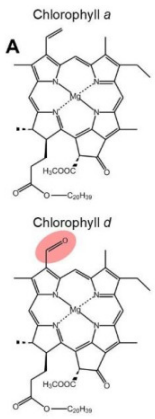
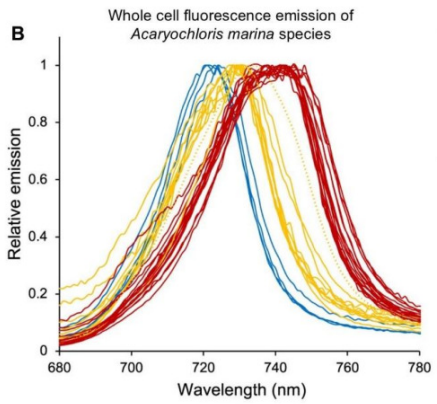
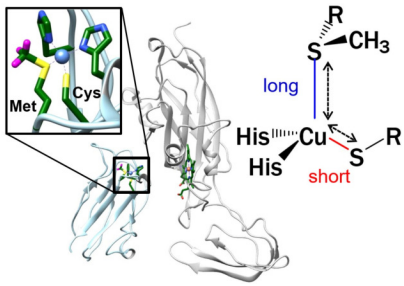
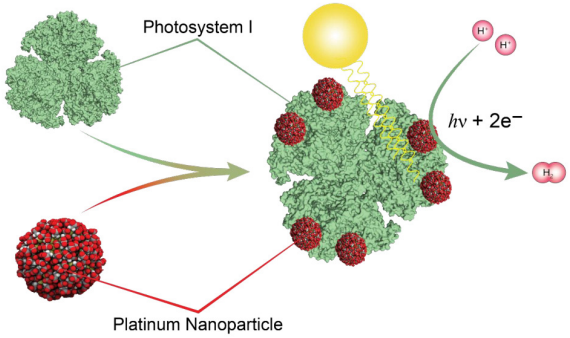
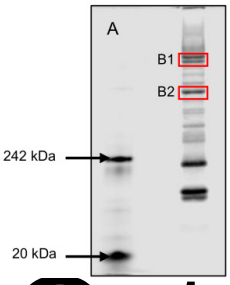
51st Midwest/Southeast Photosynthesis Conference Agenda and Abstracts

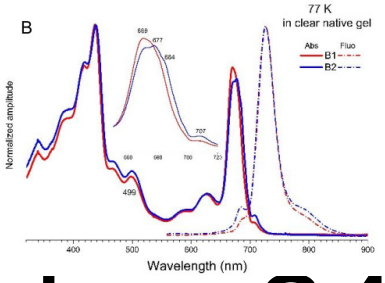


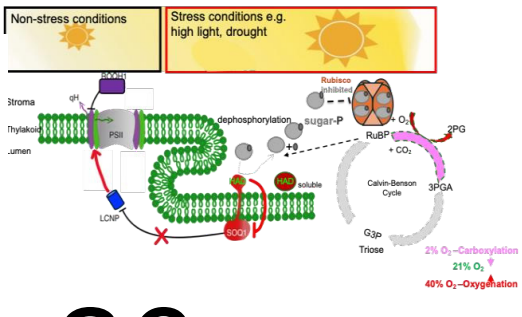


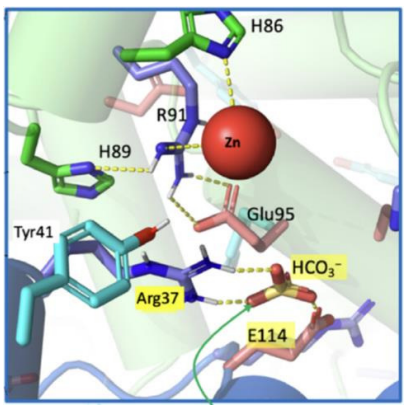






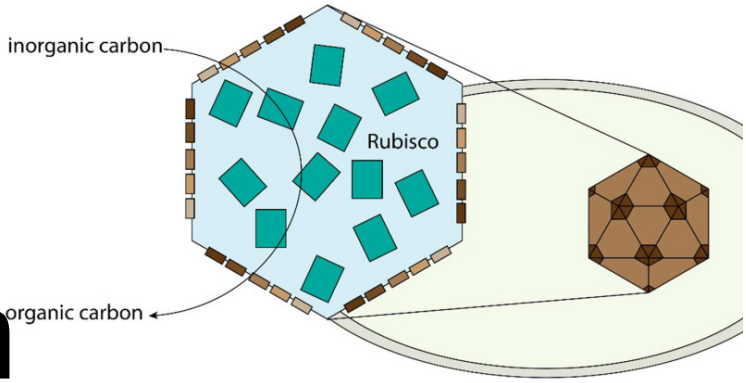






October 24-26 2025

Turkey Run Inn



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Program

Friday, October 24

4:00–6:00pm	**Registration and Poster Hanging**		
6:00pm	**Dinner**		
7:30–7:40pm	**Introduction and Welcome**		
7:40–8:20pm	Keynote	**Colin Gates** Loyola University	Strategies for Extreme Growth in *Chlorella*
8:20–8:40pm		**Nicholas Ferrari** Louisiana State University	Noisy Sunlight: Algal Acclimation to Stochastic Light
8:40–9:00pm		**Swarnali Mukherjee** Oklahoma State University	Understanding CO_2 uptake and proton pumping in cyanobacterial Photosynthetic Complex 1.
9:00–9:20pm		**Lydia Davies-Balogun** Alabama State University	Biosorption and bioaccumulation of critical metals by algae
9:30pm	**Mixer and Poster Session**		

Saturday, October 25

7:00am	**Breakfast**		
9:00–9:40am	Keynote	**Petra Fromme** Arizona State University	X-Ray and Neutron studies on Photosystem I and II
9:40–10:00am		**Chris Brininger** University of Wisconsin-Madison	Cryo-EM Structures of Photosystem I with Alternative Quinones Reveals New Insight into Cofactor Selectivity
10:00–10:20am		**Sham Vera** University of Indiana-Bloomington	Understanding the HAD domain function of the SUPPRESSOR OF QUENCHING 1 protein in photoprotection in *Arabidopsis thaliana*
10:20–10:40am	**Coffee**		
10:40–11:00am		**Audrey Short** Argonne National Laboratories	Eukaryotic Photosynthetic Thylakoid Biohybrids: Probing Fundamental Interactions in Charge Accumulation
11:00–11:20am		**Maximino Emerson** University of Wisconsin-Madison	A Structural Approach to Engineering Biohybrid Photocatalysis
11:20–11:40am		**Siva Naga Sai Damaraju** Michigan State University	Molecular Dynamics Insights into Photosystem I–Platinum Biohybrids for Photocatalytic Hydrogen Production

11:40–12:00pm		**Samuel Snyder** Argonne National Laboratory	Biohybrid Bacterial Microcompartment Shells as Nanoreactors for Photocatalytic Hydrogen Evolution
12:00–12:20pm		**Neetu Yadav** Michigan State University	Tracking Photosynthetic Metabolite Transport Through Bacterial Microcompartments Using Enhanced Sampling Simulations
12:30pm	**Lunch**		
Afternoon	**Free to explore Turkey Run State Park**		
4:00–6:00pm	**Poster Session**		
6:00pm	**Dinner**		
7:30–7:50pm		**Steven Romberger** Hiram College	Steady-State P800 Oxidation Kinetics Indicate That Heliobacterial Phototrophy is Light-Limited
7:50–8:10pm		**Himanshu Mehra** University of Wisconsin-Madison	Biochemical analysis and structural predictions of photosynthetic apparatus components in *Acaryochloris marina* spectral types
8:10–8:30pm		**Jonathan Nguyen** Michigan State University	Molecular Simulation of Photosystem II and Phycobilisome Interactions in Cyanobacteria
8:30–8:50pm		**Allison Squires** University of Chicago	Phycobilisome core architecture influences photoprotective quenching by the Orange Carotenoid Protein
9:00pm	**Campfire, Drinks, and Poster Session**		

Sunday, October 26

7:00am	**Breakfast**		
9:00–9:20am		**Nidhi Kulkarni** Louisiana State University	Enhancing the efficiency of CRISPR-Cas9 based gene editing in *Chlamydomonas reinhardtii*
9:20–9:40am		**Ignacio Sparrow Munoz** University of Illinois	Continuous directed evolution of soybean (*Glycine max*) SBPase
9:40–10:00am		**Grant Steiner** Loyola University	*Chlamydomonas reinhardtii* growth is accelerated by constitutive overexpression of a glutathione S-transferase
10:00–10:20am		**Alexzandria Stewart** Alabama State University	Engineering strategies for continuous biomass collection of Anabaena 33047
10:20–10:45am	**Career Panel**		
10:45–11:00am	**Coffee**		
11:00am	**Awards Presentations**		

Talk Abstracts

Cryo-EM Structures of Photosystem I with Alternative Quinones Reveals New Insight into Cofactor Selectivity

Christian M. Brininger[1], Jimin Wang[2], Vasily Kurashov[3], John H. Golbeck[3], David J. Vinyard[4], K. V. Lakshmi[5], Christopher J. Gisriel[1]

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[2]*Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA*

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[4]*Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA.*

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Photosystem I (PSI) is a key protein involved in oxygenic photosynthesis. It is a multi-subunit membrane bound protein complex which performs light capture and electron transfer reactions, whose investigation has a rich history. In cyanobacteria, PSI is a "trimeric" protein complex with each monomer containing 10 to 12 protein subunits and many cofactors, 11 of which form its electron transfer chain. The electron transfer chain of PSI is split into two branches, and a key component of each branch is the phylloquinone (PhQ) that is involved as a permanently bound electron transfer intermediate. Previous data suggested that in the mutant cyanobacterial strain Δ*menB Synechocystis* sp. PCC 6803, which contains a mutation interrupting PhQ synthesis, each quinone site in PSI was instead occupied by plastoquinone-9 (PQ9), which is the native quinone found in PSII, and that PQ9 could be readily exchanged for alternative quinones. This model has since been used for many biophysical studies on electron transfer in PSI, but limited structural data is available to aide reliable interpretation. Here, we present two new cryo-EM structures of PSI containing alternative quinones: one in which PSI contains PQ9, and one in which the PQ9 has been exchanged in vitro for ethyl-naphthoquinone. These structures provide new insights into the selectivity of quinones in PSI, the stability conferred by central cofactors, and new evidence towards understanding the evolutionary ancestry of PSI. This investigation advances efforts to develop precisely engineered PSI-based systems for light-driven hydrogen production, offering a pathway toward sustainable alternatives to fossil fuels.

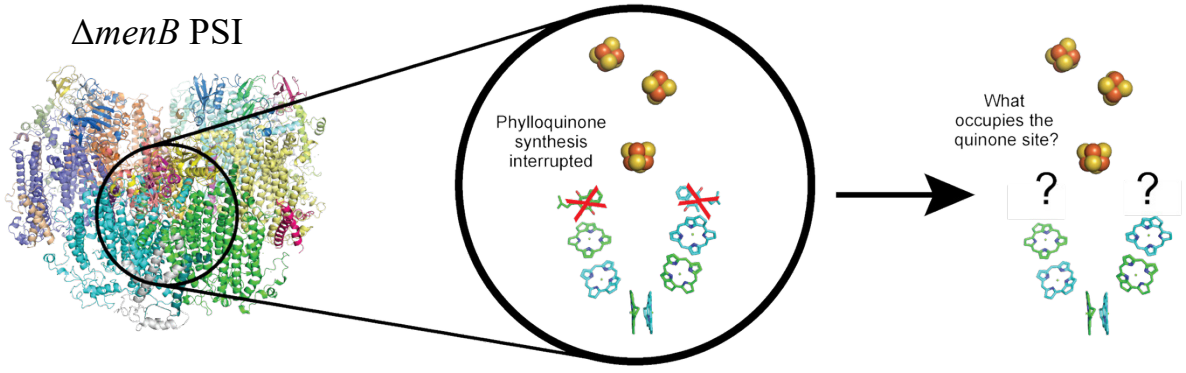


Figure. 1. The Δ*menB* mutant inhibits phylloquinone (PhQ) synthesis, a cofactor typically bound by PSI. We utilized cryo-EM structures of PSI from a newly generated Δ*menB* strain ("Δ*menB*"), and of that same PSI after EtNQ exchange ("Δ*menB*+EtNQ") to determine which quinones occupy the two quinone binding sites within each PSI monomeric unit.

Molecular Dynamics Insights into Photosystem I–Platinum Biohybrids for Photocatalytic Hydrogen Production

Siva Naga Sai Damaraju[1] , Maximino D. Emerson[2] , Christopher J. Gisriel[2], Lisa M. Utschig[3] and Josh V. Vermaas[1]

[1]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA.*

[2]*Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.*

[3]*Chemical Sciences and Engineering Division, Argonne National Laboratory, Lemont, IL, USA.*

Photosystem I (PSI) is a protein-pigment complex embedded in the thylakoid membrane of photosynthetic organisms that captures sunlight and delivers high-energy electrons to charge carriers like ferredoxin and flavodoxin to power downstream metabolism and carbon fixation. However, electrons captured by PSI have been demonstrated experimentally to transfer from iron-sulfur clusters within PSI to bound platinum nanoparticles (PtNPs). Once the electrons are transferred to the PtNP, they act as catalysts for hydrogen gas production, thus making these PtNPs attractive as biohybrids. Cryo-electron microscopy structures are available for these PSI-Pt biohybrids and indicate that these PtNPs occupy the ferredoxin and flavodoxin binding sites on PSI. However, cryoEM offers only a static picture over a population ensemble of PtNPs. So, to investigate the dynamics, we performed classical molecular dynamics simulations on PSI–Pt biohybrids from three organisms: *Synechococcus lividus* and *Thermosynechococcus vestitus* (each with two PtNPs) and *Synechococcus leopoliensis* (with one PtNP). Our simulations revealed that the interactions between the PSI and PtNPs are stable with minimal displacement throughout the simulation. The structural analysis from our study revealed that Lys27 and Lys30 of PsaA for NP1, while Lys314 of PsaB, Lys155 of PsaF for NP2, are the key residues involved in stabilizing the PtNPs on the stromal surface of the PSI monomer. The NP1 is positioned closer to the 4Fe-4S clusters in all three systems; however, in *S. leopoliensis*, where only one PtNP is bound, NP1 resides much closer to the 4Fe-4S cluster. This positioning highlights NP1 as the most promising site for efficient electron transfer and H_2 evolution. Therefore, this study provides the molecular insights that can further refine nanoparticle position on the PSI surface, which can pave the path for enhanced electron transfer efficiency and hydrogen production.

