process. The first ever solved membrane protein structure was of the bacterial reaction center which led to the thesis that the oxygen evolving photosystem II is in its reaction center structurally closely related. In the year 2001 the first structure of photosystem II and the high resolution structure of photosystem I proofed this hypothesis. The reaction center of H. modesticaldum takes a special place in between the other known structures. The H. modesticaldum reaction center is not an ancestor of any known reaction center. From the chemical nature of the Chlorophylls(three different types) as its orientation in the core of the reaction center as its adjacent integrated Chlorophyll based antenna system it is in size and composition unique. Distances between Chlorophylls in the reaction center P800 compared to P700 and to the terminal acceptor FX are different to photosystem I. In the high resolution structure of H. modesticaldum reaction center the farnesyl side chains can be assigned from electron density as the nature of the over 50 Chlorophylls in the homodimer. (Funding support ongoing by DOE grant DE-SC0010575)

### A MEMBRANE DEFECT MODEL FOR PROTEIN TRANSLOCATION ON THE TAT PATHWAY

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Over 95% of chloroplast proteins are encoded in the nucleus, synthesized on 80S ribosomes and posttranslationally imported. Many proteins residing in the thylakoid lumen cross the thylakoid membrane on the cpTat pathway, a pathway also found in bacteria and archaea and named for the twin-arginine motif in the client signal peptides. Two competing hypotheses describe the mechanism through which proteins cross the membranes on the Tat pathway. In one, pores made of the TatA subunit of the Tat translocon are proposed to form variable-sized pores which accommodate folded substrates of differing sizes. The other model posits that substrate proteins cross the membrane through defects in the bilayer structure that result in the transient formation of toroidal pores. Cell Penetrating Peptides (CPPs) are small amphipathic peptides that cross cell membranes by inducing membrane thinning that ultimately results in the induction of toroidal pores. Recognizing that thylakoid membranes are thinned by the imposition of a protonmotive force, we tested whether CPPs had any influence on Tat protein transport. Four different CPPs with vastly different primary structures were found to stimulate Tat translocation in the low micromolar range. We also found that the energetic threshold for Tat transport was lower in the presence of CPPs. Interestingly, the amphipathic helix from the TatB translocon subunit also stimulated Tat transport. Taken together, these experiments suggest a hypothesis in which protein transport on the Tat pathway operates via transient toroidal pore formation following thinning-induced breakdown of the membrane bilayer.

## SESSION VIII

### BALANCING ELECTRON FLOW AND ENERGY METABOLISM THROUGH FLAVIN BASED ELECTRON BIFURCATION

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Flavin based electron bifurcation is a fairly recently realized biochemical phenomenon that is being billed by some as a new fundamental mechanism of energy conservation. Electron bifurcation is mainly associated with aspects of anaerobic metabolism, where maximizing energy conservation is of paramount importance for survival. Electron bifurcation has also been implicated in managing electron flow for diazotrophic growth of a number of organisms. Diazotrophic growth or growth under nitrogen-fixing conditions is very energy demanding and requires large amounts of ATP and low potential reducing equivalents. Anoxygenic phototrophic bacteria grow well diazotrophically, but unlike plants and cyanobacteria, they do not generate low potential electrons during photophophorylation. Instead, some phototrophs including the purple nonsulfur bacterium Rhodopseudomonas palustris , use the FixABCX complex to couple the NADH-dependent exergonic reduction of quinone to quinol with the endergonic reduction of ferredoxin through electron bifurcation. Reduced ferredoxin is then the electron donor to nitrogenase. FixABCX has been shown Harwood group at University of Washington to be essential for diazotrophic growth in R. palustris. Coupling endergonic and exergonic reactions in this manner is a very interesting biochemical process and we are really just beginning to understand the mechanism of this process. Recent results on the mechanism of electron bifurcation will be discussed from the Biological Electron Transfer and Catalysis Program on the electron bifurcating enzyme on which most progress has been made: NADH-dependent reduced ferredoxin:NADP+ oxidoreductase. From this work we are generating testable hypotheses to probe the mechanism of FixABCX function.

## REWIRING PHOTOSYNTHESIS: FUSION OF PHOTOSYSTEM I TO HYDROGENASE

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To maximize the capture of photosynthetic electrons for algal bio-hydrogen production and minimize inactivation by oxygen, we created a strain in which PSI is fused directly to the algal [FeFe] hydrogenase. Based on the structures of PsaC in PSI and algal HydA, we inserted the HydA sequence within an outward-facing loop of PsaC. Although several versions of the psaC-hydA chimeric gene were made with different sequences at the junctions between the two polypeptides, results with the simplest one will be presented here. The chimeric gene was integrated into the chloroplast genome at the endogenous psaC locus in the hydA1 hydA2 double mutant of C. reinhardtii. Thus, after complete replacement of the psaC gene, the sole version of PSI or hydrogenase is the PSI-hydrogenase fusion. This was confirmed by immunoblots with anti-PsaC and anti-HydA antibodies. The chimeric protein exhibited detectable levels of P700 photobleaching activity at ~15% the WT level, indicating that the PsaC-HydA polypeptide had folded and co-assembled with PSI. After a brief period of anaerobic adaptation, cells expressing the PSI-HydA2 chimera produced H2 in a light-dependent fashion continuously over several days. During this time, O2 levels remained low in the culture. The HydA domain seems to block ferredoxin binding, explaining why the strain is not autotrophic. It appears that nearly all electrons from PS1 are diverted to H2 production (via the attached HydA2) or O2 reduction (via the Mehler reaction).

## PHOTOCURRENT GENERATION BY PHOTOSYNTHETIC BACTERIAL REACTION CENTERS INTERFACED WITH A POROUS ANTIMONY-DOPED TIN OXIDE (ATO) ELECTRODE

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The ability to incorporate biological components into electronic and optical devices opens the door for taking advantage of water-based, highly specific reactions and reaction pathways in a number of important applications, such as biocatalysis, synthesis, sensing, and biomedical interfaces. The bacterial photosynthetic reaction center

represents an ideal photonic component of such a system in that it is capable of modifying local chemistry via light driven redox reactions with quantitative control over reaction rates and has inherent spectroscopic probes for monitoring function. A well-characterized model system is presented, consisting of a transparent, porous electrode (antimony-doped tin oxide) which is electrochemically coupled to the reaction center via cytochrome c. Upon illumination, the reaction center performs 2-step, 2-electron reduction of a ubiquinone derivative which exchanges with oxidized quinone in solution. Electrons from the electrode then move through the cytochrome c to reoxidize the reaction center. The result is a facile platform for performing redox chemistry that can be optically and electronically controlled in time and space. These systems can be used to further explore and optimize bio-electronic interfaces and to inform the design of biohybrid and biomimetic systems for artificial photosynthesis. We are now optimizing this platform by incorporating natural and biomimetic light-harvesting systems to increase the absorption cross section. Additionally we are interfacing cyanobacterial Photosystem I, for which the terminal electron acceptor is an iron sulfur protein with a low enough redox potential to reduce NADP+ to NADPH. Such a biohybrid photoelectrochemical cell would be capable of performing useful redox chemistry in solution.

**DISTINGUISHING CHARACTERISTICS OF THE REGULATION OF CAROTENOID DEPENDENT NON-PHOTOCHEMICAL QUENCHING IN LIGHT HARVESTING PLANTS**

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Photosynthetic organisms employ various photoprotective mechanisms to dissipate excess photoexcitation as heat in a process called non-photochemical quenching (NPQ). NPQ allows for a rapid response to changes in light intensity and in higher plants is primarily triggered by a pH gradient across the thylakoid membrane. The response is mediated by the PsbS protein and various carotenoids. Time correlated single photon counting (TCSPC) measurements were performed on novel mutants of Arabidopsis thaliana to quantify the dependence of the response of NPQ to changes in light intensity on the presence and accumulation of zeaxanthin and lutein. Measurements were performed on wild type and mutants deficient in one or both of the carotenoids, as well as a mutant that accumulates lutein in an analogous manner to the accumulation of zeaxanthin in wild type and lutein deficient mutants. Changes in the response of NPQ to light acclimation in wild type and mutants that accumulate carotenoids were observed between the two successive light acclimation cycles, suggesting that the character of the rapid and reversible response of NPQ in fully dark adapted plants is substantially different than in conditions plants are likely to experience due to changes in light intensity during daylight. Mathematical models to describe the response of zeaxanthin and lutein dependent reversible NPQ were constructed that describe the observed discrepancies between the light acclimation periods. Finally, the wild type response of NPQ was reconstructed from isolated components present in mutant plants with implications for the relation between lutein and zeaxanthin in NPQ.

## SESSION IX

**MOLECULAR COATINGS FOR APPLICATIONS IN SURFACE CATALYSIS AND ARTIFICIAL PHOTOSYNTHESIS**

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Catalysts accelerate chemical transformations, but the ability to effectively interface them with surfaces for driving industrially relevant reactions using electricity or sunlight as a power source remains a major challenge. This presentation will report on recent efforts from our research group aimed at developing molecular surface coatings for photoactivating chemical transformations that include capturing, converting, and storing solar energy as a fuel. Addressing this obstacle improves fundamental understanding of catalysis in complex environments and enables technological advancements that depend on the precise control and selectivity of nanoscale components. By designing extended environments for the coordination of molecular catalysts, key features of biological enzymes, including extended ligation spheres, active-site regeneration strategies and channels for substrate delivery as well as product removal, can be integrated with the design and synthesis of human-engineered catalysts.

Functionality of these hybrid materials for applications in semiconductor photoelectrochemistry and photocatalysis are examined using electrochemical characterization techniques and an improved understanding of structure and function relationships is achieved using surface-sensitive characterization methods, including grazing angle Fourier transform infrared and X-ray photoelectron spectroscopies.

## PROTON COUPLED ELECTRON TRANSFER IN ARTIFICIAL PHOTOSYNTHETIC CONSTRUCTS

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Controlling the activity of protons around water oxidation and oxygen reduction catalytic sites is important to achieve low overpotentials of these redox processes.[1] Moreover, in natural energy coupling membranes the activity and transport of protons always play a role in the generation and dissipation of protonmotive force. Nature employs a TyrZ-His190 pair as a redox relay intrinsically coupled to proton transfer between P680 and the oxygen evolving complex in PSII. Our artificial redox relays are inspired by the TyrZ-His190 pair and comprise different benzimidazole-phenol couples (BIP, benzimidazole models HIs and phenol models Tyr)[2] with substituents designed to model the hydrogen bond network surrounding Tyrz and His190. When the substituted BIP includes a strong proton acceptor such as a primary or tertiary amine, theory predicts that upon oxidation of the phenol two proton transfers would take place. The first from the phenol to the imidazole and the second from the resulting imidazoleH+ to the amine, resulting in a translocation of protons over a distance of ca. 7 Å. These proton transfers were predicted to be concerted with the oxidation of the phenol. Electrochemical, kinetic isotope effect and spectroelectrochemical data provide strong evidence that this process is indeed observed, and as predicted is concerted. This is a powerful example of the predictive potential of theory, which can be used to guide the rational design of artificial systems for energy conversion. Our artificial system is a step towards controlling the chemical activity and proximity of protons involved in water oxidation. **References:** [1]Llansola-Portolés, M. J. et al., Artificial photosynthesis: from molecular to organic-inorganic nanoconstructs, in From molecules to materials—pathways to artificial photosynthesis. (eds. E. Rozhkova & K. Ariga) 71–98 (Springer, 2015). [2]Megiatto, J. D. Jr., et al., Nature Chem. 2014, 6, 423–428.

## LARGE-SCALE CULTIVATION OF THE CYANOBACTERIUM SYNECHOCOCCUS SP. PCC 7002 AT THE AZCATI/ATP3 ALGAL TESTBED

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The model euryhaline cyanobacterium Synechococcus sp. PCC 7002 has been identified as an excellent platform for biotechnological applications because of its fast growth and ability to thrive in salt water. Under optimal laboratory growth conditions it has a doubling time of 2.6 h, one of the fastest reported for any cyanobacterium. The species was isolated from mud sampled in fish pens on Magueyes Island, Puerto Rico, and thrives in marine estuaries and tidal zones where dramatic fluctuations in nutrient availability, salinity, irradiance, temperature and moisture occur. Synechococcus tolerates high light irradiation (equivalence of two suns), temperatures up to 42°C, and thrives at high salinities and pH. The acquisition of such extraordinary phenotypic plasticity allows rapid acclimation to environmental fluctuations, which are important characteristics of a robust industrial strain. During the summer of 2015 we conducted the first large-scale growth trials of Synechococcus in flat-panel photobioreactors and outdoor raceway ponds at the Arizona Center for Algae Technology and Innovation (AzCATI). The purpose of this work was to validate the potential of Synechococcus as a robust industrial strain, which we tested by cultivation in 110 L flat-panel photobioreactors and 1,025 L outdoor raceway ponds. Modifications to the traditional A+ growth media for Synechococcus included the use of urea as a nitrogen source, and CO2 sparging for automated pH control in the ponds. Here, we report on biomass productivity metrics and predation risks for Synechococcus when cultivated at scale and provide comparisons to traditional industry-standard algal production strains.