Biosorption and bioaccumulation of critical metals by algae

Lydia DavieBalogun[1,2], Eric Schaedig[2], Jacob Sebesta[2], Harvey J.M. Hou[1,*], and Jianping Yu[2,*]

[1]RENEW Cyanobacteria Collection Center, Department of Physical and Forensic Sciences, Alabama State University, Montgomery, Alabama 36104; [2]Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401. *Email: jianping.yu@nrel.gov, hhou@alasu.edu

The United State has identified a list of 50 critical minerals that are essential to support the current technologies in our high-tech economy. We target to develop effective recycling technologies and processes to recover material already in circulation. Our work in Summer 2025, supported in part by the Science Undergraduate Laboratory Internship program, explored effective recovery methods for metals from wastewater systems using a phosphorus-rich algae strain previously isolated from a wastewater treatment system. Algae can grow in wastewater and take up phosphorus, usually well above their nutritional need, allowing algae to store the phosphorus as polyphosphate. Polyphosphate is known to bind to metals. This led the team to explore the alga's ability to effectively uptake different metals. These metals included cobalt (Co^{2+}), dysprosium (Dy^{3+}), neodymium (Nd^{3+}), praseodymium (Pr^{3+}), and samarium (Sm^{3+}). To analyze the alga's metal compatibility, we tested several concentrations of metal with the alga. We were then able to identify the toxicity of the metals to the alga. We found that several of the metals tend to precipitate from the solution, preventing binding with the alga. Which then led to exploring different conditions to avoid precipitation. The future direction will be placed on the understanging each strain and conditions on selectivity for metal ions and identifying whether polyphosphate matrix is causing the algae and metals to bind.

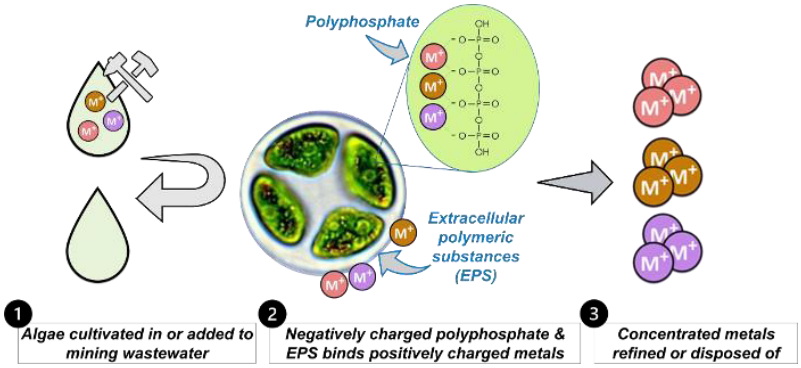


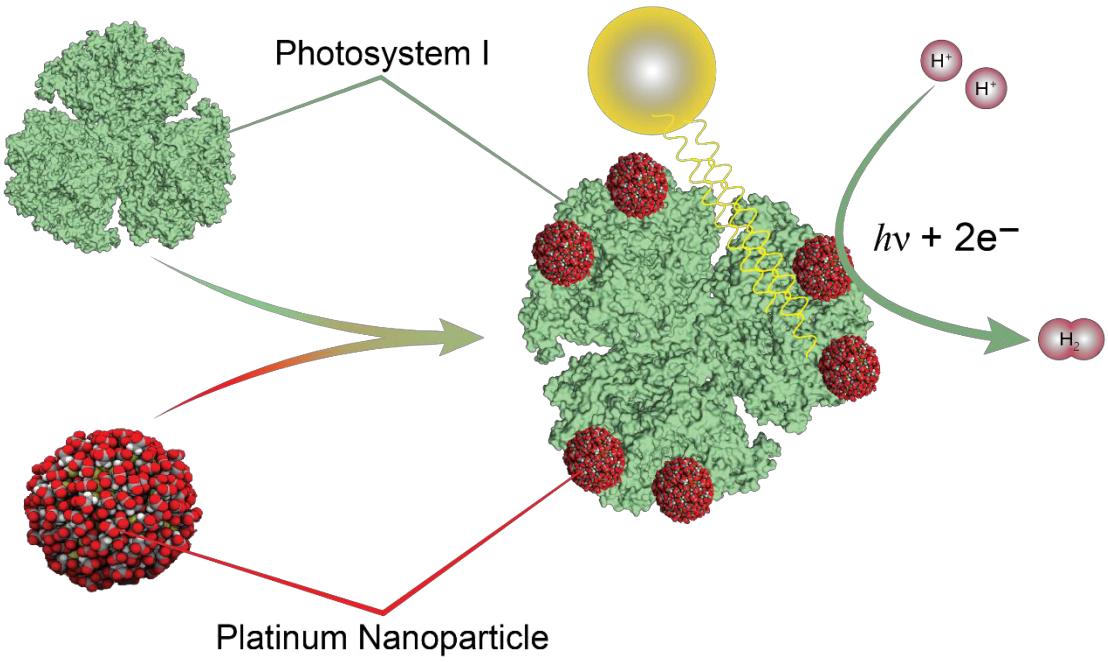
Figure 1. Concept diagram of the experiment on biosorption and bioaccumulation of critical metals with microalgae.

A Structural Approach to Engineering Biohybrid Photocatalysis

Maximino D. Emerson,[1] Audrey M. Short, [2] Siva naga Sai Damaraju,[3] Joshua Vermaas,[3] Lisa M. Utschig,[2] Christopher J. Gisriel[1]

[1]*Department of Biochemistry, University of Wisconsin-Madison. Madison, WI 53706, United States.* [2]*Argonne National Laboratory, Lemont, IL 60439, United States. Chemical Sciences and Engineering Division.* [3]*Biochemistry and Molecular Biology Department, Michigan State University, East Lansing, Michigan 48824, United States.*

In oxygenic photosynthesis, two membrane protein complexes called photosystem I (PSI) and photosystem II perform solar-to-chemical energy conversion by using light to generate low potential electrons. When PSI is excited by light, its primary electron donor has a very low reduction potential, approximately −1.2 V. Platinum nanoparticles (PtNPs) designed to bind the surface of PSI are capable of harvesting these low potential electrons and catalyzing redox chemistry. These PSI-PtNP biohybrids have been shown to perform photocatalytic hydrogen production. Recently, structural data from one such biohybrid containing PSI from the cyanobacterium *Synechococcus lividus* provided indications of the specific interactions involved in PtNP binding while also highlighting inefficiencies in PtNP usage and placement on the PSI surface. We have since determined two additional PSI-PtNP biohybrid structures containing PSI from the cyanobacterium *Thermosynechococcus vestitus* and a chemically treated PSI-core from another cyanobacterium *Synechococcus leopoliensis* to further elucidate the nature of PSI-PtNP interactions. We are now using these structures to develop engineering strategies to most efficiently use PtNPs and maximize the rate of redox activity.



Noisy Sunlight: Algal Acclimation to Stochastic Light

Nicholas Ferrari[1] and David J. Vinyard[1]

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nferr11@lsu.edu.

Natural sunlight exhibits not only dramatic shifts in intensity but also a background of rapid, irregular perturbations. While the physiological response to large, periodic fluctuations has been identified as a target for improving photosynthetic efficiency, the consequences of unpredictable fine-scale variation remain poorly understood. To address this gap, we created growth environments with stochastic light inputs such as unpredictable changes in frequency, mean, and variance. We were then able to assess the impact on photosynthetic performance through chlorophyll fluorescence and growth studies.

Wild-type *Chlamydomonas reinhardtii* and mutants deficient in various non-photochemical quenching mechanisms were grown under three regimes: (1) constant low light at 50 µmol m^{-2} s^{-1}, (2) a 12/12 diurnal sinusoidal cycle, and (3) the same diurnal cycle with added stochastic perturbations every 5 seconds, drawn from a normal distribution with mean 0 and standard deviation 10 µmol m^{-2} s^{-1}. Organisms exposed to stochastic perturbations showed lower Fv/Fm and increased NPQ induction. These results suggest that irregular, high-frequency light variation can drive acclimation not only to dynamic environments but also to unpredictable ones.

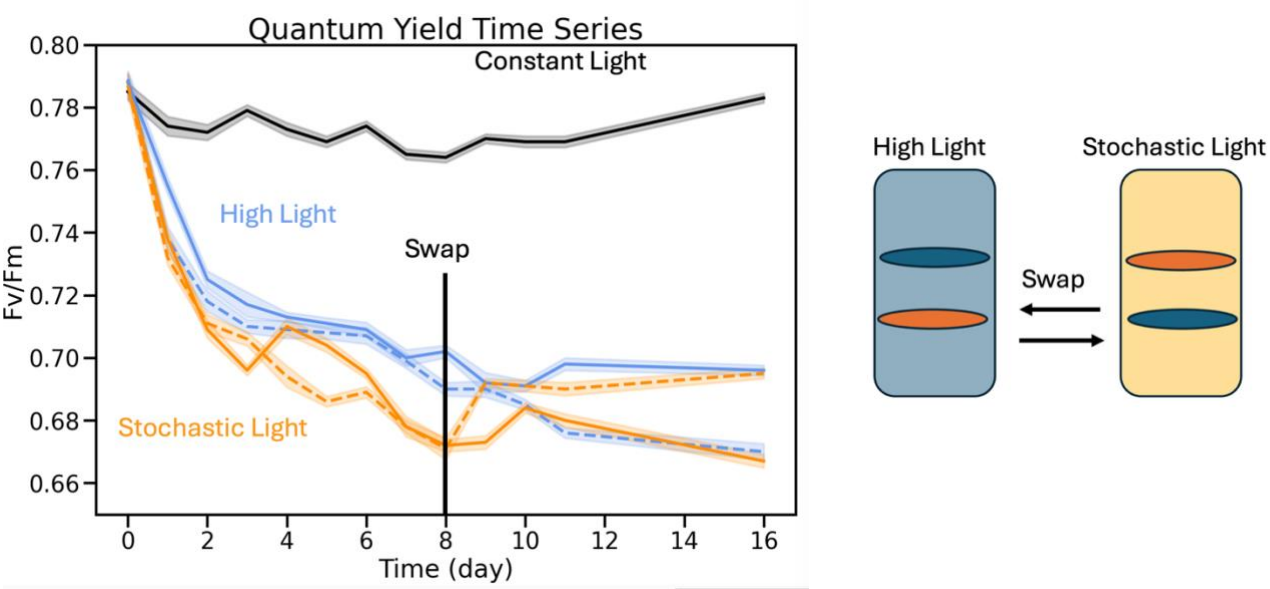

Figure 1: Time series of maximum quantum yield of PSII reveals distinct physiological responses. Dashed lines represent samples that underwent a treatment swap halfway through the experiment. Both high light and stochastic light reduce F_v/F_m relative to constant light, but the stochastic treatment drives a stronger decline. Following the treatment swap on day 8, populations transition toward the phenotype associated with the new light environment, indicating photosynthetic state plasticity and differential acclimation pathways.

Strategies for Extreme Growth in *Chlorella*

Colin Gates[1]

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The fastest-growing known eukaryotic phototroph, the green alga *Chlorella ohadii*, is a polyextremophile which is capable of surviving full desiccation for much of its life cycle. This extraordinary growth is achieved by careful regulation of the photosynthetic electron transport chain, including a substantial contribution from Photosystem II (PSII)-cyclic electron flow (CEF). As compared to *C. ohadii*, its near relatives *Chlorella vulgaris* sp. NIES 642 and *C. sorokiniana* normally grow under significantly lower light intensities and do not contend with the same water stress. We have acclimated both of these strains to grow under the extreme light conditions under which *C. ohadii* achieves its maximum growth rate. While each achieves a minimum doubling time of under 2 hours, making these three of the five fastest-growing reported phototrophs under ideal conditions, their photosynthetic electron transport chains are regulated differently to achieve this. Both acclimated strains lack the ability to perform PSII-CEF to the same degree as *C. ohadii* and are accordingly somewhat slower-growing. However, *C. sorokiniana* uses its photosynthetic electron transport chain almost identically to that of *C. ohadii* outside of Photosystem II, while NIES 642 has alternate photoprotective strategies in place. A major factor allowing *C. ohadii* to grow faster appears to be the ability to separate out its linear and cyclic electron flow mechanisms, spatially partitioning electron transduction from non-photochemical and recombinative mechanisms of excess energy dissipation. The differential behavior of these closely related strains informs on strategies for increasing cell growth rates and phototolerance.

Enhancing the efficiency of CRISPR-Cas9 based gene editing in *Chlamydomonas reinhardtii*

Nidhi Kulkarni[1] and David J. Vinyard[1]

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Gene editing in *Chlamydomonas reinhardtii* using the CRISPR-Cas9 system is still an emerging field. Although several successful CRISPR-Cas9 mediated mutations have been done, editing efficiency varies widely among target genes with reported efficiencies of 0.17-96%. The gene editing efficiency depends on several factors – those that inherently make certain genes difficult to target due to chromatin inaccessibility and topology, and those that can be externally modulated like recruitment of enzymes involved in double strand break repair, maintaining ion balance and recovery post electroporation. Among many factors that affect the latter, we show that magnesium is critical. We added different concentrations of magnesium to electroporated cells and tested resulting transformation efficiencies. We report that supplementing electroporated cells with 1 mM to 5 mM magnesium can increase the efficiency of CRISPR-Cas9-based gene editing up to 50%.

Biochemical analysis and structural predictions of photosynthetic apparatus components in *Acaryochloris marina* spectral types

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Photosynthetic organisms rely on pigment-protein complexes to capture sunlight and drive charge separation, with cyanobacteria exhibiting remarkable diversity in their photosystems and antenna assemblies. Among them, *Acaryochloris marina* represents a unique case, using chlorophyll (Chl) *d* in place of the more common Chl *a* (**Fig. 1A**), thereby shifting its absorption cross-section toward lower energies. The most studied strain, MBIC11017, has yielded structural insights into both photosystem I and II, yet little is known about how antenna complexes and photosystems vary across other *A. marina* strains. Recent work has revealed three distinct spectral strain types exhibiting short, intermediate, and long-wavelength spectral characteristics (**Fig. 1B**), yet the molecular basis underlying these differences remains unresolved. Here, we investigate the pigment-protein complexes of representative strains from all three spectral groups using spectroscopic, proteomic, and electron microscopic approaches. Our analyses identify the source of spectral shifting among different strain types and provide preliminary structural information on some components of the photosynthetic apparatus. These findings expand the molecular framework for understanding low-energy photosynthesis and illuminate the ecological and evolutionary strategies that may have enabled *A. marina* to diversify and thrive in varied light environments.

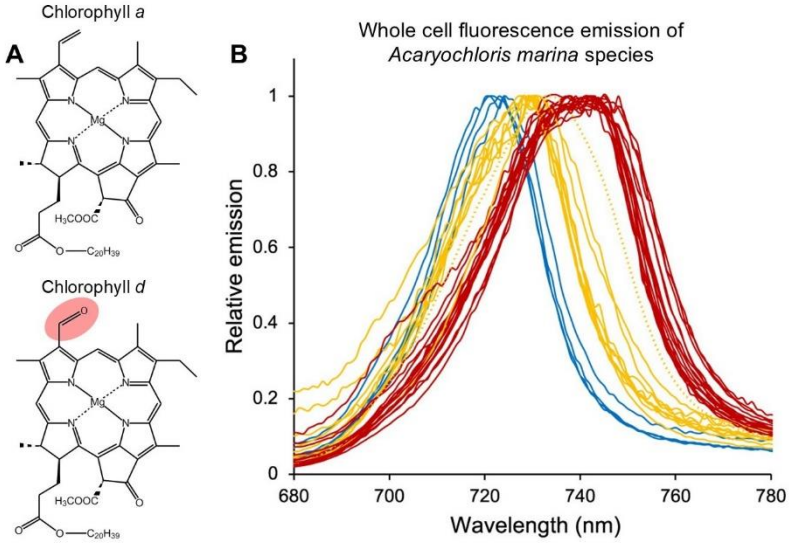

Fig. 1. Structures of chlorophylls *a* and *d* (**A**), and fluorescence emission of *A. marina* spectral types (**B**).