## PHOTOSYNTHETIC AND METABOLIC MODULATION IN CHLAMYDOMONAS, NANNOCHLOROPSIS AND SYNECHOCOCCUS

**MATTHEW C. POSEWITZ**[1], ROBERT E. JINKERSON[1], HUIYA GU[1], BRAIN VOGLER[1], AND FIONA DAVIES[1]

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The modulation of carbohydrate and/or protein biosynthesis results in a myriad of poorly characterized metabolic and photosynthetic adjustments. The loss of these photosynthate “sinks” necessitates the rerouting of metabolism and/or the alteration of photosynthetic activities. Quantification of photosynthetic $O_2$ evolution and intracellular metabolite levels indicates that photosynthetic activity is diminished in Chlamydomonas starchless strains, especially under conditions of nutrient deprivation, and that central metabolites accumulate that could be used for fatty acid biosynthesis. Moreover, enhanced respiration of $^{18}O_2$ is observed in the light in starchless strains, consistent with activation of a “water-water” cycle. Interestingly, the photosynthetic machinery is poised for carbon fixation, provided an appropriate metabolic sink is available. Additionally, malonyl-CoA levels are elevated several fold in the starchless strains, but this accumulation does not appear to be effectively utilized by the fatty acid synthase complex. Similar results are observed in other phototrophs suggesting that lipid synthesis in these organisms is regulated at many levels and preliminary data suggests a significant role for posttranslational modifications. Evaluation of the interactions between photosynthetic and metabolic activities is necessary for informed metabolic engineering strategies.

## Poster presenters and titles (listed alphabetical by last name)
## #Undergraduate; *Graduate Student; **Post-doctoral Fellow

1. ABDUREHMAN, RENA#

QUANTITATIVE AND QUALITATIVE ANALYSIS OF PROTEIN TRAFFICKING IN PEA PROTOPLAST PLASTIDS

2. ANDERSSON, BJÖRN*

PHYSIOLOGICAL AND SYSTEMS LEVEL OBSERVATIONS OF SYNECHOCYSTIS PCC 6803 GROWING IN A RAPIDLY FLUCTUATING LIGHT ENVIRONMENT

3. T.J. AVENSON

PROBING INTRA-LEAF PHOTO-PHYSICS VIA DIFFERENTIAL FLUORESCENCE INDUCTION KINETICS

4. BAILEY, SHAUN

CHARACTERIZATION OF LIGHT HARVESTING IN NANNOCHLOROPSIS

5. BRADY, NATHAN*

BIOMOLECULAR BROWNIAN DYNAMIC SIMULATION OF THE DOCKING OF CYANOBACTERIAL CYT. C6 TO THE LUMENAL SURFACE OF PSI

6. CABALLERO, MICHAEL*

DISTINGUISHING THE ROLES OF UDP-GLUCOSE DIPHOSPHORYLASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

7. CANTRELL, MICHAEL*

THE ABSENCE OF EXCITATION DEPENDENT QUENCHING IMPACTS THE FITNESS AND PHOTOPHYSIOLOGY OF CHLAMYDOMONAS REINHARDTII

8. DAY, PHILIP*

TARGETING OF AN ESSENTIAL B-BARREL PROTEIN TO THE CHLOROPLAST OUTER ENVELOPE MEMBRANE

9. DEBUS, RICHARD J.

EVIDENCE FROM FTIR DIFFERENCE SPECTROSCOPY THAT A SUBSTRATE H2O MOLECULE FOR O2 FORMATION IN PHOTOSYSTEM II IS PROVIDED BY THE CA ION OF THE CATALYTIC MN4CAO5 CLUSTER

10. DETAR, RACHAEL*

DISSECTING THE PHYSIOLOGICAL AND GENETIC SIGNIFICANCE OF PLASTID ION TRANSPORTERS BY QUANTIFY-ING THE EXPRESSION OF DIFFERENT METABOLIC AND REGULATORY PATHWAYS IN TRANSPORTER MUTANTS.

11. DOBSON, ZACHARY*

DISCOVERY AND ISOLATION OF NEW HIGH LIGHT TOLERANT PHOTOSYNTHETIC CYANOBACTERIA

12. GANESAN, INIYAN*

EVALUATING THE PORE SIZE OF CHLOROPLAST TOC AND TIC PROTEIN TRANSLOCONS

;

13. GLIBERT, PATRICIA M.

CHANGING NITROGEN FORMS THROUGH NUTRIENT POLLUTION IS ALTERING PHOTOSYNTHETIC PROCESSES IN DOMINANT PHYTOPLANKTON LEADING TO CHANGES IN COMMUNITY COMPOSITION: IMPORTANCE OF PHOTORESPIRATION

14. GISRIEL, CHRISTOPHER**

STRUCTURAL ANALYSIS OF THE HOMODIMERIC REACTION CENTER IN HELIOBACTERIUM MODESTICALDUM

15. JALLET, DENIS**

FUNCTIONAL CHARACTERIZATION OF TWO PUTATIVE PLASTID TERMINAL OXIDASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

16. JOHNSON, WILLIAM*

THE CREATION OF A PROTEOLIPOSOME SYSTEM FOR RECONSTITUTION OF THE HELIOBACTERIAL REACTION CENTER TO PROBE THE ROLE OF MENAQUINONE.

17. KANA, TODD

PROROCENTRUM MINIMUM MAINTAINS HIGH PHOTOSYNTHETIC ELECTRON TRANSPORT UNDER PCO2 STRESS (HIGH PH) BY REDUCING OXYGEN.

18. KLASEK, LAURA*

DEFINING THE MECHANISM OF PROTEIN TARGETING TO CHLOROPLAST MEMBRANES: THE 700-KDA CHAPERONIN COMPLEX

19. KOOCHAK, HANIYEH**

A METHOD FOR QUANTIFICATION OF DIFFERENT PHOTOSYSTEM II ASSEMBLY FORMS IN STACKED AND UNSTACKED THYLAKOID MEMBRANE DOMAINS

20. LASSALLE, LOUISE**

ROOM TEMPERATURE STRUCTURES OF PHOTOSYSTEM II USING X-RAY FREE ELECTRON LASER

21. LI, MENG**

CHARACTERIZATION OF LIGHT INDUCED ARCHITECTURAL CHANGES OF PLANT THYLAKOID MEMBRANES

22. LUO, LAI*

ENHANCEMENT OF CYCLIC ELECTRON FLOW IN THE RICE NPQ-DEFICIENT PSBS KNOCK OUT PLANT

23. MA, LIN

REACTIVE OXYGEN SPECIES PRODUCTION IN RICE MUTANTS WITH ALTERED NON-PHOTOCHEMICAL QUENCHING OF CHLOROPHYLL FLUORESCENCE

24. MONDAL, JYOTIRMOY*

STICKING IT TO PSI: ENHANCING E- TRANSFER THROUGH T. ELONGATUS PSI BY BIOENGINEERING THE PSI:FD INTERFACE

25. MOORE, THOMAS1;

THE END-PERMIAN EXTINCTION AS A WARNING FOR THE ANTHROPOCENE+

26. NGUYEN, JONATHAN#

OLIGOMERIZATION OF CHROOCOCCIDIOPSIS TS-821 PSI IN RESPONSE TO LIGHT LEVELS: A POTENTIAL EARLY MECHANISM FOR CAROTENOID ACCUMULATION AND PHOTO-PROTECTION

27. ORF, GREGORY**

CONSTRUCTION AND UTILIZATION OF A TRACTABLE GENETIC SYSTEM IN HELIOBACTERIUM MODESTICALDUM FOR THE MUTAGENESIS OF THE PSHA REACTION CENTER POLYPEPTIDE

28. SANDERS, ERIKA#

A HEURISTIC APPROACH TO MOTIF IDENTIFICATION AND VERIFICATION IN CHLOROPLAST TRANSIT PEPTIDES

29. SCHAFFER, ALEXANDER*

INVESTIGATING ENVIRONMENTAL EFFECTS ON THE EXPRESSION OF PSBA (D1) VARIANTS IN LEPTOLYNGBYA SP. HERON ISLAND

30. SERBAN, ANDREW*

CHARACTERIZING THE ASSEMBLY MECHANISM OF TOBACCO RCA USING A SINGLE-MOLECULE APPROACH

31. VÖLKNER, CARSTEN*

INVESTIGATING THE FUNCTIONAL LINK BETWEEN ENVELOPE ION TRANSPORT AND PHOTOSYNTHESIS

32. WILLIAMS, JONAH*

FORWARD GENETIC SCREENS FOR THE DISCOVERY OF FUNCTIONAL GLUCAN HYDROLASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

33. WU, GUANGXI*

PHOSPHORYLATION OF CP29 BY STN8 AND ITS POSSIBLE INVOLVEMENT IN THE HIGH LIGHT INDUCED STATE TRANSITION IN MONOCOT RICE PLANTS

34. YOUNG, IRIS**

INSIGHTS INTO THE OXYGEN EVOLVING MECHANISM OF PHOTOSYNTHESIS USING XFEL DIFFRACTION

35. ZHANG,, SHANGJI *

STRUCTURAL STUDY OF F-TYPE ATP SYNTHASE FROM SPINACH CHLOROPLASTS AND HELIOBACTERIUM MOD-ESTICALDUM

## NUMBERED POSTERS ABSTRACTS (WITH PRESENTER IN BOLD)

### POSTER 1: QUANTITATIVE AND QUALITATIVE ANALYSIS OF PROTEIN TRAFFICKING IN PEA PROTOPLAST PLASTIDS

**ABDUREHMAN, RENA**[1]; MONDAL, JYOTIRMOY[1]; HOLBROOK, KRISTEN[1]; BRUCE, BARRY[1,2]

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Proper subcellular protein localization is integral to the function of all eukaryotic cells. Transient yellow fluorescent protein (YFP) expression represents a simple, visible, and quantitative assay that can permit protein localization in living cells to be monitored. Using YFP as a reporter, we will be transiently expressing a chimeric RuBisCO transit peptide utilizing Pisum sativum (pea) protoplasts. Protoplasts are often used in protein trafficking studies for their easy uptake of foreign genes due to their lack of the protective cell wall. Despite being the primary system for in vitro import studies, a reliable protocol for isolation of intact protoplasts does not exist for pea. Our research focuses on developing robust and rapid methodologies for isolating pea protoplasts from the developing leaves of pea seedlings. The main parameters we will be optimizing include protoplast isolation and purity and transfection methodologies. With a highly efficient transformation protocol we can follow up using chloroplast isolation from the transfected protoplasts. With the development of these methodologies, our research will focus on the quantitative and qualitative analysis of subcellular protein localization using optical imaging (bright field and LSC microscopy), FACS, and finally immunoblotting analysis for both the precursor and mature form using antibodies. We intend on providing a highly efficient method of analysis for transient gene expression in the most relevant and pertinent in vivo plant cell system, differentiated chloroplast. Our studies can be further applied to test the subcellular signaling pathways and localization of an array of different proteins utilizing the model plant species, Pisum sativum.

### POSTER 2. PHYSIOLOGICAL AND SYSTEMS LEVEL OBSERVATIONS OF SYNECHOCYSTIS PCC 6803 GROWING IN A RAPIDLY FLUCTUATING LIGHT ENVIRONMENT

**ANDERSSON, BJÖRN**[1]; PARK, SEIJIN[2]; SHEN, CHEN[2]; DANDY, DAVID S.[2]; REARDON, KENNETH F.[2]; PEERS, GRAHAM[1];

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Aquatic phototrophs face fluctuations in light intensity in their natural environments. Growth in mass culture greatly increases the frequency of light fluctuations due to increased cell density and rapid mixing. We hypothesize that this will induce a suboptimal acclimation state, reducing the proportion of absorbed light energy used for carbon fixation. To test this hypothesis, we subjected a dense culture of the model cyanobacterium Synechocystis sp. PCC 6803 to a sinusoidal light regime in bench-top photobioreactors. We used a computational approach to estimate the daily integrated light exposure of an individual cell at 184 µmol photons per m2 s-1. However, cells oscillated between saturating irradiances (averaging 1400 µmol photons per m2 s-1) on the culture surface and limiting irradiances (<1µmol photons per m2 s-1) at the bottom, with an average frequency of 17 s, due to rapid vertical mixing and self-shading. There were no major changes in cell pigment concentrations and slight variations in photosystem II maximal efficiency did not indicate major photodamage throughout the day. We hypothesis that the kinetics of non-photochemical quenching and alternative electron transfer induction reduce the overall photosynthetic efficiency of the culture and we are currently investigating this using pulse amplitude modulated fluorescence and membrane -inlet mass spectrometry techniques. We also collected samples for analysis of the transcriptome and proteome with high temporal resolution and preliminary proteomic results will be presented. We aim to identify novel factors that reduce photosynthetic efficiency in mass culture.

### POSTER 3. PROBING INTRA-LEAF PHOTO-PHYSICS VIA DIFFERENTIAL FLUORESCENCE INDUCTION KINETICS

**T.J. AVENSON,** LICOR BIOSCIENCES, LINCOLN, NEBRASKA, USA

Probing Intra-Leaf Photophysics Via Differential Fluorescence Induction Kinetics Avenson, T.J. LICOR Biosciences, 4647 Superior St., Lincoln, NE 68504 Chlorophyll a fluorescence induction (FI) has been the topic of research for several decades and can provide quantitative information concerning exquisite photophysics controlling the emission of chlorophyll fluorescence from photosystem (PS) II, the pigment-binding protein complex in which photosynthesis is initiated. The various transitions of the fast phase (i.e. within ~300-500 ms of turning on saturating light) of FI can provide detailed information concerning how the photo-

physics occurring within PSII can be impacted by various environmental stresses. Several types of fluorometers have been used during the decades of research aimed at studying FI. Here, we report the use of a newly designed fluorometer that takes advantage of both low intensity pulsed amplitude modulation (PAM) light and high intensity continuous light, the LEDs of which, while being separate, are spectrally identical (LEDs peak at 632 nm). Together they can be used to near simultaneously measure chlorophyll a FI using PAM and continuous light. Using the instrument's standard operating procedure, modest 2-3% differences were observed between the dynamics of FI curves measured using the two procedures in dark-adapted leaves. The amplitudes of the modulation pulses were varied between 10% and 100% of the peak modulation amplitude (~130 µmol m-2 s-1) and the two types of FI kinetics were measured. Small differences (3-5%) were observed between the modulated and continuous FI signals, suggesting that the two light sources, while being significantly different in intensity, 'probed' similar phenomena throughout the depth of the leaves. By contrast, the standard fluorometer was altered such that the modulated LEDs were changed to blue LEDs (peak centered at 430 nm), while the saturating flashes continued to use the standard red LEDs. The resultant fractional differences between the modulated and continuous FI signals were about five times larger than obtained with red modulated LEDs and were characterized by a systematic bias toward negative values (i.e. the modulated FI signal was systematically higher than the continuous FI signal). Salient, oscillatory dynamics were also observed in the differences. These results suggest that being able to measure FI simultaneously with red continuous and blue modulated light may provide an interesting new tool for studying intra-leaf, photo-physical phenomena.

## POSTER 4. CHARACTERIZATION OF LIGHT HARVESTING IN NANNOCHLOROPSIS

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Most oxygenic photoautotrophs respond to changes in their light environment by altering the composition of the photosynthetic machinery, in a process known as photo-acclimation. For eukaryotic algae, changes in irradiance typically elicit adjustments in the overall levels of pigmentation, in particular, cellular chlorophyll content. Decreases in the average levels of irradiance, for sustained periods, typically result in an increase in reaction center content and an increase in the size of the associated antenna. As a consequence of the increase in chlorophyll during low light acclimation, photosynthesis is typically saturated at lower irradiance levels. Since the saturation of photosynthesis is accompanied by the onset of non-photochemical quenching of chlorophyll fluorescence (NPQ), in order to dissipate the excess absorbed excitation energy, low light acclimated photo-autotrophs are prone to squander more incident irradiance as heat in natural light environments. It is predicted that significant improvements in the overall light use efficiency and productivity of algal cultures could be achieved by decreasing the overall chlorophyll content and light harvesting efficiency of individual cells. The result for the culture would be to exchange the losses of absorbed excitation energy, through heat dissipation, for higher saturating irradiance levels of photosynthesis and increased light penetration into the culture. It has been suggested that decreased pigmentation would be most beneficial when it is associated with decreases in the PSII antenna size. To address this, we have comprehensively reduced the pigment and antenna proteins in Nannochloropsis using targeted methods and forward genetic screens. Here we describe those approaches and pick out some key findings, including the discovery of key regulators of light harvesting capacity, association of a single light harvesting protein with NPQ formation and substantial redundancy among light harvesting polypeptides.

## POSTER 5. BIOMOLECULAR BROWNIAN DYNAMIC SIMULATION OF THE DOCKING OF CYANOBACTERIAL CYT. C6 TO THE LUMENAL SURFACE OF PSI

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Cyanobacteria and some algae utilize cytochrome c6 (cyt c6) as the primary electron donor to photosystem 1 (PSI). This protein functions as an e- shuttle between the membrane complexes, cyt. b6/f and PSI. Although the structures of individual cyt c6 and PSI are known for Thermosynechococcus elongatus, the transient nature of the cyt c6 interaction with PSI has proven elusive for crystallization of the binary complex. As a result, the structural details of the binding are poorly understood with multiple proposed pathways: a simple oriented-

collision mechanism, a two-step mechanism requiring complex formation, or a complex formation with rearrangement(s) at the interface prior to electron transfer. Since the binding and electron transport between cyt c6 and PSI occurs on the order of microseconds, it is not easily investigated by molecular dynamics (MD) methods. For these reasons, we have chosen to simulate this interaction using the bimolecular Brownian Dynamics Simulation (BDS). BDS studies these binding processes by animating the intermolecular configurations through time, uncovering the mechanism of association on the diffusion relevant timescales. A PSI monomer, an assembled trimer, and a trimer embedded in native lipids of the thylakoid membrane, are modeled separately to assess the contribution of each component as often done with BDS of heterogeneous biosystems. Simulating this interaction provides potential docking conformations to be assessed for guiding rational engineering of the interface both in silico and in vitro in the hope of enhancing the electron transfer efficiency. Our end goal is to assemble a stable binary complex from the native components for applied photosynthesis and conversion of photons to electrical energy. Because we are free from the in vivo requirement to shuttle between cyt. b6/f and PSI, we may be able to build a device with much faster electron transfer rates than found in nature.

## POSTER 6. DISTINGUISHING THE ROLES OF UDP-GLUCOSE DIPHOSPHORYLASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

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Diatoms are a promising platform for biofuel production due to their high lipid content and rapid growth. Increased lipid productivity can be achieved by redirecting carbon away from storage carbohydrates and into neutral lipids. Diatoms synthesize chrysolaminarin, a storage sugar, from UDP-glucose. Two UDP-glucose diphosphorylases (UGPases) have been identified in the Phaeodactylum genome, but functional assignments of UGP1 and UGP2 to chrysolaminarin synthesis is complicated by the other roles of UDP-glucose in cell metabolism. We intend to functionally define the roles of UGPases in diatoms. We developed a UGPase activity screen and identified a novel target from a Phaeodactylum tricornutum cDNA library, which we named UGP3. Phylogenetic analysis of diatom UGPases suggests that UGP1 and not UGP2 or UGP3 are important for chrysolaminarin synthesis. We generated CRISPR/Cas9 mutants of UGP1, UGP2, or UGP3 in Phaeodactylum and determined chrysolaminarin per cell, BODIPY RFU per cell (a neutral lipid indicator), growth rates, and Fv/Fm. ugp1 strains accumulate 25 % chrysolaminarin per cell and 160 % BODIPY fluorescence per cell relative to WT, but growth rates remain the same. While ugp2 mutants accumulate less chrysolaminarin, they grow slower (0.51 d-1) and have a lower Fv/Fm (0.448) compared to WT (1.02 d-1, 0.601). A lipidomics experiment found the most abundant sulfoquinovosyldiaclyglycerol species was reduced in ugp2 relative to WT suggesting its role in sulfolipid synthesis. ugp3 knockouts do not have a significant difference relative to WT for these observations. A distinct physiological pattern for each knockout suggests different roles for each diatom UGPase and this context improves our understanding of diatom metabolism.

## POSTER 7. THE ABSENCE OF EXCITATION DEPENDENT QUENCHING IMPACTS THE FITNESS AND PHOTOPHYSIOLOGY OF CHLAMYDOMONAS REINHARDTII

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A major component of photoprotection occurs at the level of the light harvesting complexes. Photosynthetic pigments can dissipate excess light energy through processes collectively measured as non-photochemical quenching (NPQ) of chlorophyll fluorescence. A major component of NPQ is the thermal dissipation of excess light energy (excitation dependent quenching, qE). In green algae, qE is regulated by stress-related light harvesting complex proteins (LHCSRs), the xanthophyll cycle and luminal acidification. In this study we use a complete LHCSR knockout (npq4lhcsr1) to investigate the importance of qE to fitness and maintenance of photosynthetic capacity under continuous light (50, 400 and 860 µmol photons m-2 s-1) and in a photobioreactor simulating a natural light regime. The npq4lhcsr1 mutant had no observable qE, regardless of growth condition. Under 860 µmol photons m-2 s-1 continuous light, loss of qE correlated with a significant reduction in growth rate compared to wild type (WT). This reduction in growth was correlated with reductions in maximum net oxygen evolution capacity and a reduced maximum quantum yield of PSII. Surprisingly, photoinhibition in npq4lhcsr1 was not associated with increased lipid peroxidation. The npq4lhcsr1 strain had reduced growth rates compared to WT in sinusoidal light conditions. In contrast with continuous light, npq4lhcsr1 has a higher maximum net oxygen evolution rate at solar noon compared to WT and maintains a high, but significantly reduced maximum quantum yield of PSII relative to WT. Quantification of the D2 subunit of PSII and the PsaC subunit of PSI by immunoblot indicates an increase in the ratio of PSII:PSI in the mutant vs. WT. Despite equivalent carbon accumulation rates during the

day, npq4lhcsr1 cultures displayed reduced cell division at night. These findings demonstrate the importance of qE in maintaining fitness in both continuous and natural light conditions.

## POSTER 8. TARGETING OF AN ESSENTIAL B-BARREL PROTEIN TO THE CHLOROPLAST OUTER ENVELOPE MEMBRANE

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The majority of chloroplast proteins are targeted to the interior compartments by transit peptides that are removed after import. This is not the case for most of the proteins in the chloroplast outer envelope membrane (OEM), whether their transmembrane domains are β-barrels or α-helices. This is not surprising since OEM proteins should not need to enter the stroma, where the transit peptide would be removed. Toc75, a central component of the translocon at the outer envelope membrane of chloroplasts, is an exception. The Toc75 N-terminal targeting information is comprised of a chloroplast import signal and an envelope-sorting signal in tandem. In green plants Toc75 coexists with its paralog OEP80. The function of OEP80 has not been experimentally verified, but like Toc75, it is essential for plant viability. Our results using in vitro chloroplast import assays suggest that OEP80 also uses N-terminal targeting information that is removed after import. Unlike the N terminus of Toc75, OEP80's N terminus is not sufficient for import, in that the C-terminal transmembrane β-barrel is also required. OEP80 and Toc75 are comprised of three polypeptide transport-associated (POTRA) domains likely located in the intermembrane space, and a transmembrane β-barrel. A synthetic construct consisting solely of OEP80's transmembrane β-barrel can be imported into chloroplasts without the aid of the N-terminal targeting information. Our working hypothesis is that OEP80's β-barrel is imported in a similar manner as other chloroplast β-barrels and the N-terminal targeting information is necessary to accommodate the intermembrane space localized POTRA domains. This research will add to our knowledge of β-barrel targeting to the chloroplast OEM which is currently poorly understood.