Understanding CO_2 uptake and proton pumping in cyanobacterial Photosynthetic Complex 1.

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To survive under low CO_2 concentrations, cyanobacteria have evolved unique mechanisms that enhance the efficiency of photosynthetic CO_2 fixation. The CO_2 Concentrating Mechanism (CCM) in cyanobacteria includes specialized NADPH Dehydrogenase Type I complexes (a.k.a. Photosynthetic Complex-1 or PC1 or NDH-1 complexes) whose major function is to provide enough inorganic carbon (Ci) to Rubisco in low Ci conditions. These complexes possess extrinsic subunits specially evolved for high or low affinity CO_2-uptake, CupA and CupB respectively. The catalytic site at the interface between the extrinsic CupB and transmembrane NdhF4 subunits of the CO_2 uptake module of these complexes differs from regular carbonic anhydrases because of their unusual Zinc coordination. This Zn in the putative CO_2 binding site is coordinated by residues from the neighboring NdhF4 subunit; namely Arg37 and Tyr41 residues. We hypothesize that Arg37 around the active site of the Cup proteins in the NdhF4 subunit plays an important role in mediating the pumping of protons away from the CO_2 hydration active site. To test this, we generated R37 mutants and examined their growth phenotypes and carbonic anhydrase activities. Our results show that mutants exhibit severe growth defects under low Ci supporting a direct role for Arg37 in moving the generated proton away from the active site fast enough for the CO_2 hydration reaction to proceed unidirectionally and in an energized manner, preventing the reverse reaction back to CO_2.

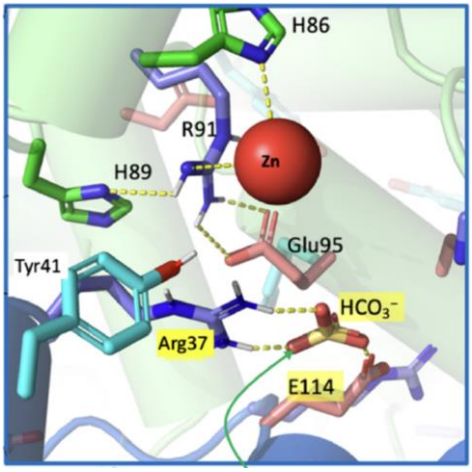

Figure1. The active site of the CO_2 concentrating CupB subunit, showing Zn-coordinating residues.

This work is supported by the United States Department of Energy, Office of Basic Energy Sciences, DE-FG02-08ER15968

Molecular Simulation of Photosystem II and Phycobilisome Interactions in Cyanobacteria

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[2]National Institute of Health, Bethesda, MD.

Phycobilisomes (PBS) are the major light harvesting antenna complexes in cyanobacteria and red algae. PBS absorb and transfer light energy to photosynthetic reaction centers, through pigment molecules known as phycocyanobilins. The transfer of energy between PBS and the photosynthetic reaction center, photosystem II (PSII) results in the production of the oxygen we breathe on Earth. Though the relationship between PBS and PSII is well-documented and atomic resolution structures exist for both, little is known about the interactions and energy transfer pathways between the two photosynthetic components. Leveraging existing structural data, we are changing this narrative and exploring the dynamics of a PBS-PSII complex through molecular dynamics simulations. Aligning resolved Synechocystis sp. PCC 6803 PBS and PSII structures from Cryo-EM and utilizing AlphaFold2, we have constructed a 3 million atom system with both photosynthetic components present. Through ~600ns of molecular dynamics simulations with 10 replicas, we observe significant structural changes within the ApcG and rod-core linker proteins. Hydrogen bonding to form the PBS-PSII complex increase over the course of the molecular dynamics simulations, primarily from the ApcE and ApcG residue pair interactions with PsbB and PsbC, respectively. We have also calculated approach distances between phycocyanobilins, chlorophylls, pheophytins, and quinones to predict the impact of thermal motion on excitation energy transfer. Through network analysis of these distances, we identified one key phycocyanobilin with a minimum distance of ~35Å to the closest chlorophyll, the only phycocyanobilin connecting the network of cofactors in the PBS-PSII complex. This minimum distance of ~35Å appears to be comparable to distances found in recent structures, though how efficient this is for energy transfer remains to be seen. This is one of the first looks at the PBS-PSII interface, and we have been able to predict possible interactions within this complex.

Steady-State P800 Oxidation Kinetics Indicate That Heliobacterial Phototrophy is Light-Limited

Steven P. Romberger[1,*], Tayleigh Price[2,3], Hallie Chavez[1,4,5], Alysa Giudici[1,2,6], Alexus Acton[1,2,7], and Meaghan Stafford[1,8]

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The heliobacteria are a family of phototrophic bacteria known for their unique production of bacteriochlorophyll *g* and for their use of the simplest known Type I reaction center. In this work, we characterize P800 oxidation kinetics in whole cells of *Heliomicrobium modesticaldum* under the continuous illumination that is more consistent with *in vivo* conditions, an area of research that remains largely unexplored. When assayed at 800 nm, whole cells display a large bleaching immediately upon illumination by actinic light, corresponding to the production of $P800^+$. The initial bleaching typically reaches a maximum intensity at 10 - 30 ms, at which point a slower, partial recovery leads to a steady-state that is smaller than the initial bleaching. The effects of charged redox reagents, in particular ferric ammonium citrate, and the cytochrome *bc* complex inhibitor azoxystrobin, demonstrate that this recovery phase is due to forward donation to $P800^+$ from cytochrome *c*. A steady-state kinetics analysis comparing the effects of actinic intensity on the rate of P800 oxidation to that of P700 oxidation in spinach chloroplasts and whole cells of *Synechococcus* sp. PCC 7002, suggest that the heliobacterial reaction center is inherently light-limited. In support of a light-limited model, light saturation profiles of untreated cells compared to those treated with ferric ammonium citrate indicated that only 32% of the P800 pool is oxidized during continuous illumination. Taken together, these results indicate that, in stark contrast to all other known phototrophs, phototrophy in the heliobacteria is light-limited.

Eukaryotic Photosynthetic Thylakoid Biohybrids: Probing Fundamental Interactions in Charge Accumulation

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Photosynthesis harnesses energy from the sun, driving electron transport and carbon fixation. Previous work in our lab has shown that negatively charged mercaptosuccinic acid capped platinum nanoparticles (PtNP) are able to self-assemble on positively charged regions of Photosystem I (PSI) near ferredoxin/flavodoxin binding locations. This targeted self-assembly strategy translates to PtNP incorporation at intrinsic PSI sites within thylakoid membranes to achieve complete solar water-splitting systems. Not only can PtNP be used as a H_2 catalyst but also as a bioinorganic probe of fundamental interactions between native electron acceptor proteins and PSI, allowing better understanding of how PSI electron transfer drives the formation of NADPH. When PtNPs are incorporated with thylakoid membranes, electron transport between Photosystem II (PSII) and PSI can be observed. Recent optical transient absorption spectroscopic results show that PtNP acts as an "electron sink" that rapidly "pulls" electrons through the linear electron transfer chain in biohybrid thylakoids. Thus, targeted binding of PtNPs to PSI can potentially be used to "switch" between cyclic and linear electron transport mechanisms. In this work, we explore how different eukaryotic thylakoid membranes across several species (Spinach, *Scenedesmus obliquus,* and *Chlorella vulgaris*) may affect H_2 production efficiencies as well as higher order complex formation between PSI and ferredoxin (Fd) and ferredoxin-$NADP^+$ reductase (FNR). Understanding the fundamental mechanisms of electron transfer between PSII and PSI as well as between PSI and its native protein and abiotic electron acceptors will inform design strategies for sustainable photosynthetic-inspired systems with efficient solar energy conversion and solar fuel synthesis capabilities.

Biohybrid Bacterial Microcompartment Shells as Nanoreactors for Photocatalytic Hydrogen Evolution

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Bacterial microcompartments (BMCs) are protein shells that encapsulate enzymes and substrates of specific metabolic pathways to augment catalytic turnover through means of confinement. Compartmentalization of reaction pathways is a ubiquitous phenomenon in biological systems, but more difficult to achieve through synthetic means. As such, in this study we have engineered biohybrid BMC shells loaded with abiotic compounds to leverage the chemistries of synthetic molecules and tune their reactivities within biological microenvironments. We encapsulated the benchmark photosensitizer, $Ru(bpy)_3$, and the molecular catalyst, chloro(pyridine)cobaloxime, to facilitate photocatalytic hydrogen evolution in confinement. The $Ru(bpy)_3$/cobaloxime loaded shells were able to successfully generate H_2, albeit at a lower turnover number (TON) and turnover frequency (TOF) than the bulk multimolecular reaction without protein. Remarkably, when $Ru(bpy)_3$ was added to solution outside of the loaded shells the TOF increased dramatically, surpassing that of the bulk reaction 4-fold. Since $Ru(bpy)_3$ outside the shell should theoretically be incapable of direct electron transfer to cobaloxime located on the inside, our results suggest an electron transport process through the ~30 Å thick shell wall, perhaps via tyrosine-mediated proton coupled electron transfer. These results highlight the potential of BMC shells as nanoreactors for energy-conserving chemistries with applications in photocatalysis, electrocatalysis, and biomaterials.

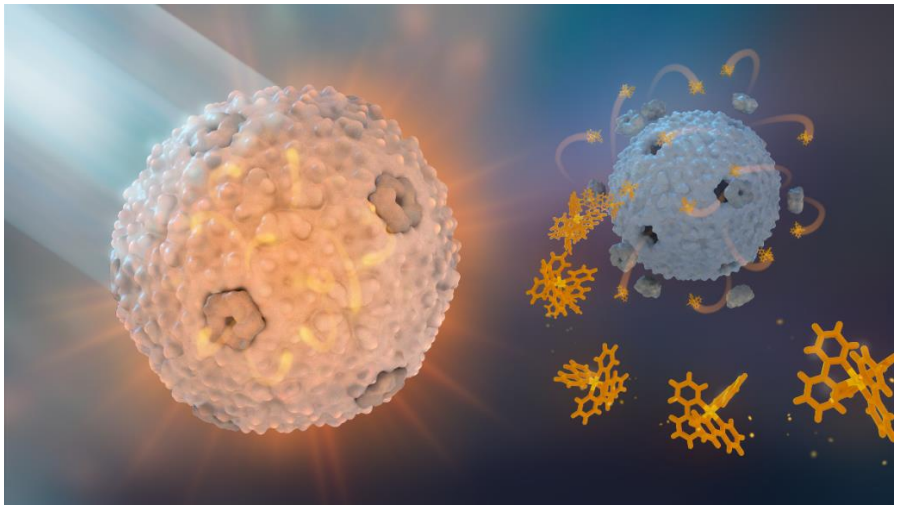

Figure. 1. Scheme showing BMC shell loaded with photosensitizers and illuminated with blue light.

Continuous directed evolution of soybean (*Glycine max*) SBPase

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Protein engineering for crop applications can be limited by a lack of understanding of the sequence-structure-function relationship. Directed evolution can overcome this by combining gene-specific hypermutation and selection. Continuous directed evolution does this within the cell by linking survival to a desired protein activity, amplifying the search of sequence space for gene variants with improved or novel functions. It therefore has the potential to guide gene editing efforts in plant or crop enzymes where little is known about their function. However, this application is currently lacking. Here, a continuous directed evolution technique, OrthoRep, is applied to soybean (*Glycine max*) SBPase, a photosynthetic enzyme implicated in the rate limiting step of carbon assimilation under current and future atmospheric CO2 concentrations and whose activity has been shown to determine plant yield.

We establish and validate an SBPase activity dependent yeast (*Saccharomyces cerevisiae*) selection platform and introduce OrthoRep into it. Ninety-six independent evolution campaigns across a range of population sizes and replicates show that SBPase is readily and rapidly evolvable, reaching native yeast SBPase-like performance in a timescale of 50-70 generations in most populations.

A well-defined set of genotypes appearing multiple times in populations with evolved improved growth was identified, with the high degree of convergence and evolution speed suggesting a smooth fitness landscape. Assays are currently underway to validate growth-enhancing mutation properties, determine causality in multi-substitution mutants, and biochemically characterize the most promising mutants. Additionally, we are working on solving the first crystal structure of a higher plant SBPase to understand the structural and mechanistic basis of improved enzymatic performance. This work showcases the power of synthetic biology for improving crops for sustainability or bioproducts.

Phycobilisome core architecture influences photoprotective quenching by the Orange Carotenoid Protein

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Photosynthetic organisms rely on sophisticated photoprotective mechanisms to prevent oxidative damage under high or fluctuating solar illumination. Cyanobacteria, which have evolved a unique, water-soluble light harvesting complex—the phycobilisome—achieve photoprotection through a photoactivatable quencher called the Orange Carotenoid Protein (OCP). Phycobilisomes are highly symmetric and modular, formed by hierarchical assembly of conserved subunits into diverse geometries ranging from simple bundles to elaborate fan- or bouquet-like macromolecular architectures. Although OCP is known to provide photoprotection across species of cyanobacteria with different phycobilisome structures, it is not known whether or how these structural variations relate to changes in the photoprotective function of OCP. For example, OCP was recently discovered to bind as a dimer at two specific instances of an abundant structural motif on the tricylindrical phycobilisome of *Synechocystis* sp. PCC 6803, yet these sites are sterically inaccessible on a more common pentacylindrical phycobilisome (*Anabaena* sp. PCC 7120). To understand how structural modularity and binding specificity contribute to conservation of OCP binding sites and function across different phycobilisome architectures, here we compare experimentally measured photophysical states accessible to these prototypical tricylindrical and pentacylindrical phycobilisomes, with and without OCP, at the single-molecule level. Together with Monte Carlo simulations of exciton transfer in OCP-quenched phycobilisomes, our results suggest that OCP binds at distinct and specific sites in each type of phycobilisome, yet provides nearly identical quenching strength to both phycobilisomes. Our findings (Ejaz et al. 2025) highlight the utility of modular phycobilisome structures in balancing robust conservation of photoprotective function with adaptability of site-specific binding across species.

Ejaz A, Sutter M, Lechno-Jossef S, Kerfeld CA, and Squires AH. (2025). Phycobilisome core architecture influences quenching by the Orange Caroteinoid Protein. PNAS 122(41). DOI: 10.1073/pnas.2420355122

Chlamydomonas reinhardtii growth is accelerated by constitutive overexpression of a glutathione S-transferase

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A genetic mutant of the green alga *Chlamydomonas reinhardtii* that constitutively overexpresses cytosolic glutathione S-transferase (CC-4610; *GSTS1*) was investigated in comparison to wild type (CC-125) by a spectroscopic overview of their photosynthetic physiology. This overexpression results in several phenotypic changes, including a significant amplification to overall growth in photoautotrophic conditions (55% higher OD_{730} at the start of death phase). The breadth of changes to the photosynthetic electron transport chain visible in the genetic mutant indicate that implementation of GST overexpression in higher order plants may prove a succinct, efficient way of optimizing their growth. When compared to CC-125 by a battery of techniques *in vitro*, several of the phenotypic changes in CC-4610 mimic those of high-light grown green algae. Observation of Q_A^- reoxidation kinetics suggest that the mutant exhibits a higher reduction and turn-over of the plastoquinone pool, with a 13.1% larger population of reaction centers performing primary electron transfer to Q_B. CC-4610 also displays a markedly larger proton motive force than the wild type when monitored by electrochromic shift studies. Comparisons of fast repetition rate fluorometry, electrochromic band-shift, Q_A^- reoxidation kinetics, and quantum yields suggest an added mechanism of electrons being removed from the photosynthetic electron transport chain and subsequently shuttled to the mitochondria, amplifying respiration in CC-4610. These mechanisms may work in parallel to increase ATP availability and storage, respectively, contributing to the substantial increase in growth.