## POSTER 9. EVIDENCE FROM FTIR DIFFERENCE SPECTROSCOPY THAT A SUBSTRATE $H_2O$ MOLECULE FOR O2 FORMATION IN PHOTOSYSTEM II IS PROVIDED BY THE CA ION OF THE CATALYTIC MN4CAO5 CLUSTER

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Evidence from FTIR Difference Spectroscopy that a Substrate H2O Molecule for O2 Formation in Photosystem II is Provided by the Ca Ion of the Catalytic Mn4CaO5 Cluster Christopher J. Kim and Richard J. Debus*, Department of Biochemistry, University of California, Riverside, CA USA The O2-producing Mn4CaO5 catalyst in Photosystem II oxidizes two molecules of water (substrate) to produce one molecule of molecular oxygen. Considerable evidence supports identifying one of the two substrate water molecules as the Mn4CaO5 cluster's oxo bridge known as O5. The identity of the second substrate water molecule is less clear. In one set of models, the second substrate is the Mn-bound water molecule known as W2. In a competing set of models the second substrate is the Ca2+-bound water molecule known as W3. In both sets of models, a deprotonated form of the second substrate moves to a position next to O5 during the catalytic step immediately prior to O-O bond formation. To differentiate between the two sets of models, FTIR difference spectroscopy was employed to identify the vibrational modes of hydrogen-bonded water molecules that are altered by the substitution of Sr2+ for Ca2+. The spectral regions examined included the carbonyl stretching modes of carboxylic acids involved in networks of hydrogen bonds surrounding the Mn4CaO5 cluster, the O−H stretching region of hydrogen-bonded water molecules, and the D-O-D bending region (D2O was employed instead of H2O because D-O-D bending modes appear in a less congested region of the FTIR spectrum). Crystallographic and computational studies have shown that only three water molecules have their positions shifted substantially by the substitution of Sr2+ for Ca2+. These are W3, the Ca2+-bound W4, and the W5 water molecule that bridges W2 and W3 by hydrogen bonds. The FTIR data presented in this study show that the substitution of Sr2+ for Ca2+ substantially alters the vibrational modes of only a single water molecule: the water molecule whose D-O-D bending mode is eliminated during the catalytic step immediately prior to O-O bond formation. These data are most consistent with identifying the Ca2+-bound W3 as the second substrate involved in O-O bond formation. It is suggested that W5 moves to replace the coordination position on Ca2+ vacated by W3, in agreement with one of the conclusions of a recent DFT study.

## POSTER 10. DISSECTING THE PHYSIOLOGICAL AND GENETIC SIGNIFICANCE OF PLASTID ION TRANSPORTERS BY QUANTIFYING THE EXPRESSION OF DIFFERENT METABOLIC AND REGULATORY PATHWAYS IN TRANSPORTER MUTANTS.

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Ion gradients across the chloroplast envelope and thylakoid membranes are crucial for organelle function and photosynthesis. These functions include regulating osmotic balance, maintaining chloroplast shape and architecture, regulating key photosynthetic enzymes, and finally the maintenance and regulation of the proton motive force (PMF) to generate ATP. While it is known that ion and pH gradients are important for the regulation of photosynthetic proteins and membranes, little is known about how ion gradients may impact gene expression, and more specifically, retrograde signaling by disruption of photosynthesis. To better understand how chloroplast ion gradients impact gene transcription, we ran an RNA sequencing experiment on kea1kea2 double mutants which lack two main K+/H+ antiporters in the chloroplast inner envelope membrane. Simultaneous loss of function in kea1 and kea2 results in reduced photosynthetic capacity, and altered PMF partitioning. The primary objective of our study is to examine to what extent the phenotype corresponds to downregulation of nucleus encoded photosynthetic genes. Our bioinformatics analysis on RNA sequencing data reveal a slew of deregulated genes in the kea1kea2 double mutants compared to wildtype controls. This includes downregulation of genes related to chlorophyll and heme biosynthesis, a pathway that plays a key role in retrograde signaling. Additionally, genes encoding proteins associated with circadian rhythm, and chloroplast rRNA processing where found to be differentially expressed in kea1kea2 mutants. Interestingly, kea1kea2 double mutant treated with 75 mM NaCl which to triggers visual phenotypic rescuing led to more wildtype-like gene expression for genes in these aforementioned pathways. Our experiment provides global insights into the wide-ranging importance of intact plastid ion flux for transcriptional control of photosynthesis, chloroplast development and signaling processes that involve the plant chloroplast. On my poster, I will present more detailed transcriptional network and pathway analysis and discuss these data along with future research directions.

## POSTER 11. DISCOVERY AND ISOLATION OF NEW HIGH LIGHT TOLERANT PHOTOSYNTHETIC CYANOBACTERIA

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In nature, Photosystem II (PSII) has a half-life between 30 minutes to 1 hour in high light conditions. This is due to the photodamage and subsequent degradation to the D1 subunit. Because PSII is vital for photosynthetic organisms, there is a massive amount of energy used to repair this protein. If this damage, and thus repair process, could be avoided, photosynthetic organisms would be able to focus more of the energy they capture from the sun on growth. We report the discovery and isolation of a new photosynthetic cyanobacteria that exhibits a high tolerance to light. In order to determine how this organism is surviving in high light conditions, we want to investigate PSII specifically. We plan to try to crystallize the PSII from this organism to determine if there is anything inherently different from either of the two known structures of PSII from *Thermosynechococcus elongates* or *Thermosynechococcus vulcanus.*

## POSTER 12. EVALUATING THE PORE SIZE OF CHLOROPLAST TOC AND TIC PROTEIN TRANSLOCONS

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Proteins are generally thought to traverse canonical proteinaceous translocons in an unfolded conformation, as is the case for the mitochondrial membrane translocons (TOM and TIM) and SecYEG. Translocation of dihydrofolate reductase (DHFR) through TOM/TIM and SecYEG is inhibited by the stabilizing DHFR inhibitor, methotrexate (MTX), which prevents DHFR unfolding by the translocons. However, MTX does not significantly inhibit import of DHFR through the translocons of the outer and inner chloroplast membranes (TOC and TIC). Chloroplast TOC and TIC are able to tolerate small folded proteins, such as internally cross-linked bovine pancreatic trypsin inhibitor, a 6.5kDa protein, but it is unclear if larger folded proteins are tolerated. Here, we show that the 22kDa DHFR is in fact translocated in complex with MTX, and is therefore translocated in a folded conformation. When DHFR was imported into chloroplasts in the presence of sub-saturating MTX concentrations, DHFR was still detected in complex with MTX within the chloroplasts. To independently measure the degree of precursor foldedness tolerated by TOC/TIC, the functional translocon pore size was probed with particles of fixed diameters conjugated to precursor proteins. Precursors conjugated to 20Å and 26.5Å particles were imported into chloroplasts, indicating the TOC/TIC maximum pore size is greater than 26.5Å. This pore size supports the idea that DHFR, with an average minor axis diameter of 27Å, is imported through TOC/TIC in a folded conformation.

## POSTER 13. CHANGING NITROGEN FORMS THROUGH NUTRIENT POLLUTION IS ALTERING PHOTOSYNTHETIC PROCESSES IN DOMINANT PHYTOPLANKTON LEADING TO CHANGES IN COMMUNITY COMPOSITION: IMPORTANCE OF PHOTORESPIRATION

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Anthropogenic activities are altering both total nutrient loads, and they are changing the dominant form of N nutrient delivered to many coastal marine and freshwater systems. While the major oxidized form of N, nitrate (NO3-), is the dominant N form contributing to eutrophication in many aquatic ecosystems, there are several reasons why high loads of chemically-reduced forms of N, such as ammonium (NH4+), urea, and dissolved organic nitrogen have increased. Differential phytoplankton community composition has been associated with different forms of N, it has also been documented that under conditions of highly elevated NH4+, typically exceeding several tens to hundreds of µM, both the total N taken up and overall growth with NH4+ enrichment can be suppressed rather than enhanced. Different mechanisms for rebalancing cellular redox under conditions of non-steady-state growth may function in diatoms under different environmental conditions. Under cool, NO3--rich conditions, dissimilatory NO3-/NO2- reduction to NH4+ serves as a major sink for excess reductant. Under warm conditions, but with NO3- as the dominant N substrate, the activity of NR is reduced (but dissimilatory NO3-/NO2- reduction to NH4+ remains important), but that of Rubisco also increases, with the result that overall rates of C fixation are proportionately higher. Under cool temperatures with NH4+ as the primary N substrate, the cell more likely balances its redox state mainly through photorespiration. Finally, under warm conditions with NH4+ as the dominant N substrate, C assimilation increases due to the higher temperature optima of Rubisco, but photorespiratory rates remain high. However, these pathways also have negative feedbacks and consequences on cell metabolism when homeostasis is not attained. Increases in NH4+ have also been related to increased polyamine synthesis. Polyamines are key precursors for the siliceous cell walls of diatoms. When polyamine synthesis is overexpressed, typically as a result of lack of reducing power, thickened cell walls may be produced, leading to enhanced cell sinking.

## POSTER 14. STRUCTURAL ANALYSIS OF THE HOMODIMERIC REACTION CENTER IN HELIOBACTERIUM MODESTICALDUM

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Heliobacteria are anaerobic bacteria that employ a Type I homodimeric reaction center (RC) to perform light-driven transfer of an electron from the periplasm to the cytosol to drive metabolic processes. To date, the RCs for which high-resolution structures are available are of heterodimeric RCs (purple bacterial RC, PSI, PSII). No structures of any homodimeric type I RCs from anoxygenic phototrophs (heliobacteria, green sulfur bacteria, acidobacteria) have been solved. Like its closest homolog, Photosystem I (PSI), the HbRC has 22 transmembrane helices per homodimer. The two PshA polypeptides are related by a C2 symmetry axis normal to the membrane plane, along which the electron transfer cofactors are coordinated. A dense network of BChl g, a pigment unique to Heliobacteria, serves as an antenna system for the delivery of energy to the electron transfer cofactors, similar to that observed in PSI. Specifically, the HbRC coordinates more than 40 BChl g, 2 BChl g', 2 81 -OH Chl a, 2 carotenoids, and 1 [4Fe-4S] cluster. The homodimeric complex has a molecular weight of ~180 kDa. Although there are significant differences in known energy transfer and electron transport pathways, the HbRC maintains structural homology to PSI and other RCs. Because the HbRC is also the simplest of all RCs, this analysis provides significant insight into the how the common ancestor of all RCs may have evolved. (Supported by DOE grant DE-SC0010575)

## POSTER 15. FUNCTIONAL CHARACTERIZATION OF TWO PUTATIVE PLASTID TERMINAL OXIDASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

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Alternative Electron Transport (AET) is the transfer of electrons from photosystems to molecular oxygen. Flavodiiron proteins and Plastid Terminal Oxidases (PTOXs) mediate AET in cyanobacteria and eukaryotic algae, respectively. PTOXs not only play a photoprotective role in Chlorophyte algae but also contribute to chlororespiration. The role of PTOXs in Stramenopile algae - like diatoms - has not been studied to date. We present the first characterization of putative PTOX (pPTOX) function in the model diatom Phaeodactylum tricornutum. Phaeodactylum possesses two pPTOXs: pPTOXA and pPTOXB. Both pPTOXs retain all conserved domains required for PTOX activity and are predicted to be targeted to chloroplasts. The pPTOXB transcript appeared ten times more abundant than the pPTOXA transcript 6h after dawn under a 12h:12h light:dark cycle. We generated pPTOXA as well as pPTOXB knock-outs using the recently developed CRISPR-Cas9 system. The pPTOXA KO lines grew normally compared to wild-type (WT) when cultivated under continuous low light or high light with air sparging.

Previously studied Chlamydomonas reinhardtii PTOX deficient mutants displayed a post-illumination dark fluorescence rise linked to non-photochemical plastoquinone reduction. We observed such a fluorescence rise in WT Phaeodactylum cells only when applying PTOX inhibitors such as propylgallate or octylgallate. The pPTOXA KO strains behaved like WT, suggesting that pPTOXA plays a minor role in chlororespiration. The characterization of pPTOXB KO lines is still in progress and will also be presented.

**POSTER 16. THE CREATION OF A PROTEOLIPOSOME SYSTEM FOR RECONSTITUTION OF THE HELIOBACTERIAL REACTION CENTER TO PROBE THE ROLE OF MENAQUINONE.**

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This investigation utilizes membrane mimics (liposomes) infused with menaquinone (MQ). Purified heliobacterial reaction center (HbRC) is then reconstituted into the liposomes. The liposomes are decorated with recombinant cytochrome c553 with a hexahistidine tag that binds via an Ni(NTA)-lipid. The system was used to test the hypothesis that the HbRC can photoreduce MQ to MQH2, as suggested by experiments in our lab using crude heliobacterial membrane suspensions. In fact, the role of MQ in the HbRC is not fully understood. There is evidence that supports, as well as contradicts, MQ involvement in electron transfer through the HbRC. This system provides a controlled mock-native environment to test the ability of the HbRC to photoreduce MQ to MQH2. Although the heliobacterial photosynthetic apparatus is a type I reaction center, the data in this study suggests that the HbRC can act in a way that was previously thought to be restricted to type II RCs. The reduction of P800+, by cytochrome c553 within the liposome system, was monitored using pump-probe absorption spectroscopy. The photoreduction of MQ to MQH2 was analyzed using high performance liquid chromatography.