Engineering strategies for continuous biomass collection of *Anabaena 33047*

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Cyanobacteria provide an accessible system for studying sustainable biotechnology because of their roles in nitrogen fixation, CO -capture, and nutrient cycling. A persistent challenge in experimental work, however, is biomass collection, which is often performed in labor-intensive batch processes that interrupt growth and increase the risk of contamination. This project explores a small-scale rotating biofilm reactor designed to continuously collect *Anabaena 33047* biomass under laboratory conditions. The reactor uses a simple vertical filter paper substrate mounted on a rotating frame, allowing cells to attach, grow, and be harvested with minimal handling. Trials were conducted to assess substrate stability, growth consistency, and ease of biomass removal in a controlled bench-top environment. Results show that this setup can reliably maintain cultures while simplifying collection, making it a useful tool for laboratory teaching, training, or preliminary research studies. By reducing complexity and cost, this small-scale system provides a practical entry point for learning about attached growth systems and continuous biomass collection in cyanobacteria.

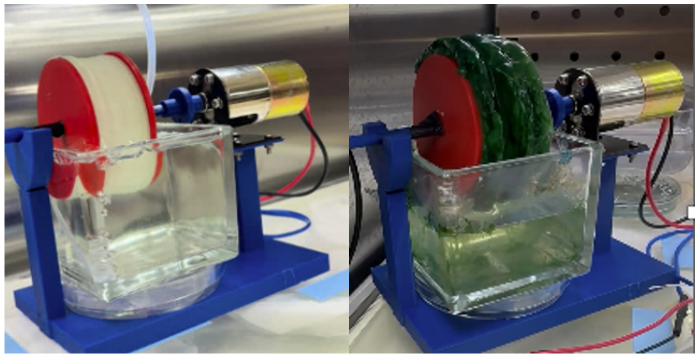

Figure 1. A small-scale biofilm reacter for continuously biomass collection of *Anabaena 33047*.

Understanding the HAD domain function of the SUPPRESSOR OF QUENCHING 1 protein in photoprotection in *Arabidopsis thaliana*

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Abstract

Photosynthesis converts light into chemical energy, essential for food production and climate change mitigation. Yet, excess light can be detrimental, producing reactive oxygen species that damage plant cells and triggering photorespiration by prompting ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to react with O_2 instead of CO_2. Additionally, Rubisco activity can be impaired by sugar phosphate inhibitors. Plants employ non-photochemical quenching (NPQ) to dissipate excess light as heat. One form, qH, is a slowly reversible NPQ process mediated by the protein LIPOCALIN IN THE PLASTID (LCNP). The protein SUPPRESSOR OF QUENCHING 1 (SOQ1) negatively regulates qH, while RELAXATION OF QH1 (ROQH1) deactivates it. Loss of LCNP makes plants vulnerable to photobleaching in excess light, while constitutive qH activation in *soq1 roqh1* mutants severely restricts growth, highlighting the need to balance light harvesting and energy dissipation. SOQ1 has multiple isoforms and domains in the chloroplast stroma and lumen. While the lumenal domain inhibits qH, the function of the stromal haloacid dehalogenase-like hydrolase (HAD) domain remains unknown. This domain, unique to land plant SOQ1 proteins, is not required for qH inactivation but is closely related to CbbY, a sugar phosphatase protein that dephosphorylates xylulose-1,5-bisphosphate, a Rubisco inhibitor. We propose that under excess light or photorespiratory conditions, accumulating sugar phosphates may activate SOQ1-HAD sugar phosphatase activity, leading to downstream modulation of the lumenal domains of SOQ1, thereby triggering qH (Fig.1). Here, using chlorophyll fluorescence, we observed qH-dependent increase in NPQ during photorespiration. We will further investigate Rubisco activity and identify the SOQ1-HAD domain's substrates in qH transformants via gas exchange and mass spectrometry. These studies will enhance our understanding of SOQ1 domain crosstalk in photoprotection, offering insights for engineering resilient crops.

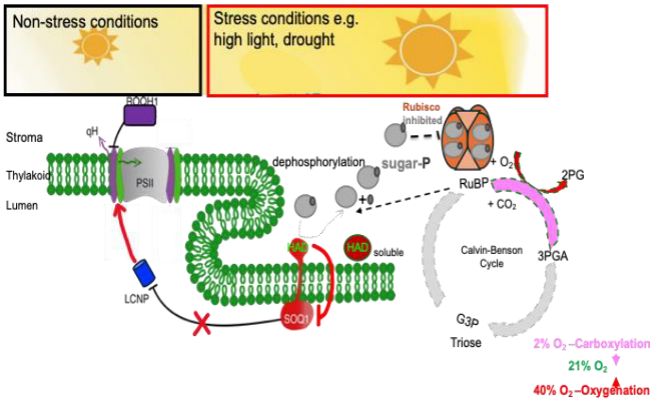

Fig.1. SOQ1-HAD domain functions as a sugar phosphatase

Tracking Photosynthetic Metabolite Transport Through Bacterial Microcompartments Using Enhanced Sampling Simulations

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Bacterial Microcompartments (BMCs) are proteinaceous organelles that sequester key metabolic reactions to increase enzymatic efficiency and prevent the loss of volatile or cytotoxic intermediates. Among them, carboxysomes (CBs) are of particular interest, as they sequester ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) to enhance carbon fixation by elevating the local CO_2 concentration and thereby improving rubisco's catalytic efficiency. The BMC shell functions as a selective, semi-permeable barrier, and its permeability is central to maintaining metabolite gradients between the organelle interior and the cytoplasm. While prior studies have demonstrated selective transport of Calvin cycle metabolites into CB hexameric pores, the precise mechanisms governing molecular influx and efflux through both the pores remain unclear. Here, we employed enhanced sampling molecular dynamics simulations to quantify the permeability coefficients of key metabolites—HCO_3^-, CO_2, O_2, ribulose bisphosphate (RuBP), and 3-phosphoglycerate (3-PGA)—through the hexameric and trimeric pores of the BMC shell. Our results reveal that the pores preferentially facilitate the passage of HCO_3^-, 3-PGA, and RuBP, while providing lower permeability for CO_2 and O_2. These findings offer mechanistic insight into metabolite transport across BMC shells and establish a predictive framework for tuning shell permeability. This approach enables the rational engineering of BMC-based metabolic modules, opening new opportunities in synthetic biology for enhancing carbon fixation, bioenergy production, and sustainable biodesign.

Poster Abstracts

UNREVELAING PHOTOPROTECTION MECHANIESMS IN THE FENNA-MATTHEWS-OLSON (FMO) LIGHT-HARVESTING COMPLEX

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Photoprotection is a critical biochemical process that helps organisms to survive cellular damage caused by excessive sunlight. Under sunlight excitation bacteriochlorophyll molecules can undergo intersystem crossing to long living triplet state. In aerobic conditions, the triplet-state (bacterio)chlorophylls can transfer energy to molecular oxygen. This spin-allowed process generates highly reactive singlet oxygen that irreversibly reacts with nearby organic molecules, eventually leading to oxidative stress and damage to living cells.

Many photosynthetic proteins mitigate the damaging effect of excess solar radiation by incorporating carotenoid molecules whose low-lying triplet states become a sink for the excitation energy from which it safely dissipates into heat.

The Fenna-Matthews-Olson (FMO) pigment protein is essential in transferring excitation energy from the chlorosome antenna to the reaction center in green sulfur bacteria. Surprisingly, FMO complex doesn't contain any carotenoids, yet it's found to be exceptionally stable in aerobic conditions. Therefore, our goal is to understand the photoprotection mechanisms of this photosynthetic complex. Implications of this project are a rational design of bio-inspired light-harvesting antennas and the redesign of natural photosynthetic systems, opening new possibilities for regulating these systems in response to excess light.

We explore three possible mechanisms contributing to the photoprotection of FMO. One possibility is a physical barrier photoprotection mechanism that in which the protein backbone acts as a shield to prevent molecular oxygen from reaching BChl sites. To this end, we investigated the distribution of molecular oxygen in molecular dynamics trajectories and computed the binding free energy between O2 and BChls. Furthermore, we studied the energy transfer mechanism between the triplet BChl and O_2 utilizing quantum mechanical calculations, because the fast energy transfer depends on factors such as strong coupling and energy matching between donor and acceptor. Finally, we are investigating whether the photoprotection chemistry in FMO can be attributed to a redox-dependent excitation quenching mechanism and whether specific key amino acids play a role in this redox chemistry.

Probing Metabolite Overflow Mechanisms of Cyanobacterium *Synechocystis* sp. PCC 6803 under High Light and High Salt Conditions

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Metabolic overflow in cyanobacteria may play a crucial role in managing excess energy and redirecting carbon flow during stressful conditions (Cano et al 2018). We have previously reported the metabolite overflow of cyanobacteria using HPLC, LCMS, and FTIR (Alamin et al 2024). We identified 56 targeted and 170 untargeted metabolite end-products of overflow metabolism in cyanobacteria. The 226 metabolites include organic acids, amino acids, organic bases, esters, alcohols, ketones, aldehydes, and sugars. HPLC data showed that pyruvate overflow is sensitive to the presence of nitrate and lower pH in the growth medium. FTIR analysis showed the changes in overflow metabolites end-products in cyanobacterium *Synechocystis* 6803 at high light condition. In this work we focus on the overflow metabolism of Synechocystis sp. PCC6803, when exposed to high light and high salt environments, which are significant abiotic stressors that can disrupt cellular balance. The cultures were cultivated in BG-11 medium and subjected to increased photon flux densities along with elevated NaCl concentrations. The research monitored changes in growth and the excretion of various metabolites. A major overflow product with mM scale of glycerol with minor other metabolite was observed at the late growth phase (Figure 1). The findings highlight that heightened light conditions enhance photosynthetic electron flow, leading to an increased accumulation and release of overflow metabolites such as glycerol, acetate, and ethanol. Additionally, high salt stress adds an energetic burden due to the need for ion homeostasis and the biosynthesis of compatible solutes, further amplifying the overflow responses. Overall, these insights enhance our understanding of how Synechocystis sp. PCC 6803 reallocates energy and carbon during environmental stress, contributing valuable information for developing metabolic engineering approaches aimed at improving biofuel and bioproduct synthesis. Future work will access the mechanistic details using genetically engineering mutants associate salt stress in cyanobacteria.

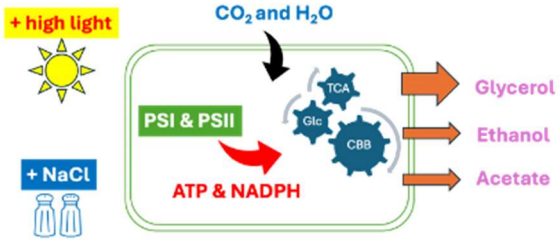

Figure 1: Metabolite overflow profiles of cyanobacteria at high light and high salt conditions

Cano et al (2018) Manipulation of glycogen and sucrose synthesis increases photosynthetic productivity in cyanobacteria, Front Microbiol. 14: 1124274.

Ibrahim Alamin, Jacob Sebesta, Arnav Deshpande, Vida A Dennis, Lieve Lauren, Jianping Yu, and Harvey J.M. Hou, "HPLC, LCMS, and FTIR analysis of cyanobacterial metabolite overflow for energy management," 50th Midwest/southeast Photosynthesis Meeting, Turkey Run Inn, Marshall Indi, October 25-27, 2024

Photoprotective capacity under dynamic light conditions varies across the maize canopy

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Crops in the open field grow in extremely dynamic light environments: cloud cover, sun angle, wind, and self-shading within dense canopies can alter light intensity by orders of magnitude within seconds. When light intensity is too high for all the energy to be used for photosynthesis, photoprotective mechanisms are induced to prevent damage to the photosynthetic machinery and subsequently relaxed when light becomes limiting. Nonphotochemical quenching (NPQ), wherein excess light energy is dissipated as heat, is one of the most universal and important photoprotective mechanisms in plants. NPQ responses are fast but not instantaneous, often lagging behind light fluctuations and temporarily limiting photosynthetic efficiency and resulting in substantial losses to carbon assimilation. Within the complex structures of plant canopies, there is significant spatial variation not just in light availability, but also in the frequency, duration, and amplitude of light fluctuations. Leaves at different levels have distinct anatomical, structural, and metabolic characteristics, and whilst our understanding of NPQ responses to dynamic light has increased substantially, how those responses vary within the leaf canopy is not yet fully understood. We studied variation in photoprotective and photosynthetic capacity across the maize canopy by comparing NPQ induction and relaxation dynamics and carbon assimilation under steady and fluctuating light conditions. Top leaves not only had higher photosynthetic capacity in steady state but also exhibited greater adaptability to changing light conditions – NPQ induction and relaxation were faster in top than in bottom leaves, suggesting more effective photoprotection at that canopy position. Concomitantly, top leaves had higher assimilation relative to steady state after light-step changes, further indicating faster responses to fluctuating light. These findings underscore the importance of considering canopy position for models of photoprotective responses and whole-canopy photosynthesis.

FTIR Difference Spectroscopy for the Study of Photosystem I from *Chroococcidiopsis thermalis* PCC 7203 Cells Grown Under Far-Red and White Light

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Photosystem I (PSI) is a membrane protein complex in oxygenic photosynthesis that mediates photoinduced oxidation-reduction in the thylakoid membrane. Certain types of cyanobacteria adapt and grow under far-red light, where a small fraction of chlorophyll *a* (Chl *a*) in PSI is replaced by chlorophyll *f* (Chl *f*) through a process called far-red light photo acclimation (FaRLiP). The absorption maximum of Chl *f* is around 740 nm, making photosynthesis possible using lower-energy, far-red photons.

Here we are studying PSI from *C. thermalis* cells grown under far-red light (FRL) and white light (WL). FTIR difference spectra (DS) shows vibrational bands associated with both neutral and oxidized P700. Time-resolved FTIR DS experiments show distinctly different kinetics for FRL- and WL-PSI, suggesting modified charge-separation dynamics. Photo accumulated and time-resolved FTIR DS reveal distinct features associated with $P700^{+}$ and A_1^{-} formation that allow a comparison of the structure of these pigments in FRL- and WL-PSI.

Keywords: Photosystem I, chlorophyll *f*, far-red light, *Chroococcidiopsis thermalis*, FTIR difference spectroscopy.