**POSTER 17. PROROCENTRUM MINIMUM MAINTAINS HIGH PHOTOSYNTHETIC ELECTRON TRANSPORT UNDER PCO2 STRESS (HIGH PH) BY REDUCING OXYGEN.**

**KANA, TODD**

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Prorocentrum minimum Maintains High Photosynthetic Electron Transport Under pCO2 Stress (High pH) by Reducing Oxygen. Todd M. Kana, University of Maryland Center for Environmental Science Horn Point Laboratory, Cambridge, MD 21613 and Bay Instruments, Easton, MD 21601 The dinoflagellate, Prorocentrum minimum, was found to sustain high rates of oxygen cycling in the light under conditions of low pCO2 (induced by high pH). Irradiance curves conducted under ambient pCO2 (pH 8) exhibited oxygen uptake rates that were 20-30% of the gross oxygen evolution (E0) rate over most irradiances. However, under pCO2 stress conditions with E0 at 30% of maximum, O2 reduction accounted for 100% of the electron flow from PSII at all irradiances. During pH titration experiments, E0 remained high until pCO2 declined to 10 ppm, whereas a comparative experiment with the cyanobacterium, Synechococcus, showed a gradual decrease in E0 to full inhibition at 25 ppm CO2 and minimal increase in oxygen uptake. Measurements of oxygen isotope discrimination under pCO2 replete conditions, with and without KCN, showed a shift from e=-20 (-KCN) to e=-27 (+KCN), suggested that Alternative respiration was present but limited in importance under such conditions. Inhibition by DCMU and DBMIB rules out chlororespiration. The importance of downstream reactions (Rubisco and mitochondrial) for O2 uptake is suggested by high (50%) residual uptake under DCMU treatment. We speculate that photorespiration may play an important role in a Form 2 Rubisco organism. P. minimum is a bloom-forming species that can drive the ambient pH to >9 and therefore survives pCO2 stress by maintaining high electron transport rates. That ~100% of photosynthetic electron transport under such conditions leads to oxygen reduction suggests that there is little photosynthetic growth potential in intense blooms depite high PSII activity. The known H2O2 producing capability of this species may be linked to the photosynthetic oxygen cycling described here.

**POSTER 18. DEFINING THE MECHANISM OF PROTEIN TARGETING TO CHLOROPLAST MEMBRANES: THE 700-KDA CHAPERONIN COMPLEX**

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Title of abstract: Defining the Mechanism of Protein Targeting to Chloroplast Membranes: the 700-kDa Chaperonin Complex Laura Klasek1*, Joshua K. Endow1, Steven M. Theg2, Kentaro Inoue1 1Department of Plant Sciences, University of California, Davis, Davis, California, USA 2Department of Plant Biology, University of California, Davis, Davis, California, USA Body of abstract: Chloroplasts house oxygenic photosynthesis, which is essential for life on earth. Photosynthetic electron transfer and ATP

synthesis occur at the thylakoid membrane. Thylakoid development requires coordinated synthesis, delivery, and assembly of its components, and we do not fully understand the mechanism by which this occurs. Plastidic type I signal peptidase 1 (Plsp1) is a useful marker for thylakoid development. Plsp1 functions in removal of targeting signals at envelope and thylakoid, and its abundance in each membrane correlates with thylakoid development. Plsp1's thylakoid targeting occurs via cpSec1; however, a second membrane-bound product occurs spontaneously during in vitro targeting. Data suggests it arises from premature folding of the catalytic domain that then binds to the stromal face of thylakoids. Plsp1's stromal intermediate is found in a 700-kDa complex that co-migrates with chloroplast chaperonins (Cpn60). We hypothesize that the 700-kDa complex keeps Plsp1 unfolded in the stroma. In the present work, we began characterization of the 700-kDa complex by in vitro chloroplast import and pull-down assays. Deletion of Plsp1's stromal N-terminus affected neither 700-kDa complex formation nor targeting, while deletion of its transmembrane domain (TMD) resulted in greater soluble localization and increased 700-kDa complex formation. His-mPlsp1ΔTMD pulled down Cpn60 from a stromal extract. These results indicate Plsp1 directly binds Cpn60 at a region within Plsp1's C-terminus. Cpn60 has established roles in folding, but our data suggest participation in protein sorting. Future work will define the role of Cpn10/20 and the mechanism of capture and release of Plsp1 by Cpn60, extending understanding of the interface of import, sorting, and assembly of proteins.

## POSTER 19. A METHOD FOR QUANTIFICATION OF DIFFERENT PHOTOSYSTEM II ASSEMBLY FORMS IN STACKED AND UNSTACKED THYLAKOID MEMBRANE DOMAINS

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In higher plants thylakoid membranes are sub-compartmented into stacked grana core and unstacked grana margins and stroma lamellae. The majority of the water-splitting photosystem II (PSII) is located in the tightly stacked core granal regions. In grana PSII is organized as a core dimer with minor light harvesting complexes (LHCII) and four major trimeric LHCII forming the massive so-called C2S2M2 holocomplex (C=core, S=strongly bound LHCII, M=moderately bound LHCII). But also unstacked grana margins and stroma lamellae contain PSII most likely involved in the repair of photodamaged PSII and biogenesis. The PSII assembly forms in unstacked thylakoid regions, however, are less well characterized. Knowledge of the assembly forms and their quantification in all thylakoid domains is prerequisite for full understanding of PSII repair and biogenesis. In fact, the present methods are not adequate for an elucidation and quantification of various PSII assembly forms occurring in thylakoid membranes under different conditions. In this research, quantifying different PSII assembly forms in different sub-compartments of thylakoid were performed using a new method based on Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) and dot immunoblotting. To add, in order to study the impact of thylakoid lipids on supercomplex assembly and the supramolecular protein arrangement in grana membrane, Arabidopsis lipid mutants will be compared with wild type with regards to the quantification of PSII assembly forms in stacked and unstacked thylakoid domains. Our results on grana core, grana margins and stroma lamellae quantification reveal distinct sets of PSII assembly forms in the different domains, underlying the spatial and temporal separation of PSII repair reactions and components in the thylakoid membrane. It was recognized that the grana margin could be a place of protein degradation and the stroma lamellae is the site of de novo protein synthesis and PSII reassembly. Funding: This research was supported by the National Science Foundation (MCB-30376375).

## POSTER 20. ROOM TEMPERATURE STRUCTURES OF PHOTOSYSTEM II USING X-RAY FREE ELECTRON LASER

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Photosynthesis converts light energy into biologically useful chemical energy vital to life on Earth. This process involves photosystem II (PSII), a homodimeric membrane protein complex. PSII catalyzes the photo oxidation of water into dioxygen at the Mn4CaO5 cluster in the oxygen-evolving complex (OEC). Under illumination, the OEC cycles through five intermediate S-states (S0 to S4) (1), where S1 is the dark-stable state and S3 is the last semi-stable state before O-O bond formation and O2 evolution (2). The structure of PSII in the dark state at cryogenic temperatures has been solved using X-ray diffraction at X-ray free electron laser (XFEL). To understand the O-O bond formation mechanism, elucidating the structures of the OEC in the different S-states is a prerequisite. We recently determined the room temperature structures of Photosystem II in the illuminated S3-state at 2.25 Å and

the dark S1-state at 3.0 Å resolution obtained using an XFEL (3). Major structural changes are not observed either in the peptide backbone or the Mn4CaO5 cluster between the dark and illuminated states, precluding mechanisms that require large changes in the S3 state. The potential binding sites for NH3, a water analog, are also discussed, based on the 2-flash illuminated state data, which has important implications for the mechanism of water oxidation.

## POSTER 21. CHARACTERIZATION OF LIGHT INDUCED ARCHITECTURAL CHANGES OF PLANT THYLAKOID MEMBRANES

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Characterization of Light Induced Architectural Changes of Plant Thylakoid Membranes Meng Li1* and Helmut Kirchhoff1 1Institute of Biological Chemistry, Washington State University, Pullman WA 99164 Plant thylakoid membranes form tightly stacked grana that are connected by stoma lamellae. The two different compartments harbor different membrane protein complexes involved in photosynthesis, with most functional PSII found in grana whereas PSI and the ATPase are confined to unstacked regions. In the dark, the grana have a relatively constant diameter in the range of 400-600 nm, however, the structure of grana is highly dynamic. Reports have shown grana structural differences between light versus dark adapted plants: with bigger lumen space and extension of grana margins in the light. This structural changes of the thylakoid system facilitates electron transport and the PSII repair cycle. However, the current understanding of thylakoid membrane architectural changes is restricted mainly to two light intensities only. To understand the full dynamic range of thylakoid membrane alterations, thylakoid membrane ultrastructure will be investigated under different light intensities by state-of-the art electron microscopy (high pressure freezing and freeze substitution of Arabidopsis leaf discs followed by thin sectioning). One focus will be the correlation between the photoprotective high energy quenching and the thylakoid membrane ultrastructural changes. In addition, so far no information exists about how fast thylakoid membranes change their shape. Therefore, we will study the kinetics of architectural changes in response to light. The preliminary results of those investigations will be presented. Funding: DOE-BES #127673-001

## POSTER 22. ENHANCEMENT OF CYCLIC ELECTRON FLOW IN THE RICE NPQ-DEFICIENT PSBS KNOCK OUT PLANT

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Rice PsbS knock out (KO) plant (OsPsbS-KO) leaves lack energy-dependent part of non-photochemical quenching (NPQ) and is therefore sensitive to photoinhibition. As compensation for the lack of an important protection mechanism, NPQ, against high light stress, the efficiency of the cyclic electron flow around PSI was enhanced in OsPsbS-KO, while there is no noticeable difference in linear electron flow between the mutant and wild-type (WT) plants. This aroused our interest to investigate the relation between NPQ and cyclic electron flow around PSI using OsPsbS-KO and WT. With dark adaptation, the activity of PGR5-dependent route was significantly high in mutant comparing with WT, when comparing the rate of P700 photooxidation. In addition, the infiltration of leaves with 150 mM sorbitol, 40 μM DCMU plus 2 mM hydroxylamine to induce the electron transport pathway switched to cyclic mode led to a remarkable difference between dark-adapted WT and PsbS-KO rice leaves. However, the difference disappeared when leaves were infiltrated with antimycin A to inhibit the PGR5-dependent route. A rise in a chlorophyll fluorescence parameter, Fo after turning off actinic light is a sign of NDH-dependent route, and to achieve the Fo level in the mutants comparable to that of the WT, pre-illumination at a certain dose of light was required in the mutant. Take together, these results lead us to propose that, in the absence of qE, cyclic electron flow (CEF) around PSI can be as alternative pathway for protection from excess energy absorbed by C3 plants. Moreover, that the strong activity of PGR5-dependent CEF is probably responsible for the slower activation of the NDH-dependent CEF in the mutant.

## POSTER 23. REACTIVE OXYGEN SPECIES PRODUCTION IN RICE MUTANTS WITH ALTERED NON-PHOTOCHEMICAL QUENCHING OF CHLOROPHYLL FLUORESCENCE

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Photosynthesis uses light energy to convert it to chemical energy. However, light is also dangerous when it's exceeding the capacity of the electron transport in chloroplasts. Therefore plants evolved several protective mechanisms, including non-photochemical quenching (NPQ) of chlorophyll fluorescence. When NPQ is inhibited, one might expect more reactive oxygen species (ROS) to be produced in chloroplasts. Therefore in this research we

have used two different rice mutants, OsPsbS-knock out (KO) and OsStn8-KO mutants, with decreased NPQ to investigate types of ROS produced and their production sites. Photosystem (PS) II in detached leaves of both OsPsbS-KO and OssStn8-KO mutants were more sensitive to photoinhibitory illumination compared with wild type (WT) plants. Using histochemicalassay and spectrophotometric methods monitoring absorbance and fluorescence changes, we determined the levels of ROS, including singlet oxygen, superoxide and hydrogen peroxide in leaves and thylakoids as well. Both of thetwo NPQ-less rice mutants interestingly generated more superoxide and hydrogen peroxidein their chloroplasts. However, singlet oxygen production was not enhanced in the two rice mutants under our experimental conditions. To clarify the site of ROS production, we have isolated different pigment-protein complexes of thylakoids. We observed that PSII complexes isolated via different methods from both mutants produced more superoxide compared with the wild type. Also time-course experiments using isolated thylakoids showed that superoxide production was the initial event that production of hydrogen peroxide proceeded from that. However, the production sites of superoxide in PSII seem to be different between the two mutants according to protein oxidation assays.