Spectral Evolution under Iron Stress in Two Model Cyanobacterial Cells

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Iron is the second most abundant element and an essential micronutrient for plant growth. The solubility of iron in water is low, which limits its availability, especially in aquatic environments. Global iron levels for most marine bodies are <1 nM, while for freshwater, they generally do not exceed 18 µM. This suggests that aquatic photoautotrophs experience a constant iron deficiency. Iron is essential for maintaining photosynthetic activity, and many cyanobacteria have evolved a specialized protein called IsiA (Iron-stress-induced). The role of this protein continues to be investigated. Interestingly, unlike *Synechocystis* 6803, the diazotrophic cyanobacterium *Anabaena* 7120 has multiple copies of IsiA that form a unique complex with monomeric PSI that lacks the usual PsaL subunit. In this study, we followed the changes in spectral properties from iron surplus to iron deficiency in whole cells. Based on our analysis, we determined that under iron stress, the levels of PSI declined at a significantly faster rate than those of PSII. In fact, the levels of PSII did not decrease considerably under iron starvation. We also observed that in *Synechocystis* 6803, the response to iron stress was immediate. In comparison, in *Anabaena* 7120, the response to iron stress was gradual and slow. Upon comparing the IsiA-fluorescence decay profiles between the two strains, we observed almost no change in *Synechocystis* 6803; however, the decay lifetime slowed down under prolonged iron stress in *Anabaena* 7120. We propose that the additional copies of IsiA proteins in *Anabaena* 7120 are responsible for exhibiting characteristics that differ from those of *Synechocystis* 6803. Further work is being conducted to elucidate the expression and roles of these additional IsiA proteins under iron stress in *Anabaena* 7120.

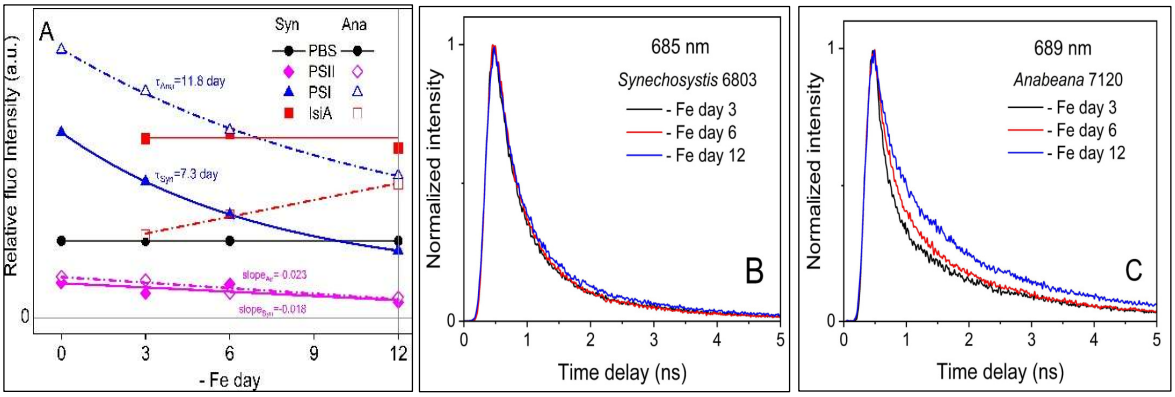

Figure 1. (**A)** Comparison of relative fluorescence emissions from each pigment-protein complex (excitation at 410 nm) for *Synechocystis* 6803 and *Anabaena* 7120 at various stages of Fe deficiency. **(B, C)** Dynamics of IsiA fluorescence emission decay in whole cells at various stages of Fe deficiency. IRF, instrument response function; FWHM, full width at half maximum.

This study is supported by funding from the U.S. Department of Energy (DOE), Office of Basic Energy Sciences (DE-FG02-99ER20350) and from the DOE Office of Science Energy Earthshot Initiative (DE-SC0024702).

Interrelated Roles of Chloride and Bicarbonate in Regulating Photosystem II Function in *Limnospira maxima*

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Efficient charge separation and electron transfer in Photosystem II (PSII) depend on small inorganic cofactors that maintain redox balance and catalytic stability. Chloride facilitates oxygen evolution by stabilizing the electrostatic and hydrogen-bonding network surrounding the water oxidizing complex, thereby supporting proper turnover and minimizing charge recombination. Bicarbonate, coordinated to the non-heme iron that bridges the primary and secondary quinones, plays a key role on the acceptor side by facilitating electron transfer and maintaining redox balance within the plastoquinone pool. While traditionally viewed as independent, previous studies suggest that chloride and bicarbonate may act cooperatively to coordinate proton and electron transfer across PSII. To examine this possible relationship, the hypercarbonate-requiring cyanobacterium *Limnospira maxima* was studied due to its high tolerance for dissolved inorganic carbon and halide substitution. This work investigates how bicarbonate depletion impacts PSII activity in bromide substituted cells, in comparison to the native chloride containing system, probing potential interdependencies between chloride and bicarbonate. In 77K fluorescence spectra, bromide substituted cells retain a higher PSII:PSI emission ratio and relatively elevated F685:F695 ratio, consistent with greater antenna association under depletion. Fast repetition rate fluorometry (FRR) fluorescence revealed a loss of stable PSII centers in bromide depleted samples. Q_A^- reoxidation kinetics showed fewer active centers overall, with the remaining centers transferring electrons rapidly to Q_B or the non-heme iron but unable to sustain downstream turnover. Cytochrome b_6f and plastocyanin measurements indicate enhanced PSI cyclic electron flow, suggesting compensatory energy balancing when linear flow through PSII is restricted. P700 oxidation signals further support stronger and faster PSI activity under depletion. Together, these studies indicate that chloride and bicarbonate work together to sustain PSII turnover and their combined depletion disrupts the balance of electron transfer across the photosynthetic apparatus in *Limnospira maxima*.

Investigating the role of PsbR phosphorylation in photosystem II disassembly and repair

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Photosystem II (PSII) is vulnerable to photodamage from reactive oxygen species generated during photosynthesis, with damage intensified under abiotic stresses such as high light, drought, and elevated temperatures. To mitigate this damage, PSII undergoes a dynamic disassembly and repair cycle, in which the PSII supercomplex dissociates into a repair-competent submonomeric form, allowing for the selective replacement of damaged subunits. Our previous work showed that core protein phosphorylation facilitates this disassembly by promoting the dissociation of peripheral antenna complexes and the monomerization of the dimeric PSII core. However, the specific roles of individual phosphorylation sites remain poorly understood. Our recent phosphoproteomic analyses have identified several novel PSII phosphoproteins, including CP47 and PsbR. PsbR, a nuclear-encoded protein, contains up to four phosphorylation sites, with serine 58 (S58) being the most abundantly phosphorylated. We hypothesize that phosphorylation of PsbR promotes the disassembly of dimeric PSII supercomplexes spanning adjacent thylakoid membranes, with potential implications for thylakoid stacking and the mobilization of PSII for repair. To investigate this, we have employed Phos-tag gel electrophoresis to identify the physiological conditions under which PsbR is phosphorylated and to determine the protein kinase responsible. We are currently generating transgenic *Arabidopsis thaliana* lines expressing phosphorylation-deficient and phosphomimetic variants of PsbR to assess the functional consequences of its phosphorylation on PSII disassembly and repair.

Building an Understanding of Epoxidase Enzymes in Xanthophyll Cycles

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Photoprotection is an essential counterbalance to light harvesting to avoid damage from excess absorbed light energy and is regulated by two different cycles. While the violaxanthin (VAZ) cycle is well-studied, the lutein epoxide (LxL) cycle is less understood due to its absence in model species. Previous studies have shown that the LxL cycle has the potential to improve photosynthetic efficiency when transgenically engineered into Arabidopsis. Our work focuses on characterizing the genes involved in these cycles – zeaxanthin epoxidase (ZEP) and lutein epoxidase (LEP) – from *Glycine max* (soybean) and examining whether the molecular interactions between these proteins influence substrate specificity. Additionally, we are exploring what effect swapping protein domains, e.g., the forkhead-associated (FHA) domain, will have on enzyme function to further uncover the function of these genes and their xanthophyll cycles in agriculturally significant plants.

Figure. ##. A descriptive figure may be included. If including a figure, please add a figure caption here.

Isolation and characterization of cyanobacteria and algae strains in Alabama, Missouri, Colorado, and Gulf coast regions

Maazeera Fatima[1,2], Ibrahim Alamin[1,2], Lydia DaviesBalogun[1], Victory Obele[1], Khadijah Taite[1,3], and Eric Schaedig[4], Jacob Sebesta[4], Anindita Banejee[5], Michelle Liberton[5], Vida A Dennis[2], Himadri Pakrasi[5,*], Jianping Yu[4,*], and Harvey J.M. Hou[1,2,*]

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We have previously isolated fast-growing cyanobacteria that can be utilized for biofuel and biochemical production (Yu et al 2015). Recently we used bioprospecting to discover natural strains of microalgae with desired traits (Schaedig et al 2024). Our hypothesis is that Alabama and the Gulf coast region may host unique microalgae and cyanobacteria strains for bioenergy application. In this work, we collected samples from freshwater and coastal locations in Alabama, Missouri, Colorado, and the Gulf Coast to establish pure cultures of cyanobacteria and algae (Figure 1). Characterization of the samples includes physiological assays to evaluate growth and development under diverse light and nutritional circumstances. The cellular protein and pigment abundance in samples were accessed using UV-vis spectroscopy and Fourier transform infrared (FTIR) method. Future work will be placed on 16S rRNA sequencing to verify taxonomic identity. This work has laid a foundation to establish database of cyanobacteria and algae strains at Alabama State University. The data show the ecological diversity of cyanobacteria and algae and offer potential use in biofertilizer production, oil degradation, plastic digestion, and forensic application.

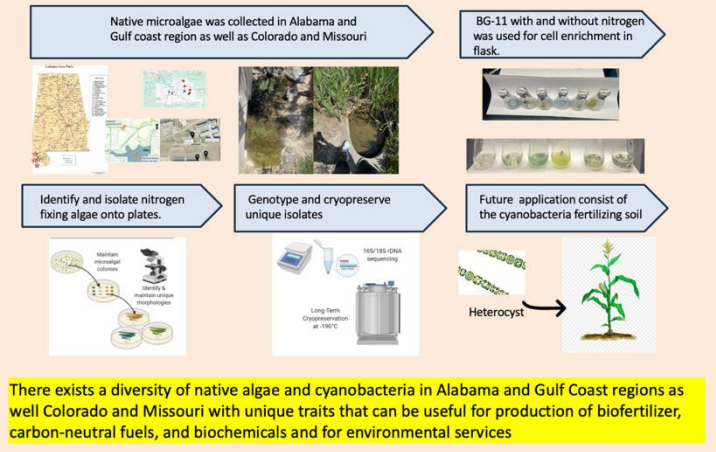

Figure 1: Brief diagram for collection and analysis of cyanobacteria and algae strains.

Yu, J., Liberton, M., Cliften, P. F., Head, R. D., Jacobs, J. M., Smith, R. D., Koppenaal, D. W., Brand, J. J. and Pakrasi, H. B. 2015 "Synechococcus elongatus UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO2." Scientific Rep., 5: 8132

Eric Schaedig, Michael Cantrell, Chris Urban, Jacob Sebesta, Katherine J. Chou, and Jianping Yu* (2024) Isolation & bioprospecting of wild microalgae for biotechnology applications, Front. Sci. Technol. Eng. Math, 8: 17-18

A shady business in legumes: Identification of a lutein epoxidase in soybean

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Leaves rapidly adjust their xanthophyll pool in response to changes in light to funnel absorbed excitation energy toward either photochemistry or photoprotective energy dissipation. In roughly 60% of species surveyed, α-xanthophylls are converted between shade-enriched lutein epoxide (Lx) and photoprotective lutein (L), forming the lutein epoxide cycle (LxL cycle). Despite its prevalence, the enzyme involved in the epoxidation reaction of the LxL cycle is unknown. Using pigment profiling and canopy-specific RNA-Seq, we detect Lx throughout the middle and lower canopy of soybean and link its accumulation to the expression of a mono-oxygenase we call *lutein epoxidase* (*LEP)*. We confirm LEP activity in a transient assay in *Nicotiana benthamiana*. Lastly, we show that *LEP*s are distinct from *zeaxanthin epoxidases* (*ZEP*s) and are conserved across many legumes. The discovery of an enzyme involved in α-xanthophyll biosynthesis provides us with new opportunities to explore how plants optimize photosynthesis in deeply shaded environments.

Development of a pipeline for the *in situ* assembly dynamics of a carbon-fixing bacterial nanocompartment

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Despite being a dominant and integral part of the carbon cycle throughout the domains of life, the signature enzyme of carbon fixation (Ribulose-1,5-bisphosphate carboxylase/oxygenase or **Rubisco**) is compromised by promiscuous reactions with other substrates. This promiscuity has stimulated the evolution of various carbon-concentrating mechanisms (**CCMs**) to enhance Rubisco activity. A prominent CCM in bacteria is the alpha carboxysome (**α-CB**). α-CBs are large (~150 nm), self-assembling, polyhedral protein shell assemblies which sequester the encapsulated Rubisco enzymes away from competing substrates. While the α-CB is an attractive tool for heterologous enhancement of carbon fixation, its assembly process has not been characterized *in vitro*. Our research utilizes the powerful tools of cryo-electron tomography (**cryo-ET**) and subtomogram averaging (**STA**) to interrogate the *in vivo* molecular interactions that govern the life cycle of the α-CB. We currently are developing an environmentally driven pipeline for producing synchronized *in vitro* α-CB expression events in the autotroph *Halothiobacillus neapolitanus*. Our preliminary work suggests that environmental manipulation and resource availability results in widespread changes in both α-CBs and the surrounding cell ultrastructure. Functional reconstitution or recombinant expression of this nanocompartment is an emerging research field with a wide array of biomedical and bioengineering applications. This work provides critical insights into optimization of these processes.

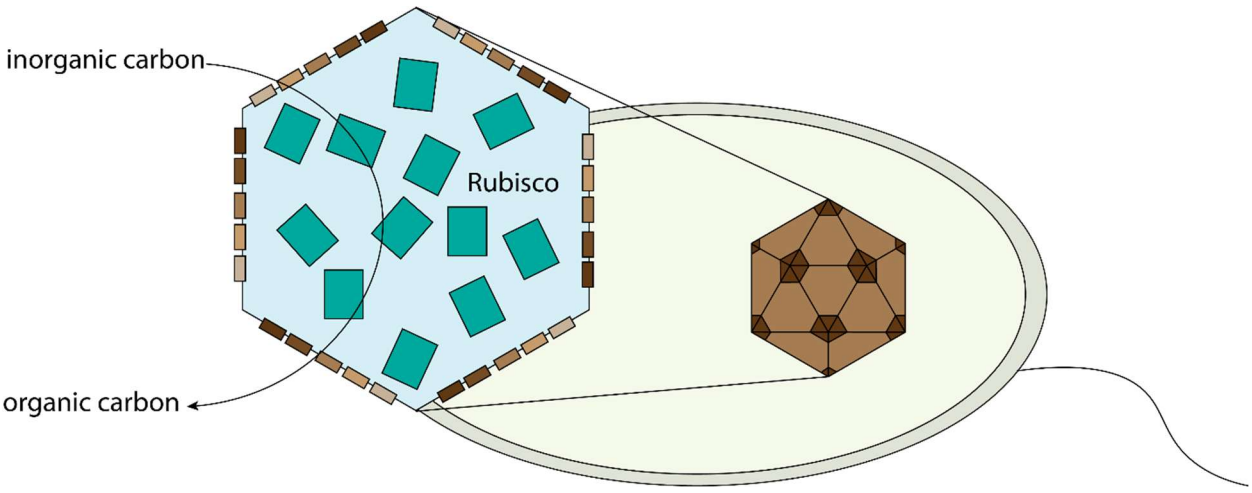

Figure 1. Schematic of Rubisco encapsulation inside polyhedral α-CB inside *Halothiobacillus neapolitanus*.

Protein-protein interactions within the diatom chloroplast three-component system

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Phaeodactylum tricornutum is a unicellular marine alga belonging to the diatoms, a diverse group of photosynthetic eukaryotes adapted to fluctuating aquatic environments with variable light and nutrient availability. Despite these environmental challenges, diatoms exhibit high photosynthetic productivity, making them promising candidates for biofuel production and bioengineering. Diatom plastids originated through secondary endosymbiosis with a unicellular red alga, retaining genetic material from both red algal and cyanobacterial ancestors. One example of conserved prokaryotic genes in diatoms is the two-component system (TCS), which consists of a sensor histidine kinase and a response regulator. In diatom plastids, the sensor kinase is the chloroplast sensor kinase (CSK), while two paralogous response regulators, chloroplast response regulator 1 (CRR1) and chloroplast response regulator 2 (CRR2), likely arose from a gene duplication event. The function of this "three-component" system in diatom plastids remains unclear. We hypothesize that CRR1 and CRR2 regulate plastid-encoded heat shock genes and are activated in response to changes in temperature and light. Using yeast two-hybrid analysis, we present preliminary evidence of interaction between CSK and CRR1, as well as between CRR1 and CRR2. These findings contribute to understanding the protein-protein interactions within the diatom chloroplast two-component system.

Modulation of Photosystem II Cyclic Electron Flow by State Transition Regulation in *Chlamydomonas reinhardtii*

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As global temperatures rise and drought conditions become more frequent, agriculture faces increasing strain, necessitating new strategies for crop acclimation to environmental hazards. In some photosynthetic organisms, the poorly characterized process of photosystem II-cyclic electron flow (PSII-CEF) plays a crucial role in protecting the PSII D1 protein under extreme light conditions and bypassing production of reactive oxygen species that cause photoinhibition. State transitions, a well-established process, regulate the exchange of light-harvesting complex (LHC) proteins between PSII and photosystem I (PSI), optimizing energy distribution to their respective reaction centers. By comparing the absorbance and fluorescence spectra between the green alga *Chlamydomonas reinhardtii* and its *stt7* mutant strain, CC-5681, which is trapped in state 1 with its LHC associated to PSII, we can further elucidate the conditional regulation of PSII-CEF. Here we report that linear electron flow through the photosynthetic electron transport chain and the activity of PSI is disrupted by the lack of state transitions, leading to the return of electrons to PSII via backward transitions characteristic of elevated PSII-CEF. Oxygen yield is diminished beyond what is expected from visible center activity, indicating significant PSII inactivation. PSI is expressed at lower levels in the mutant. Electrochromic shift studies indicate that despite conditions favoring minimal PSI-cyclic electron flow activity, the mutant has a stronger pH gradient across the thylakoid membrane, supporting the idea of PSII-CEF as a proton-coupled, energy-transducing process in addition to its photoprotective role.

TLP18.3 and Psb27-H1 binding to luminal CP43 and two Rubredoxins binding to stromal PsbE in higher plant Photosystem II as revealed by a structural mass spectrometry pipeline

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Cross-linking mass spectrometry (XL-MS) is a structural proteomics technique used to study protein–protein interactions and protein conformations at residue-level resolution. It has become a powerful complement to cryo-EM, X-ray crystallography, and NMR, particularly for probing dynamic or heterogeneous complexes. Here, we present a pipeline of locating protein subunits in large protein complexes by integrating isotopically encoded cross-linking, identification, evaluation, structural prediction, modeling, and structure validation. Using Photosystem II (PSII) as a model, the structural location of TLP18.3, Psb27, and two Rubredoxins in Photosystem II on the luminal side and stromal sides respectively in higher plants were established. Protein structure prediction, including AlphaFold 3, was performed if structures were not available. Structural modeling was performed using HADDOCK and ClusPro and importantly justified by using the chemical restraint, i.e., the intrinsic feature of the cross-linker arm span (11 Å). The resulting models of TLP18.3-Psb27-PSII and Rubredoxin-PSII provide a solid foundation for understanding their molecular functions during PSII dynamic life cycle and steady-state photoprotection in higher plants.

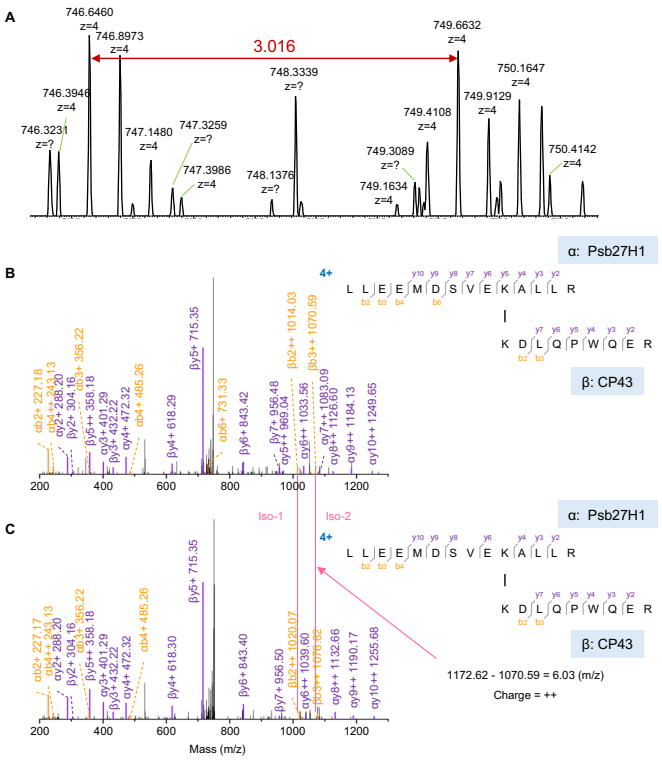

Figure. 1. Cross-links between Psb27 and CP43.

a, Precursor ion (MS1) spectra of cross-linked Psb27 and CP43 peptides showing the light and heavy cross-linked species (BS_3-h_{12}/d_{12}). The characteristic isotopic fingerprint appears as a peak doublet of similar intensity, separated by m/z 3.016.

b, Product-ion spectra (MS2) of the cross-linked peptide with BS_3-h_{12}.

c, Product-ion spectra of the cross-linked peptide with BS_3-d_{12}.

Exemplary isotopic fingerprints are indicated by the product ion pair Iso-1 and Iso-2 in panel c, corresponding to two identical ions.

Regulation of Cyclic Electron Flow in *Synechocystis* via NdhO and Bidirectional Hydrogenase *Synechocystis* 6803.

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Cyanobacteria are gram-negative oxygenic photosynthetic microorganisms that have evolved a unique CO_2 Concentrating Mechanism (CCM) to overcome the shortcomings of RuBisCO. The CCM consists of specialized NADPH Dehydrogenase Type I complexes (NDH-1 complexes). The NDH-1 is a multi-subunit protein complex that functions in respiratory electron flow and is also the main return path for photosynthetic cyclic electron flow in cyanobacteria. In both cases, reduced ferredoxin (Fd) from Photosystem 1 donates its electron to the trans-membrane plastoquinone pool (PQ) via the NDH-1 complex. One subunit of this complex is NdhO, which is an Oxygenic Photosynthetic Subunit (OPS), is located at the periphery of the NDH-1 complex and has been hypothesized to be regulating the cyclic electron flow through the complex. Additionally, an enzyme known as the bidirectional NiFe hydrogenase is present in *Synechocystis 6803*. It is a hetero pentameric enzyme and has Hox EFU (diaphorase) and Hox YH subunits. The Hox EFU consists of Fe-S clusters and oxidizes NADPH which the cyanobacterial NDH-1 complex lacks and thus unable to oxidize NADP.

We hypothesize that under conditions of stress (such as high light) Hox oxidizes NADPH. As a result of the oxidation electrons are transferred to ferredoxin which reduces it. The reduced ferredoxin transfer electrons to NDH-1MS (which likely lacks NdhO) through the PSI and are returned to Fd. Conversely, we also hypothesize that in the presence of NdhO, cyclic electron transfer through the NDH-1 complex will be lower as compared to the deletion of NdhO, because of NdhO blocking (acting as a brake or as an inhibitor) the electron transfer from ferredoxin to NDH-1MS.

The hypothesis is currently being tested in vivo with strains: the Wild-type *Synechocystis 6803*, M55 (insertionally inactivated NDH-1 complex) and ΔNdhO (mutant lacking the *ndhO* gene), OxndhO, ΔHox ΔNdhO, ΔHox OxndhO, and ΔHox (mutant lacking the bidirectional hydrogenase).

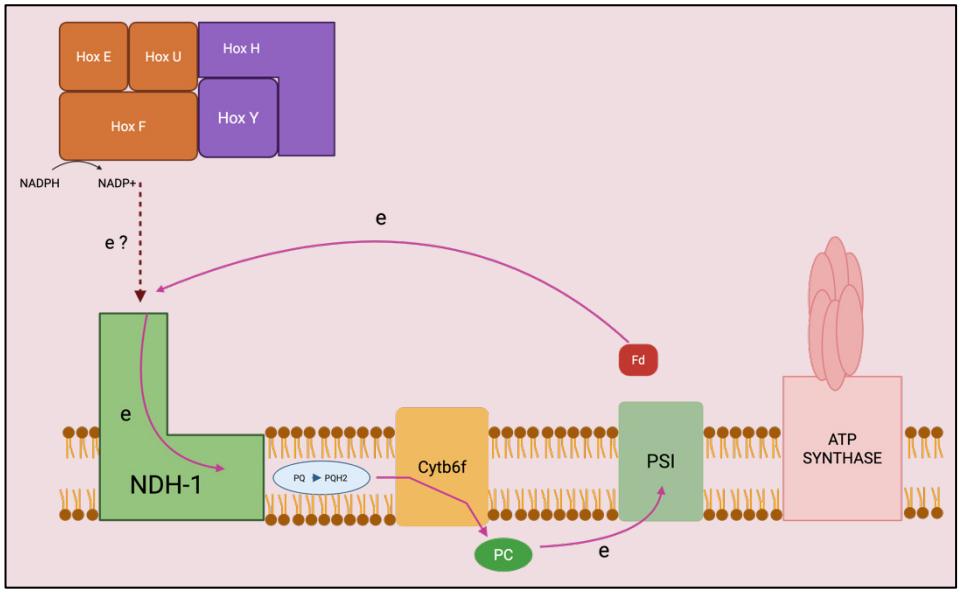

Figure 1. A schematic of the suggested movement of the electron movement from the NADPH oxidation to the NDH-1 complex directly.

Emerging functional roles for Chloroplast Sensor Kinase in land plants

Chloroplast Sensor Kinase (CSK) is an evolutionarily conserved bacterial-type kinase present in photosynthetic organisms. In cyanobacteria, the CSK homologue, Hik2, functions within a canonical two-component signaling system with its response regulator Rre1 to mediate transcriptional responses to changes in plastoquinone redox state. In non-green algae, the CSK homologue is thought to function with a homologue of the Rre1 termed Chloroplast Response Regulator (CRR). In contrast, CSK in green algae and plants has lost both the conserved histidine autophosphorylation site and the cognate response regulator partner. Nevertheless, CSK in these lineages retains the capacity to bind a 3Fe-4S cluster essential for redox sensing of plastoquinone pool. CSK is ubiquitously found in land plants, and a recent report shows selection for high CSK gene expression in modern cultivars of *Manihot esculenta* – a C3-C4 intermediate crop. However, the mechanistic basis of this broader functional role for CSK remains unresolved, apart from some earlier reports of altered plastid gene expression in CSK mutants. Furthermore, CSK knockout plants in *Arabidopsis thaliana* exhibit no visible phenotype under standard growth conditions. We hypothesize that CSK has evolved new functional roles within land plants by acting as an atypical serine/threonine kinase with phosphorylation targets across the photosynthetic metabolism. To investigate this, we are employing a mass spectrometry–based approach to define the CSK proxiome, proteome, and phosphoproteome using *Arabidopsis thaliana csk* mutants and wild type plants. Additionally, screening for photosynthetic phenotypes under high light and fluctuating light conditions will further unravel CSK's functional significance for C3 model and C4 crop plants.

Spectroscopic Studies of the IsiA-Photosystem I protein supercomplexes from the filamentous cyanobacterium *Anabaena* sp. PCC 7120

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Cyanobacteria, if exposed to iron-deficient conditions prevalent in nature, express large quantities of Iron-stress-induced-A proteins (IsiA). In the model cyanobacterium *Synechocystis* 6803, IsiA protein is highly homologous to CP43 inner antenna protein of PSII. Typically, IsiA forms tight rings around PSI trimer, forming new PSI-IsiA supercomplexes, though it could also produce PSI-free ring-like assemblies. However, some cyanobacteria, such as *Anabaena* 7120, produce multiple different versions of IsiA, which are structurally distinct from the IsiA seen in *Synechocystis* 6803 and interact differently with PSI. The photophysical properties and functional roles of such supercomplexes have not been investigated in detail.

Here, we present a set of spectroscopic studies on two distinctly different PSI-IsiA supercomplexes from *Anabaena* sp. PCC 7120. The investigations focused on determination of how alterative assemblies of PSI and IsiA influence the process of excitation energy transfer between antennas and core complex. For that purpose, we employed a broad range of static and time-resolved optical spectroscopies such as absorption, fluorescence, fs-time-resolved transient absorption, and ps-time-resolved fluorescence decay mapping. The studies were performed at 77 K. Low temperature enhances spectral differences between both separations, but also greatly improves detectability of some signals (as PSI-mediated fluorescence emission), which are extremely low and difficult to evaluate at room temperature. Additionally, we employed a unique sample handling method. All measurements were done on samples obtained via clear native gel separation (gel cuts with bands, B1 and B2).

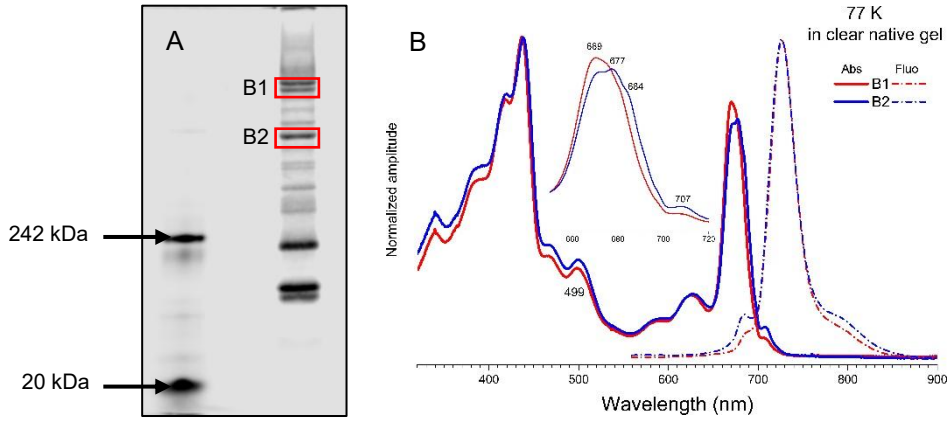

Figure 1. A) Clear-Native PAGE showing the pigment-protein bands B1 and B2. **B)** Absorption and fluorescence emission spectra of two species of IsiA-PSI supercomplexes (B1, B2) in polyacrylamide gel. The spectra were recorded at 77 K. For better comparability all spectra were normalized at their maxima.