## POSTER 24. STICKING IT TO PSI: ENHANCING E- TRANSFER THROUGH T. ELONGATUS PSI BY BIOENGINEERING THE PSI:FD INTERFACE

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Ferredoxin (Fd) shuttles electrons from photosystem I (PSI) to Fd-NADPH reductase (FNR) within the stroma of chloroplasts and cyanobacteria. This role requires delicate balance between affinity for PSI/FNR and diffusion between these two binding partners in the stroma. However, unlike the in vivo process, our work on applied photosynthesis does not require a tradeoff between binding affinity and stromal diffusion, with the goal of building a near solid state system with maximum rates of forward electron transfer. The atomic level interaction between Fd and PSI is still unclear and not much is known about the binding partners in this interaction, neither we have a crystal structure of the complex itself. This interaction is crucial to understand for assembling our model biohybrid solar cells. Previously we have computationally shown that Fd interacts with the stromal subunits of PSI (PsaC/PsaD/PsaE) in three different possible conformations of which two of them have Fd in a nearly 180° rotation. Nevertheless, these conformations have highly 'frustrated' regions that are involved in there binding, but the interaction is not tight enough for them to remain as a complex as the biological significance of this interaction is limited to shuttling of reduced Fd from PSI to FNR. For the purpose of building our biohybrid solar cells we have engineered Fd with a TiO2 binding peptide, hence a modified Fd (LSTB1-Fd), attaching Fd in vitro to the TiO2 nanoparticles. Firstly, we have computationally generated single (S63D/E/W and F38A/W) and double mutants (combination of both) of LSTB1-Fd, and looked into the interaction with PSI by rigid body docking and molecular dynamic simulation. These mutations can possibly lead to an enhanced or 'sticky' interaction between the two proteins. To confirm this, we have experimentally generated these mutants and begun to perform in vitro binding assays with PSI including chemical crosslinking, back-scattering interferometry, and SPR. Our goal is to have an enhanced affinity for a sticky binding of Fd with PSI to increase the electron transfer in diffusion-free fashion.

## POSTER 25. THE END-PERMIAN EXTINCTION AS A WARNING FOR THE ANTHROPOCENE+

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Photosynthesis operating at ca. 156 TW net primary production (NPP) powers most of the biosphere and on the geological time scale has produced large accumulations of reduced carbon collectively known as fossil fuels.[1,2] Over multiple glacial/interglacial periods photosynthesis has been the major driver of the "fast" global carbon cycle in which the production of CO2 from respiration and decay of organic material is balanced by photosynthetic CO2 reduction. Human activity transfers carbon from the "slow" to the "fast" carbon cycle, which has overwhelmed the capacity of photosynthesis to control CO2 levels. As a consequence, CO2 levels are rising in the atmosphere and oceans and are higher than ever experienced by human societies. Worryingly, a new theory posits that 252 million years ago methanogens could have carried out a similar transfer of carbon resulting in the end-Permian extinction (3rd mass extinction event) in which 90+ % of species became extinct.[3] Our poster points out inconvenient parallels between the behavior of methanogens and humans when requisite factors are present including a seemingly limitless supply of chemical potential in the form of reduced carbon. A similar abstract was submitted to the 79th Harden Conference, Innsbruck, Austria, 16 – 20 April 2016. **References:** [1]Schramski, et al., Human domination of the biosphere: Rapid

discharge of the earth-space battery foretells the future of humankind, PNAS, 2015, 112, 9511 [2]Sherman, et al., Photosynth. Res. 2013, 120, 59-70; Llansola-Portoles, et al., "From Molecules to Materials" 2015, Springer ISBN 978-3-319-13800-8 [3]Rothman et al., Methanogenic burst in the end-Permian carbon cycle, PNAS, 2014. 111, 5462-5467

## POSTER 26. OLIGOMERIZATION OF CHROOCOCCIDIOPSIS TS-821 PSI IN RESPONSE TO LIGHT LEVELS: A POTENTIAL EARLY MECHANISM FOR CAROTENOID ACCUMULATION AND PHOTO-PROTECTION

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Photosystem I (PSI) has been reported to mainly form trimeric complexes in cyanobacteria. However, a tetrameric form of PSI has since been discovered in several cyanobacteria including the thermophile *Chroococcidiopsis* sp. TS-821. Under lab culture conditions, PSI in these cyanobacteria has been predominately in equilibrium between a tetrameric and dimeric form. Cryo-TEM indicates that the tetramer is really a dimer of dimers with different subunit interfaces with the dimer and between dimers in the tetramer. However, we have also shown that under very low light conditions this organism can also form a trimeric form as well. The formation of tetrameric PSI is thought to be caused by high light conditions. To investigate the assembly and functional role of this tetrameric form we have begun looking at how the tetrameric form dissociates into a PSI dimer. We find that increasing the level of surfactant of B-DDM to 5% completely disrupts an isolated tetramer into only dimers, yet does not yield any trimers. Following this disruption, we observe that when the PSI dimer is loaded on to a sucrose density gradient for ultracentrifugation we observe a new, deep orange fraction that stays at the top of the gradient. This fraction has been analyzed for both pigment and protein content. We are now investigating the potential role of a novel carotenoid-binding protein using tandem mass spectrometry and proteomic analysis. Extraction of these carotenoids and performing proteomic analysis on them potentially gives us the statistical ability to identify and assign a gene product to this fraction of carotenoids. These carotenoids are generally known for photo-protection under conditions of high light stress for cyanobacteria.

## POSTER 27. CONSTRUCTION AND UTILIZATION OF A TRACTABLE GENETIC SYSTEM IN HELIOBACTERIUM MODESTICALDUM FOR THE MUTAGENESIS OF THE PSHA REACTION CENTER POLYPEPTIDE

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The type I reaction center from photosynthetic Heliobacteria (HbRC) is the simplest known chlorin-protein complex capable of converting solar energy into chemical energy. It is composed of only two copies of the PshA polypeptide and has no peripheral antenna proteins. Because of this simplicity, the HbRC is likely the extant reaction center with most similarity to the last common ancestor of all reaction centers. Therefore, mutagenesis of the PshA polypeptide would be beneficial for determining the base requirements for a functioning reaction center, as well as which structural changes are necessary for the evolution of type II and heterodimeric reaction centers. Unfortunately, a tractable genetic system in Heliobacteria has not yet been fully developed. However, some techniques useful for the genetic manipulation of other members of phylum Firmicutes, such as the Clostridia, are directly applicable to Heliobacteria. Here, we will provide an update on our progress towards a tractable genetic system in Heliobacterium modesticaldum using these techniques, including data on transformation procedures, plasmid isolation, chromosomal gene knock-outs, promoter design, and expression of heterologous reporter proteins. We will also present use of this tool set for point mutation of the PshA polypeptide, as well as the use of differential tag affinity chromatography to purify mutant PshA heterodimers.

## POSTER 28. A HEURISTIC APPROACH TO MOTIF IDENTIFICATION AND VERIFICATION IN CHLOROPLAST TRANSIT PEPTIDES

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Despite over forty years of investigation the functional elements of chloroplast transit peptides (TP) has evaded elucidation. This elusivity is in part due to the high variability of the targeting sequence and possibly the fundamental plasticity of the organelle itself. Recently, our lab has identified two conserved elements in TPs: an N-terminal Hsp70 recognition domain and a central FGLK motif shown to interact *in vitro* with one or more of the Toc GTPases. How-

ever, this work has focused on Rubisco (SS) and Ferredoxin (Fd). Both of these precursors share many traits including a similar length TP (~54 aa), high protein abundance, central role in photosynthesis, targeting to green plastids, stromal localization, and a similarly small mature domain. To explore the presence of these motifs in a larger and unbiased set of plastid precursors we have taken an agnostic and heuristic approach to identifying critical targeting elements *in vivo*. Using computational tools we have identified a subset of 912 highly confidently predicted chloroplast precursors in the *Arabidopsis* genome. This set was further reduced to 327 to include only TPs of a similar length to Fd and SS. Finally; this set was reduced to 231 precursors that were found to include at least one FGLK motif. From this subset, a smaller group of 7 preproteins were selected to represent a diverse set of functional roles. The transit peptides of these 7 preproteins were then subjected to 6 different heuristic changes that remove either one or both of these domains as well as removing basic residues alone. These 42 targeting sequences were then fused to YFP and evaluated and quantified for *in vivo* targeting using a biolistic transformation and fluorescent imaging method developed in our lab.

## POSTER 29. INVESTIGATING ENVIRONMENTAL EFFECTS ON THE EXPRESSION OF PSBA (D1) VARIANTS IN LEPTOLYNGBYA SP. HERON ISLAND J

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Photosystem II has a functional half-life of approximately 30 minutes and as a repair mechanism, it has been shown the central D1 subunit is degraded and replaced with a newly synthesized subunit. Leptolyngbya sp. Heron Island J has been reported to have eight variants of this gene, significantly more than most other characterized organisms1. Two of these genes fit a previously described “rogue” category2 and lack many of the necessary ligands to assembly a manganese cluster, critical for the water-splitting reaction. Recent work has suggested these rogue D1 proteins may serve to provide an alternative function to the reaction center under specific environmental conditions3. With PCR, we have confirmed the presence of all eight variants in the genome, and developed primers suitable for quantitative PCR to determine gene expression levels from cDNA generated from RNA extractions. Further work entails subjecting the organism to a variety of environmental stressors ranging from O2 and nitrate concentrations to light intensity and color. We aim to connect environmental factors and stimuli to the expression of these rogue D1 to characterize possible function. 1. Cardona T, Murray JW, Rutherford AW. Origin and Evolution of Water Oxidation before the Last Common Ancestor of the Cyanobacteria, MOLECULAR BIOLOGY AND EVOLUTION, Vol: 32, Pages: 1310-1328, ISSN: 0737-4038 2. Murray JW. Sequence variation at the oxygen-evolving centre of photosystem II: a new class of 'rogue' cyanobacterial D1 proteins, **References**: Photosynth Res. 2012 Feb;110(3):177-84. doi: 10.1007/s11120-011-9714-5. Epub 2011 Dec 21. 3. Ho MY, Shen G, Canniffe DP, Zhao C, Bryant DA. Light-dependent chlorophyll f synthase is a highly divergent paralog of PsbA of photosystem II. Science. 2016 Aug 26;353(6302). pii: aaf9178. doi: 10.1126/science.aaf9178. Epub 2016 Jul 7.

## POSTER 30. CHARACTERIZING THE ASSEMBLY MECHANISM OF TOBACCO RCA USING A SINGLE-MOLECULE APPROACH

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Most life on Earth depends on the utilization of sunlight to drive the reduction of CO2 needed for the synthesis of biological compounds with high energy densities. To predict how climate change will impact the fitness of photosynthetic organisms, we must understand how atmospheric carbon assimilation is regulated. Rubisco is considered to be the most abundant protein on Earth due to its critical role in fixing atmospheric CO2 in photosynthetic organisms. However, an activator protein discovered in the late 80’s termed Rubisco Activase (Rca), has been shown to be necessary for Rubisco reactivation and subsequently essential for plant survival. Rca belongs to the AAA+ protein superfamily, and like most proteins in this family, is believed to self-assemble into a hexamer and be most active in Rubisco reactivation when in this state. Despite observing hexameric assemblies in different conditions, there is no clear self-assembly mechanism purposed for Rca and very little information is known about the factors governing assembly. As most researchers are aware, stromal conditions (ADP and ATP ratios, and Mg2+ concentrations) in higher plants vary with changing environmental conditions and may serve to regulate Rca assembly and activity in vivo. Understanding how these variables are able to provide such fine regulation is essential in understanding how to engineer Rca for more efficient Rubisco reactivation. Consistent with our previously published Fluorescence Correlation Spectroscopy (FCS) work with cotton β Rca, tobacco β Rca also appears to assemble in a step-wise fashion, however, has been observed to assemble far more rapidly, with Kd values in the sub micro-molar range, and achieve maximal hexameric oligomer population at much lower concentrations (3 μM for tobacco, 23 μM for cotton). Also consistent with previous work, tobacco β Rca assembly

changes in response to varying nucleotides and Mg2+ ratios, suggesting a possible mechanism for regulating Rca activity. When compared with previously published activity data, the self-assembly model seems to suggest that the hexamer, not the dimer, has highest ATPase activity. We have also generated the famous R294V tobacco β Rca mutant, which based on preliminary measurements, appears to also assemble in a step-wise fashion and not always assume the hexameric state, as stated in the literature. In addition to Tobacco β Rca work, a great effort was put into studying spinach β Rca assembly, however, the β isoform appears to be in equilibrium with much larger oligomers and may require the α isoform to properly assemble. Understanding the mechanism behind Rca self-assembly is critical in advancing the field of Rubisco/Rca bioengineering and generating more efficient crops for energy and food production.