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Light-Induced Electron Spin Qubit Pair States in Type I and Type II Photosynthetic Reaction Centers

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Photosynthetic Reaction Center (RC) proteins are complex yet well-defined and tunable systems, which can serve as ideal model systems for investigating spin dynamics and coherences, since long-lived electron spin entanglement - manifested as light-induced spin-correlated radical pairs (SCRPs) - can easily be generated and observed. Since discovery of SCRPs in our group in the 1980s, they have been extensively used to enhance our understanding of structure-function relationships in photosynthetic RC proteins. More recently, SCRPs have also been utilized as tools for quantum sensing where they act as electron spin-based quantum bit (qubit) pairs. Decoherence poses a significant challenge in realizing practical applications of electron spin qubits. Despite their importance, critical aspects like coherence spatial lengths, lifetime, decoherence mechanisms, and their interaction with the local and global protein structure, remain poorly understood, limiting our understanding of decoherence. Our work utilizes both experimental and theoretical strategies, including deuteration, biochemical modification of photosynthetic RCs, advanced EPR techniques, and semiclassical, atomistic modeling to describe decoherence processes in Photosystem I and type II photosynthetic RCs of purple bacteria. In particular, high-frequency electron paramagnetic resonance (EPR) spectroscopy operating at 130 GHz and 4.6 T was used to study coherence times through the decay of two-pulse electron spin echo signals and Rabi oscillations at various temperatures. The coherence times show only minimal dependence on biological species, deuteration, biochemical treatment, and paramagnetic species. To interpret these observations, we carried out large scale simulations of spin dynamics utilizing existing RC crystal structures. Comparison with the experiments shows that “classical” nuclear spin diffusion and instantaneous diffusion mechanisms alone cannot explain the observed decoherence times. We suggest that the low-temperature dynamics of methyl and amino groups surrounding the unpaired electron spin centers are the main factor governing loss of coherence in photosynthetic RCs. Understanding the intricate dynamics can enhance our knowledge of photosynthetic processes and their potential applications in achieving more efficient solar energy conversion.

Optimization of PSII particle preparation from *Arabidopsis thaliana* using different detergents ratio of TX-100

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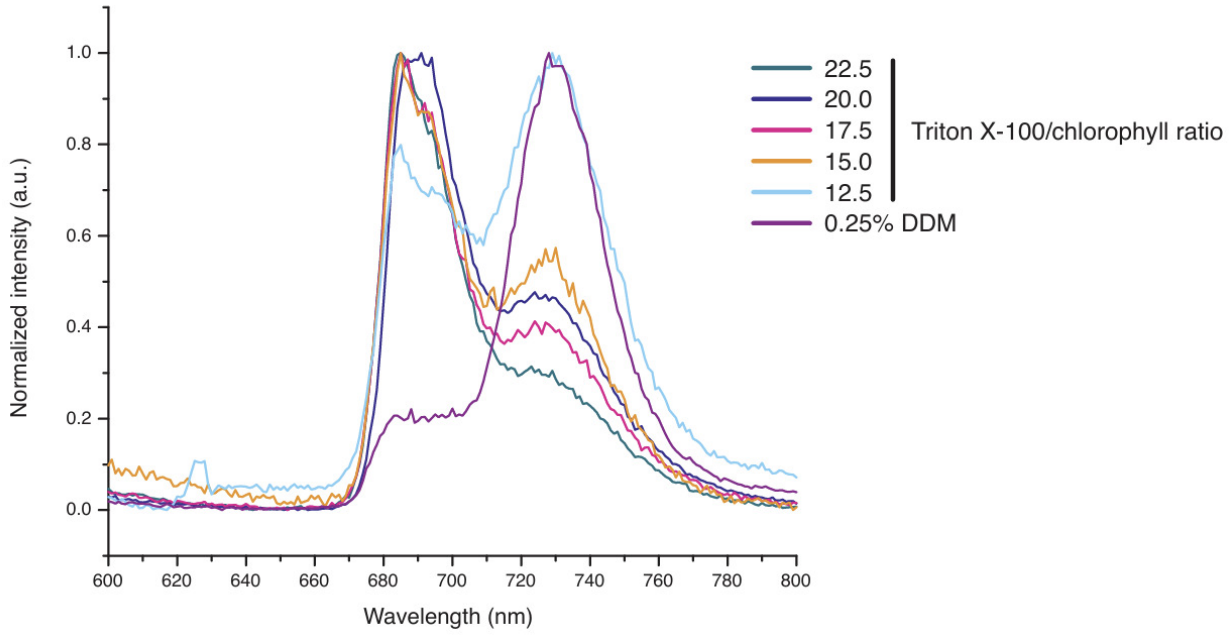
PSII particle (BBY, Berthold, Babcock, and Yocum, the three scientists who developed the method in 1981) preparation worked robustly using commercially available spinach leaves. We have evaluated the protocol using research model plant *Arabidopsis thaliana*. Five Triton X-100/chlorophyll ratios (22.5, 20, 17.5, 15, 12.5) were used to isolate BBY from relatively low light grown plants. The total PSII (chlorophyll) yield, photochemistry monitored using Q^{A-} reoxidation in the absence and presence of DCMU and fast fluorescence induction were measured using UV-Vis spectrophotometry, chlorophyll fluorometer respectively. 77K fluorescence spectrophotometry was used to monitor the contamination of Photosystem I (PSI) peaking around 725 nm. We found that decreased TX-100/chlorophyll ratio treatment of the thylakoids didn't produce significantly high yield of PSII. However, PSI contamination in our PSII samples became serious if the TX-100/chlorophyll ration decreased below 15. Thylakoids treated with 0.25% β-DDM didn't produce any significant PSII preparation. We also used SDS-PAGE gel to evaluate the protein profile in each sample. Spinach BBY was used as a control throughout the whole experiment. We will next to evaluate the excitation energy transfer using ultrafast laser spectroscopy.



Impact of Strontium Substitution on Electron Transport in Photosystem II of *Limnospira maxima*

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The role of calcium in the water-oxidizing complex (WOC) of Photosystem II (PSII) is established as facilitating the structure and function of the manganese oxidation cycle, with strontium (Sr^{2+}) being the only known functional substitute. *Limnospira maxima* was chosen as the model organism for this study due to its robust PSII activity, making it ideal for determining the physiological impacts of metal substitution *in vivo*. Biosynthetic substitution of Ca^{2+} with Sr^{2+} was investigated in *L. maxima* to determine its effects on photosynthetic electron transport. 77K fluorescence spectroscopy revealed that Sr^{2+} substitution alters the stoichiometry of the photosystems, resulting in fewer overall photosystems but with a higher proportion of healthy PSII and stronger antenna association compared to the control. Despite this, fewer excitons reached the PSII reaction center, suggesting impaired energy transfer. Fast repetition rate fluorometry demonstrated reduced oscillation quality and increased accumulation of the S_2 state, suggesting Sr^{2+} rate-limits the opening and closing of the WOC. Q_A^- reoxidation kinetics demonstrated that Sr^{2+} does not limit the acceptor side of PSII in *L. maxima*, possibly due to bicarbonate mitigation, contrasting prior findings in *Thermosynechococcus elongatus*. Cytochrome b_6f redox kinetics revealed greater oxidation and faster electron resupply under Sr^{2+}, while plastocyanin was less oxidized by PSI. P700 kinetics demonstrated that PSI draws electrons more rapidly than PSII can supply under Sr^{2+} substitution. These findings indicate that Sr^{2+} substitution shifts the balance of PSII and PSI electron dynamics by altering redox potentials, altering WOC efficiency, and potentially promoting reactive oxygen species production.

Molecular insights into *Leptolyngbya* sp. JSC-1 photoacclimation

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The cyanobacterium *Leptolyngbya* sp. JSC-1 was isolated from a floating microbial mat in an iron-rich thermal spring. Initially studied for its tolerance to oxidative stress and thermotolerance, JSC-1 was later discovered to undergo far-red light (FRL) photoacclimation, the process by which oxygenic phototrophs remodel their photosynthetic apparatus to synthesize Chls *d* and *f* and perform photosynthesis with photons up to ~800 nm. Unlike other FRL-acclimating cyanobacteria, JSC-1 uniquely combines high iron tolerance and thermotolerance, traits advantageous for extraterrestrial environments such as Mars. Despite being the first organism in which FRL photoacclimation was described, no high-resolution molecular structures exist for its photosystems or associated antenna proteins. Toward this goal, here we (a) establish cell growth conditions and (b) perform initial characterizations of the photosynthetic apparatus in JSC-1. Our results begin to establish JSC-1 as a model for understanding the structural and biochemical bases of FRL photoacclimation, quantify its physiological resilience to environmental stressors, and evaluate its potential role in extraterrestrial biomanufacturing systems.

Chemical Characterization of a Resazurin-Based Method to Detect Dissolved Oxygen

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Resazurin is a redox-active molecule with three states: a colorless reduced form called dihydroresorufin, a reversibly-oxidized red-colored form called resorufin, and an irreversibly-oxidized purple-colored form called resazurin. Because of this redox activity, resazurin has long been used to test for oxygen. In the classic assay, a sample is combined with an indicator solution containing the colorless dihydroresorufin; the presence of oxygen will result in a purple color while anoxic samples remain colorless. While this assay is widely noted, there has been almost no reported characterization of the underlying chemistries. Contrary to common assumptions, UV-visible spectroscopy indicates that, in the classic assay, the dihydroresorufin form is oxidized to a mixture of resazurin and resorufin. Experiments using different ratios of aerobic and anerobic water show a classic spectrophotometric titration curve with a clear end-point. Stability studies show that, once formed, the absorbance was stable for at least 30 minutes. While the reaction between oxygen and dihydroresorufin is nearly instantaneous, the reverse reaction in which resazurin is reduced by sodium dithionite was surprisingly slow. Kinetic analysis indicates the reaction is 1st order with respect to both dithionite and resazurin (2nd order overall), but that secondary reactions involving the reduction of resorufin to dihydroresorufin occur and follow more complex kinetics. Consistent with the slow reaction, the 1 M half-life of the first step is only 1.3 min^{-1}. Addition of the dihydroresorufin-containing indicator to whole cells of *Heliomicrobium modesticaldum* results in negligible effects on the UV-visible spectrum of the cells and only small effects on light-driven P800 oxidation. Conversely addition of resazurin results in a dramatic increase in the amount of P800 oxidation, although this effect was lost after sufficient dark incubation due to reduction of the resazurin by whole cells. Future work is directed towards continued characterization of the indicator and of its interaction with the cellular electron transfer chain.

Reevaluation of RubA in *Synechococcus* PCC 7002 Confirms Its Essential Role in Photosystem II

Clayton Robinson[1], Brandon P. Russell[1,2], Nicholas Ferrari[1], Terry Rodney[1], K. V. Lakshmi[2], and David J. Vinyard[1]

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The maturation of photosynthetic reaction centers requires multiple assembly factors. RubA in *Synechocystis* sp. PCC 6803 and its homolog RBD1 in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* are required for Photosystem II (PSII) assembly, likely by stabilizing the non-heme iron (Calderon et al. 2013; García-Cerdán et al. 2019). In contrast, Bryant and coworkers reported that RubA was essential for Photosystem I (PSI) assembly in the cyanobacterium *Synechococcus* (*Picosynechococcus*) sp. PCC 7002 (Shen et al. 2002). To clarify the role of RubA specifically in PCC 7002, we performed whole-genome sequencing of the original Δ*rubA* mutant generated in the Bryant laboratory. This revealed the expected *rubA* insertional inactivation along with an unrecognized deletion of *psaC*. We then reconstructed a clean Δ*rubA* mutation in a wild-type PCC 7002 background and analyzed photosystem function. The new Δ*rubA* strain retained functional PSI but had low levels of PSII, consistent with the role of RubA/RBD1 in other photosynthetic organisms. Our results resolve a long-standing discrepancy regarding RubA function and illustrate the utility of modern whole-genome sequencing in reassessing historical mutants.

Rubredoxin in PSII of *Synechocystis* sp. PCC 6803

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Rubredoxin A (RubA) is a small nonheme iron protein located on the stromal side of thylakoid membranes in cyanobacteria. In *Synechocystis* sp. PCC 6803 (*Syn* 6803), removal of RubA from PSII prevents supercomplex formation and leads to significantly inhibited growth. RubA has been proposed to be involved in the delivery and/or stabilization of the nonheme iron on the acceptor side of PSII. To test this hypothesis, we aim to alter the reduction potential of RubA by creating selected point mutations. Based on homology to bacterial rubredoxins, we have generated RubA variants predicted to have more positive (A58V) and more negative (V55L) reduction potentials compared to the native form. Truncated forms of these proteins have been expressed and purified from *E. coli* and are being studied *in vitro* using electrochemistry and NMR. We are also complementing a *Syn* 6803 Δ*rubA* mutant with RubA variants and will analyze growth, photosynthetic efficiency, and photosynthetic protein complex accumulation. Together, these studies will inform the mechanisms of PSII acceptor side assembly and regulation.

Mechanism of Vipp1 aggregation in the green alga *Chlamydomonas reinhardtii*

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The thylakoid membrane, found within most cyanobacteria and all chloroplasts, is the site of the light reactions of oxygenic photosynthesis and is essential for the creation of the proton gradient which powers ATP synthesis. Therefore, its integrity is required to ensure the optimal function of both photosynthetic electron transport and downstream carbon fixation. Vesicle-inducing protein in plastids 1 (Vipp1), an ESCRT-III family protein, regulates thylakoid remodeling and homeostasis, but its mechanisms of action and aggregation are poorly understood in vivo. While we have observed aggregation kinetics and regulation in a relatively simple cyanobacterium, eukaryotic systems have more complex thylakoid architecture and we sought to investigate domain effects on aggregation kinetics. By inducing controlled light damage to the thylakoid membrane in the green alga *Chlamydomonas reinhardtii* and immediate spatiotemporally resolved confocal imaging of GFP-tagged Vipp1, we were able to resolve repair priorities. In this system, Vipp1 aggregates to damage sites within the thylakoid membrane over timescales of minutes, much as in cyanobacteria. However, when multiple damage sites are stimulated, the protein prioritizes repair to a certain location depending on the damage site's distance to the pyrenoid. This research demonstrates the importance of the pyrenoid as a major regulator for the spatiotemporal distribution of Vipp1 and how damage repair is prioritized within the thylakoid membrane. The protection of the pyrenoid from proton leakage, and thus the protection of carbonic anhydrase-mediated carbon fixation, is prioritized over other regions of the chloroplast.

Comparative Effects of Anion Supplementation on the Acceptor Side of Photosystem II

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In Photosystem II (PSII), the non-heme iron (NHI) stabilizes Q_A and Q_B redox states, which mediates the electron transfer between plastoquinone acceptors, and regulates recombination pathways. Because the NHI is ligated by bicarbonate and amino acid residues, its coordination environment is strongly influenced by ionic composition, making it a key regulatory site for photosynthetic electron transport. *Picochlorum oklahomense,* a halotolerant green alga capable of thriving across a wide salinity range, provides a model system to examine how anions influence PSII function. To study ion-specific effects, cultures were supplemented with anions spanning the spectrochemical series (HCO_3^-, Cl^-, Br^-, I^-, F^-, NO_3^-, SO_3^{2-}) and their impact on Q_A^- redox kinetics and charge recombination were analyzed using chlorophyll fluorometry. Q_A^- redox analysis showed that addition of halides caused a decrease in the Q_A^--Q_B^- transfer time, suggesting a facilitation of electron transfer due to modulation of either protonation of the semiquinone or local redox poise. Nitrite and sulfite slowed electron transfer, with sulfite additionally limiting the Q_A^--Q_B transition. Fast repetition rate fluorometry studies suggest that strong field-directing ions caused limitation of quinone access to the Q_B site. The mildly beneficial halide effect on electron transfer may be localized to the depletable bicarbonate side responsible for protonation of Q_B. Nitrite appears to limit Q_B turnover, while sulfite fully inhibits the operation of PSII. The totality of sites of activity of sulfite are not clear, but Q_A^- redox modulation supports that it is affecting the acceptor side.