## POSTER 31. INVESTIGATING THE FUNCTIONAL LINK BETWEEN ENVELOPE ION TRANSPORT AND PHOTOSYNTHESIS

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Investigating the Functional Link Between Envelope Ion Transport and Photosynthesis Increased temperatures have globally led to more drought stress and higher rates of irrigation. This is usually followed by elevated soil salinity which plants experience as salt stress in their habitat. The overwhelming majority of plants are so-called glycophytes, i.e. plants that are not well-adapted to salt stress. In glycophytes, salt stress exerts a highly detrimental effect on photosynthesis and plant performance which diminishes global crop yield and biomass production. Plant function relies heavily on high internal potassium (K+) and low levels of toxic sodium (Na+) ions. Under salt stress, Na+ accumulates in the plant body, the cells and in cell organelles resulting in a drain of K+. Loss of K+ in the chloroplasts was shown many times to negatively impact photosynthesis. The recent discovery of envelope ion carriers and ion channels may open new avenues to suppress plastid loss of K+ during salt stress. Interestingly, loss-of-function mutants for both systems are strongly compromised under control conditions. However, the dramatic phenotypes can be rescued by exposing mutants to salt stress. The goal of my work is to study the functional linkage between the so far only characterized plastid ion transport mechanisms across the envelope membrane; K+/H+ efflux antiporters (KEA) and mechanosensitive ion channels MSL. I will further explore the physiological significance of plastid KEAs and MSL members for photosynthesis under control and abiotic stress conditions. On my poster, I will present some exciting early data from my PhD research and will discuss their relevance and my future project directions.

## POSTER 32. FORWARD GENETIC SCREENS FOR THE DISCOVERY OF FUNCTIONAL GLUCAN HYDROLASES IN THE DIATOM PHAEODACTYLUM TRICORNUTUM

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Diatoms store sugars as chrysolaminarin, a polymer of glucose connected by β-1,3 bonds with minimal β-1,6-branching. Presumably, these polymers are hydrolyzed by unknown endo- and exo-1,3-β-glucanases. To identify these putative hydrolases, this project employed a forward genetic screen with a cDNA library made from the model diatom Phaeodactylum tricornutum using Escherichia coli as a surrogate host. We first established that E. coli was not able to utilize laminarin, a practical equivalent of chrysolaminarin, as a sole carbon source. We then transformed E. coli with the cDNA library to pick clones that were able to grow on media with laminarin as the sole carbon source within 4 days. The clones presumably contained the candidate β-glucanases of P. tricornutum. Overall, we identified 435 positive clones out of a total of 27.5 x106 transformants. We were unable to identify the cDNA inserts in 30 screened clones. Further efforts will focus on rescreening the phenotypes on liquid media and employing reverse genetics techniques to identify chrysolaminarin glucanase enzymes.

## POSTER 33. PHOSPHORYLATION OF CP29 BY STN8 AND ITS POSSIBLE INVOLVEMENT IN THE HIGH LIGHT INDUCED STATE TRANSITION IN MONOCOT RICE PLANTS

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STN8 kinase mainly phosphorylates photosystem II (PSII) core proteins including D1, D2 and CP43, and light harvesting complex II (LHCII) is phosphorylated by STN7 in higher plants. Unlike Arabidopsis, the phosphorylation of CP29, a minor LHCII, was blocked in rice STN8 knock out (KO) mutant (Osstn8), but not in rice STN7 KO mutant. The KO of PPH1, a phosphatase for phosphor-LHCII, did not affect the phosphorylation and dephosphorylation of CP29. Dephosphorylation of CP29 is blocked by a PSII core phosphatase, PBCP in in vitro experiments (Nico et al., accepted in Plant J). Unlike rice, in Arabidopsis, spinach

and cucumber, CP29 phosphorylation was not easily observed in a condition where CP29 is strongly phosphorylated in rice wild type (WT) leaves under HL illumination. When state transition (ST) was monitored by the changes in maximum fluorescence (Fm), the blue light (BL) with low intensity-induced ST was shown in both WT and Osstn8 mutants. However, the HL-induced ST shown in WT was not observed in Osstn8 mutants. This result agree with our result that shows LHCII phosphorylation during BL illumination both in WT and Osstn8 mutants and suggests the possible involvement of CP29 phosphorylation in the HL-induced ST. The HL induced PSII supercomplex mobilization was also blocked in Osstn8 mutants. When PSII particles and PSI particles were separated by sucrose density gradient ultracentrifugation, the PSI preparation seems to have CP29, which suggest the possible involvement of CP29 in the migration of LHCII nearby PSI under HL illumination, although this remains for further careful investigation.

## POSTER 34. INSIGHTS INTO THE OXYGEN EVOLVING MECHANISM OF PHOTOSYNTHESIS USING XFEL DIFFRACTION

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The oxygen-evolving complex (OEC) in photosystem II (PSII) has recently been the focus of time-resolved studies using X-ray free electron lasers (XFELs), sources of extremely high-intensity, short-duration X-ray pulses that allow data collection before the onset of radiation damage. Studies using XFELs may also be conducted at room temperature, enabling the advancement of the OEC to a desired stage in the oxygen-evolving cycle prior to data collection. In order to probe the structure of the OEC in multiple illuminated states and evaluate the possible oxygen-evolving mechanisms consistent with these structures, we conducted a simultaneous X-ray emission spectroscopy (XES)/X-ray diffraction (XRD) experiment at the XFEL facility at LCLS. Room temperature PSII crystals containing a native-like packing of PSII dimers were illuminated with visible lasers and delivered to the XFEL beam. Structures of PSII in multiple illuminated states were derived from the XRD data using recently developed data processing tools in cctbx.xfel, PRIME, and Phenix. Examination of the electron density at the OEC, coordinating residues and waters, and adjacent water channels indicates that no large-scale rearrangement during oxygen evolution is accommodated, favoring mechanisms in which the OEC remains relatively compact and surrounding residues do not shift dramatically. A detailed comparison of OEC structures and locations of coordinated waters is now possible with recently-obtained high resolution diffraction data in several illuminated states.

## POSTER 35. STRUCTURAL STUDY OF F-TYPE ATP SYNTHASE FROM SPINACH CHLOROPLASTS AND *HELIOBACTERIUM MODESTICALDUM*

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The proposed project aim to use a micro-focus beamline on PETRA III to collect a full data set from crystals of ATP-synthase from chloroplasts (CF1F0) and *Heliobacteria* (HF1F0), and determine its intact structure. ATP synthase, one of the most important enzymes on earth, can be found in nearly all organisms from bacteria to humans. The ATP synthase is consisting of a hydrophilic F1 sub-complex and a membrane-bound FO sub-complex. Driven by the electrochemical gradient generated by the respiratory or photosynthetic electron transport chain, the rotation of the FO domain drives movements of the central stalk in response to conformational changes in the F1 domain, in which the physical energy is converted into chemical energy through the condensation of ADP and Pi to ATP. Structural information is available from the hydrophilic head (the F1 domain, bovine heart and yeast mitochondria, chloroplast, and thermophile *Bacillus* PS3) [1], sub-complexes of the peripheral stalk (*Escherichia coli*) [1] and the FO integral rotor ring (yeast, chloroplast, Spirulina platensis, and thermophilic bacteria *Ilyobacter tartaricus* and *Acetobacterium woodii*[1]. There is no structure available of the intact ATP-synthase nor the integral FO subcomplex and the exact mechanism of how the ATP synthesis is coupled to proton translocation is not known. We have successfully crystallized the intact ATP-synthase from spinach chloroplasts and *Heliobacteria.* The study of Heliobacterial ATP synthase is extremely interesting since the *H. Modesticaldum*, a thermophilic anoxygenic phototrophic bacterium, has played a key role in the evolution of phototrophic bacteria and photosynthesis in general. **References:** [1]Jay-How Yang, Iosifina Sarrou, Jose M. Martin-Garcia, Shangji Zhang, Kevin E. Redding, Petra Fromme. Purification and biochemical characterization of the ATP-synthase from *Heliobacterium modesticaldum*. Protein Expression and Purification 114 1-8 (2015) (doi: 10.1016/j.pep.2015.05.006).

# 2017 Western Photosynthesis Conference
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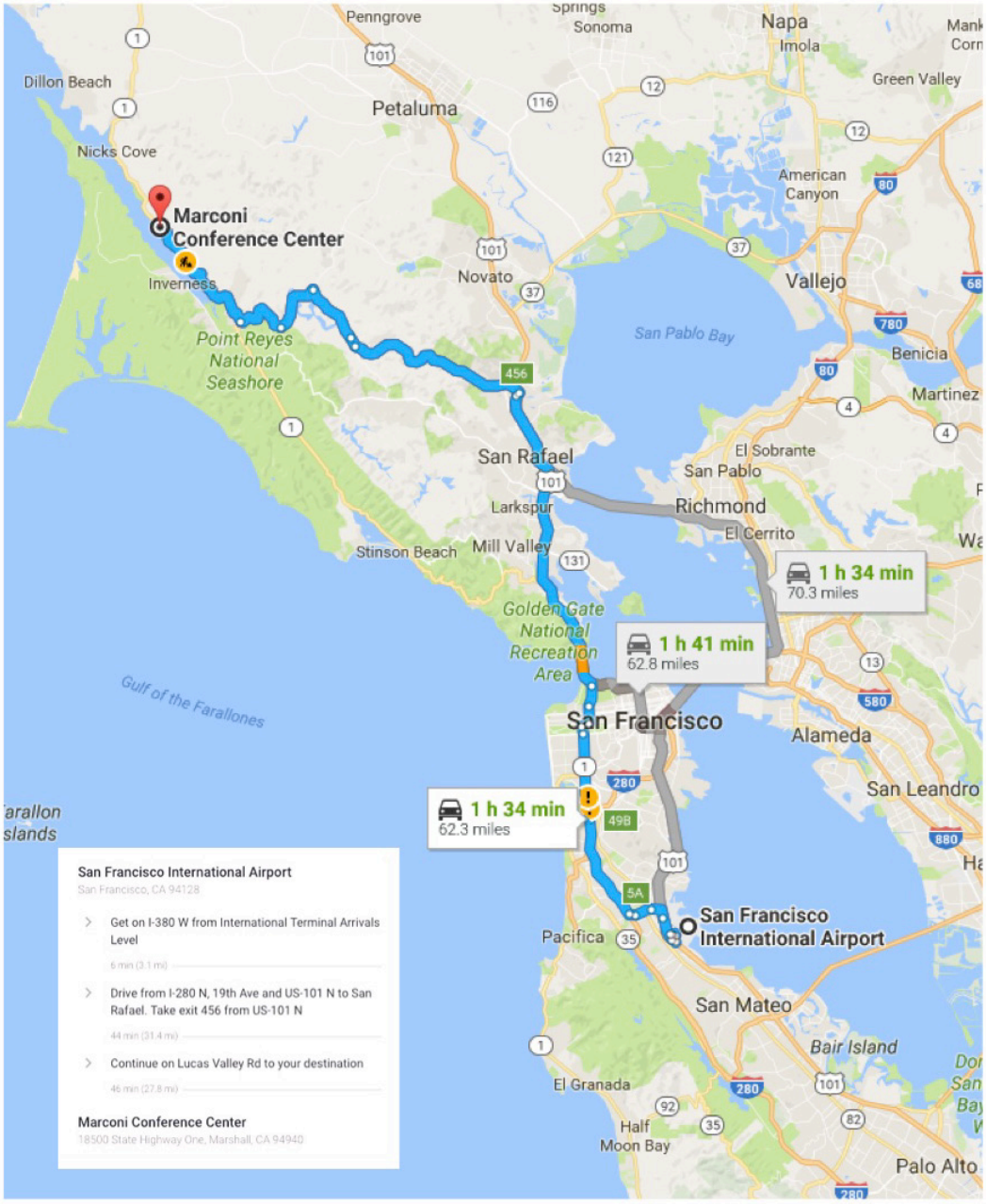
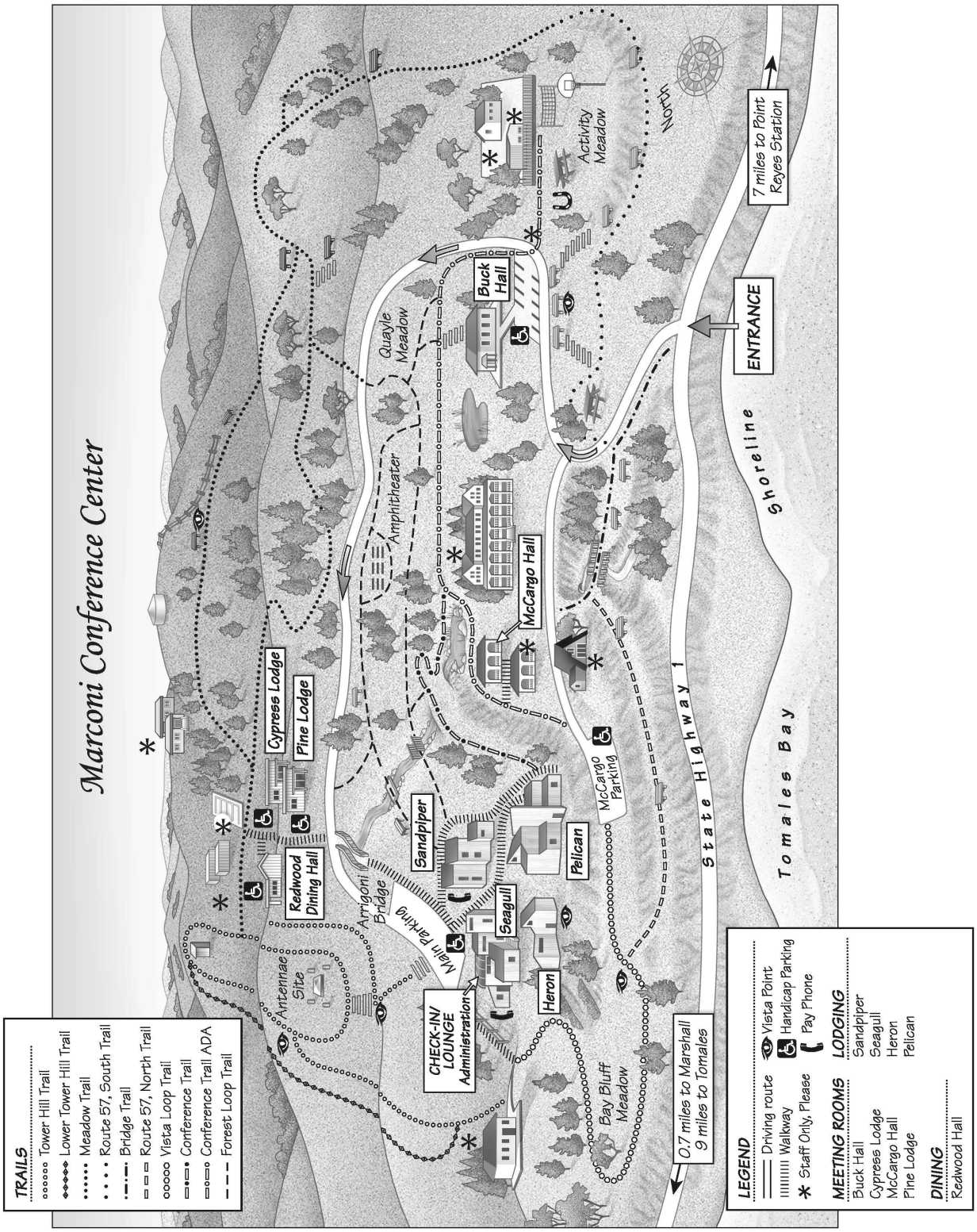
**Driving Map and Instructions from SFO**

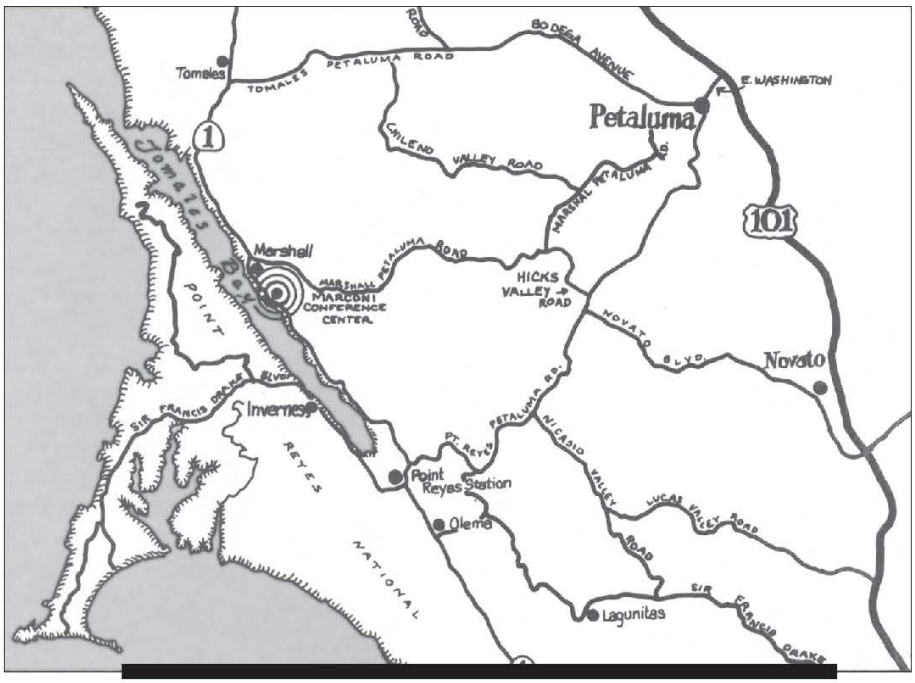


# Map Of Marconi Conference Center



Marconi Conference Center
CALIFORNIA STATE PARKS

# LOCAL AREA GUIDE




## ◆ ACTIVITIES

***Marshall:***

**Blue Waters Kayaking**
19225 Highway One
(415) 663-1743

***Point Reyes Station:***

**Point Reyes Outdoors**
11401 Highway One
(415) 663-8192

**West Marin Fitness**
65 3rd St.
(415) 663-1762

***Tomales:***

**Tomales Regional History Center**
26701 Highway One
(707) 878-9443

***Olema:***

**Point Reyes National Seashore**
Bear Valley Visitor Center
1 Bear Valley Rd.
(415) 464-5137
(415) 663-8522

**Five Brooks Stables**
9001 Highway One
(415) 663-1570

***Inverness:***

**Blue Waters Kayaking**
12938 Sir Francis Drake Blvd.
(415) 669-2600

**Jack Mason Museum**
15 Park Ave.
(415) 669-1099

**Point Reyes Lighthouse**
See *Olema* – Point Reyes Nat'l Seashore

**Tomales Bay State Park**
(415) 669-1140

***Lagunitas:***

**Samuel P. Taylor State Park**
8889 Sir Francis Drake Blvd.
(415) 488-9897

***San Geronimo:***

**San Geronimo Valley Golf Course**
5800 Sir Francis Drake Blvd.
(415) 488-4030

***Novato:***

**Indian Valley Golf Club**
3035 Novato Blvd.
(415) 897-1118

***Bodega Bay:***

**Bodega Harbor Golf Links**
21301 Heron Drive
(off S. Harbor Way)
(707) 875-3538

## ◆ BAKERIES

***Point Reyes Station:***

**Bovine Bakery**
11315 Highway One
(415) 663-9420

***Tomales:***

**Tomales Bakery**
27000 Highway One
(707) 878-2429

***Inverness Park:***

**Busy Bee Bakery**
12301 Sir Francis Drake Blvd.
(415) 663-9496

## ◆ CHURCHES

EPISCOPAL

**St. Columba's Episcopal Church**
12835 Sir Francis Drake Blvd.
***Inverness***
(415) 669-1039

***Continued on back***

P.O. Box 789, Marshall, California 94940 • www.MarconiConference.org
Phone (415) 663-9020 • Fax (415) 663-1731 • e-mail address: Reservations@MarconiConference.org

Revised 12/13

PRESBYTERIAN

**Point Reyes Presbyterian Church**
11445 Highway One
***Point Reyes Station***
(415) 663-1349

**Tomales Presbyterian Church**
11 Church St.
***Tomales***
(707) 762-4924

ROMAN CATHOLIC

**St. Helen's Church**
Marshall-Petaluma Road
& Highway One
***Marshall***
(707) 878-2208

**Church of the Assumption**
26825 Highway One
***Tomales***
(707) 878-2208

**Sacred Heart Church**
10189 Highway One
***Olema***
(415) 663-1139

*Other major denominations can be found in either San Rafael or Petaluma*

## ◆ GROCERY/DELICATESSEN

***Marshall:***

**Marshall Store**
19225 Highway One
(415) 663-1339

***Point Reyes Station:***

**Palace Market**
11300 Highway One
(415) 663-1016

**Cowgirl Creamery at Tomales Bay Foods**
80 Fourth St.
(415) 663-9335

**Whale of a Deli**
997 Mesa Road
(415) 663-8464

***Tomales:***

**Diekmann's General Store**
27005 Highway One
(707) 878-2384

**Tomales Deli & Café**
27000 Highway One
(707) 878-2732

***Olema:***

**Farm House Deli**
10003 Highway One
(415) 663-8615

***Inverness Park:***

**Perry's Delicatessen**
12301 Sir Francis Drake Blvd.
(415) 663-1491

***Inverness:***

**Inverness Store**
12784 Sir Francis Drake Blvd.
(415) 669-1041

## ◆ OYSTER SALES

***Marshall:***

**Hog Island Oysters**
20215 Highway One
(415) 663-9218

**Tomales Bay Oyster Co.**
15479 Highway One
(415) 663-1242

***Inverness:***

**Drake's Bay Oyster Co.**
17171 Sir Francis Drake Blvd.
(415) 669-1149

## ◆ RESTAURANTS & BARS

***Marshall:***

**Tony's Seafood Restaurant**
18863 Highway One
(415) 663-1107

**Nick's Cove**
23240 Highway One
(415) 663-1033

***Point Reyes Station:***

**Café Reyes**
11101 Highway One
(415) 663-9493

**Pine Cone Diner**
60 Fourth & B Street
(415) 663-1536

**Osteria Stellina**
11285 Highway One
(415) 663-9988

**Old Western Saloon**
11201 Highway One
(415) 663-1661

**Station House Café**
11180 Highway One
(415) 663-1515

***Tomales:***

**William Tell House Restaurant**
26955 Highway One
(707) 878-2403

***Olema:***

**Farm House Restaurant**
10005 Highway One
(415) 663-1264

**Sir and Star at the Olema**
10000 Sir Francis Drake Blvd.
(415) 663-1034

***Inverness:***

**Blackbird**
12781 Sir Francis Drake Blvd.
(415) 669-7195

**Drakes Beach Café**
1 Drakes Beach Rd.
(415) 669-1297

**Saltwater Oyster Depot**
12781 Sir Francis Drake Blvd.
(415) 669-1244

**Vladimir's Czech Restaurant**
12785 Sir Francis Drake Blvd.
(415) 669-1021

## ◆ SERVICES

AUTO REPAIR

**Bracken Auto**
11401 Highway One
***Point Reyes Station***
(415) 663-0633

**Cheda's Garage**
11225 Highway One
***Point Reyes Station***
(415) 663-1227

BANK

**Wells Fargo Bank**
11400 Highway One
***Point Reyes Station***
(415) 663-1713

DRUG STORE

**West Marin Pharmacy**
11 Fourth St.
***Point Reyes Station***
(415) 663-1121

GAS STATION

**Green Bridge Gas**
11401 Highway One
***Point Reyes Station***
(415) 663-8654

LAUNDROMAT

**Olema Campground**
10155 Highway One
***Olema***
(415) 663-8001

POST OFFICE

19200 Highway One
***Marshall***
(415) 663-8388

# Map of Pt. Reyes National Seashore

**--NOTES—**