Cross-species analysis of temperature-dependent phosphoglycolate phosphatase activity and dynamics

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Photorespiration occurs when Rubisco catalyzes the fixation of O instead of CO , producing the toxic metabolite 2-phosphoglycolate (2-PG). This compound must be rapidly recycled into 3-phosphoglycerate (3-PGA) to reenter the Calvin Benson Bassham (CBB) cycle. In most plants, Rubisco oxygenation occurs once for every four to five carboxylation reactions, making photorespiration the second-highest flux pathway on Earth, surpassed only by carbon fixation and CBB itself. The pathway is thought to have originated in ancestral cyanobacteria and has been conveyed to plants and algae via endosymbiosis. Individual photorespiratory enzymes have diversified to suit distinct ecological and environmental niches. One of the most conserved and essential enzymes in this pathway, phosphoglycolate phosphatase (PGPase), catalyzes the dephosphorylation of 2-PG to glycolate. Because this reaction is both energetically demanding and thermally sensitive, PGPase presents a key target for improving the robustness of the photorespiratory cycle under heat stress. Here, we surveyed PGPases from a phylogenetically and environmentally diverse panel of organisms, including mesophilic species such as *Arabidopsis thaliana* and *Brassica rapa*, as well as thermotolerant taxa from extreme habitats, such as *Rhazya stricta* (desert plant) and *Cyanidioschyzon merolae* (acidothermophilic red alga). Through heterologous expression and temperature-dependent kinetic assays, we identified isoforms exhibiting distinct thermal performance profiles that correlate with their native environmental regimes. To probe the structural underpinnings of these differences, we performed molecular dynamics simulations and quantified the flexibility of different regions of the enzymes using root mean square fluctuation (RMSF) analyses. Complementary evolutionary analyses identified residues under positive selection at the base of a flexible loop region on the periphery of the PGPase. Site-directed mutagenesis of this residue in *R. stricta* supports its role in enhancing structural stability via a hydrogen-bonding network that potentially contributes to elevated thermotolerance. Together, these data highlight adaptive strategies by which photorespiratory enzymes achieve thermotolerance and provide mechanistic insights to guide engineering of more heat resilient carbon recycling pathways in plants.

Tracking Metalloprotein Dynamics at the Plastocyanin–Cytochrome *f* Interface with Infrared Probes

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A comprehensive understanding of photosynthetic metalloprotein electron transfer (ET) requires insight into how encounter-complex dynamics and the interfacial hydration network enable charge flow between metal sites. We focus on the interaction between cytochrome *f* (Cyt *f*) and plastocyanin (Pc) to probe how protein motions shape ET. In prior work, introducing C–D vibrational probes at the Pc Cu-site ligands Cys89 and Met97 revealed a pronounced change in the Cu–Cys89 interaction upon binding to Cyt *f*, indicating that ET-active metal sites are tuned not only within individual proteins but also by protein–protein complex formation. Here, we investigate how association with Cyt *f* remodels the inner coordination sphere of Pc's central copper when the Cu center is substituted with redox-active or redox-inactive metals (Figure 1). To assess how metal identity influences Pc–Cyt *f* association and ET, we employ site-specific C–D probes in infrared (IR) spectroscopy.

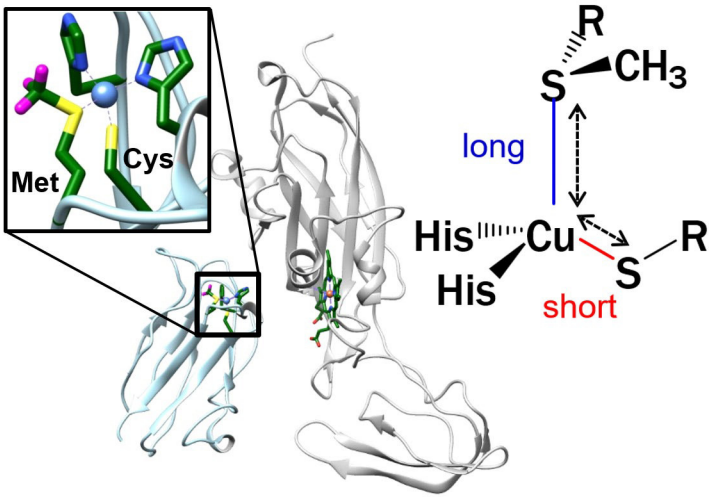


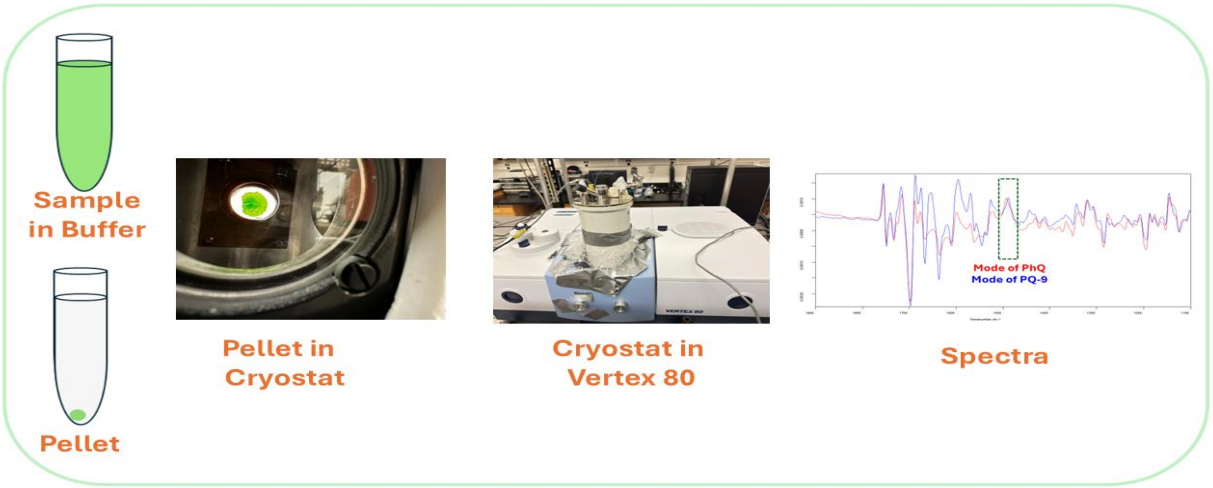
Figure. 1. Structure of the Pc (blue)–Cyt *f* (gray) complex (PDB *1TU2*). The inset highlights the plastocyanin Cu center (Cys/Met/His2 ligation). The diagram at right illustrates the characteristic distorted-tetrahedral geometry in blue-copper proteins: a short Cu–S(Cys) bond (red), a longer Cu–S(Met) interaction (blue), and two His ligands.

Light-Induced FTIR Difference Spectroscopy for the Study of the Cofactors in Photosystem I.

Sabiha Haque Tamanna[1], Muhammad Asjad[1], Michael Nelson[1], Gary Hastings[1]

[1]*Georgia State University, Department of Physics and Astronomy*

Fourier-transform infrared (FTIR) difference spectroscopy (DS) is widely used to study the structure and binding environment of electron transfer cofactors in photosynthetic complexes. Here, photo accumulated FTIR DS has been used to examine the photo-oxidation of the primary electron donor, P700, in Photosystem I (PSI) from *Synechococcus* sp. PCC 7002 (S7002) and in menB$^-$ mutant PSI from *Synechocystis* sp. PCC 6803 (S6803).In PSI the secondary radical pair state, $P700^+A_1^-$, forms within ~50 ps after excitation and decays within ~300 ns at room temperature, or ~300 µs at 77 K, making it inaccessible to photo accumulation FTIR DS methods. To overcome this limitation, we used time-resolved step-scan FTIR DS to study the short-lived A_1^- state in PSI from S7002 and in menB$^-$ mutant PSI from S6803. Photo accumulated FTIR difference spectra reveal distinct features of $P700^+$ formation, while transient A_1^- signals provide complementary insight into electron transfer recombination reactions. Together, these spectra offer information on cofactor binding and redox chemistry, enabling comparison of PSI variants. Preliminary data show reproducible signals associated with the quinones and chlorophyll molecules that act as donors and acceptors, forming the basis for a more detailed kinetic and structural analysis.



Graphical Abstract

Toward the structural basis of far-red light photoacclimation in Photosystem I of *Fischerella thermalis* PCC 7521

Shimo Tang[1], Nikki Cecil M. Magdaong[1], Maximino Emerson[1], Himanshu S. Mehra[1], Christian M. Brininger[1], Gaozhong Shen[1], Christopher J. Gisriel[1]

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Photosynthesis is one of the most important biological processes on earth; it is the foundation of the generation of energy and releases Oxygen as a byproduct for humans to breathe. Most organisms that do oxygenic photosynthesis use chlorophyll *a* to absorb visible light from 400 nm to 700 nm in the light spectrum. While certain cyanobacteria adapt to a shaded environment by additionally synthesizing chlorophyll *d* and *f*, which absorb far-red light and enrich the organism's absorbance cross-section. Gaining understanding of the molecular bases of how cyanobacteria use far-red light and the structure of the enzymes that bind to chlorophyll *d* and *f* has agricultural significance. Increasing the absorption cross-section of a crop can lead to an increase in yield. The lab aims to provide an architectural basis for engineering the cyanobacteria's properties to crops to maximize photosynthesis rate to improve the biomass. Previous cryo-EM work has revealed the molecular structure of far-red light-absorbing photosystem I from the thermophilic cyanobacterium *Fischerella thermalis* PCC 7521, but it is difficult to distinguish between chlorophyll *a* and *f*, whose structures are very similar. Due to the challenges in distinguishing these chlorophyll types in cryo-EM, we plan to perform X-ray crystallography. The photosystem I from this organism was specifically chosen because it has been shown to be especially stable. Here, we further characterize the far-red light-absorbing photosystem I complex from *Fischerella thermalis* PCC 7521 using SDS-PAGE, LC MS/MS, and negative stain transmission electron microscopy. The results suggest that the sample is at a purity appropriate for crystallization. Using this sample, we were able to identify crystallization conditions and acquire low-resolution diffraction data. Future experiments aim to enhance diffraction resolution.

Spectroscopic Evaluation of Orange Carotenoid Protein (OCP)-Mediated Fluorescence Quenching of Light Harvesting Antenna Phycobilisome in Cyanobacteria with Highly Accumulated Iron Stress Inducible Protein A (IsiA)

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Many cyanobacteria utilize the orange carotenoid protein (OCP) to bind the phycobilisome (PBS), dissipating excess absorbed light energy as heat. The canonical model of this non-photochemical quenching mechanism emphasizes PBS coupled to both photosystems (PSI, PSII), yet it remains unclear how OCP functions under nutrient stress. Under iron limitation and stress conditions, Synechocystis sp. PCC 6803 accumulates the CP43 homolog, IsiA, that assembles into oligomeric rings around PSI or forms independent aggregates, raising the question of whether OCP-mediated quenching extends to non-canonical PBS-IsiA-PSI supercomplexes. Here, we combined steady-state and time-resolved fluorescence (TRF) spectroscopy at room temperature and 77K with whole-cell analyses of Synechocystis sp. PCC 6803 wild type, OCP-deletion, and OCP-overexpression strains. Distinct fluorescence bands were attributable to PSII (~693 nm), PSI (~724 nm), and IsiA (~688 nm), indicating that under these stress conditions, PBS can coexist with both PSII and IsiA. Blue-light activation and TRF demonstrated that OCP quenching acts by depleting the amplitude of APC660/680-associated components rather than altering decay lifetimes, revealing that OCP remains an effective photoprotective mechanism, even upon IsiA accumulation. Moreover, OCP-overexpression cells enhanced IsiA quenching compared to wild type, showing a quantitative link between OCP abundance and IsiA suppression. Together, these results reveal that OCP-mediated quenching remains robust when antenna architecture is remodeled by stress conditions, extending its functional role to IsiA-containing supercomplexes and highlighting the adaptability of light-harvesting systems in fluctuating environments.

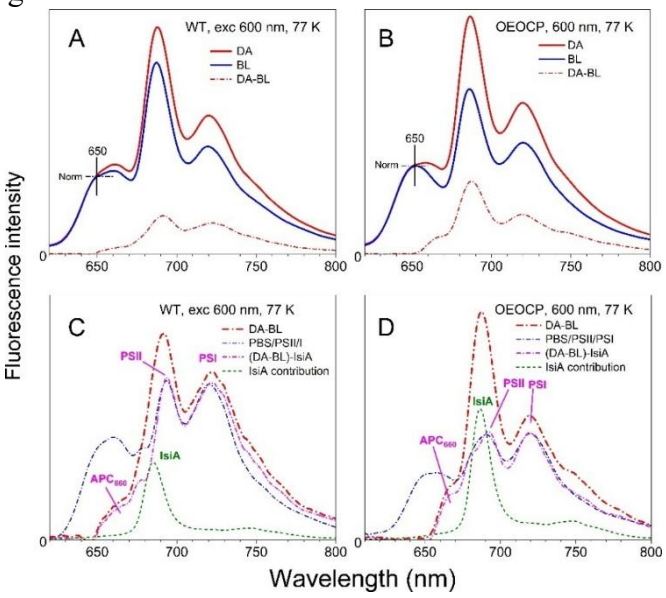

Figure 1. Blue-light induced difference spectra of WT and OEOCP *Synechocystis* 6803 at 77 K after PBS excitation (600 nm). (A–B) Fluorescence emission and difference spectra. (C–D) Spectral reconstructions showing contributions from IsiA, APC660, PSII, and PSI.

Useful Information

The conference will be held at the Turkey Run Inn, just inside Turkey Run State Park in Marshall, Indiana October 24-26. **Meals** will be in the main dining area, near the check-in desk. **Talks, coffee breaks, and posters** will be in the lower level, past the cozy lobby and down the stairs in the meeting area. Forwarned is forarmed, the Wi-Fi can be spotty at Turkey Run, so please be prepared with flash drives to transfer presentations as needed.

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6. Sandeep Biswas, Washington University
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52. Nicole Seliga, Loyola University Chicago
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61. Sivanujan Suthaharan, Purdue University
62. Sabiha Haque Tamanna, Georgia State University
63. Shimo Tang, University of Wisconsin-Madison

64. Lisa Utschig, Argonne National Laboratory
65. Michael Vaughn, SpectroLogix
66. Sham Vera, Indiana University Bloomington
67. Josh Vermaas, Michigan State University
68. David Vinyard, Louisiana State University
69. Anna Williams, Saint Louis University
70. Neetu Yadav, Michigan State University
71. Kathleen York, Bay Instruments

Code of Conduct

The Midwest/Southeast Photosynthesis Conference is committed to providing a welcoming, inclusive, and harassment-free environment in all interactions regardless of race, age, ethnicity, national origin, language, gender, gender identity, sexual orientation, disability, physical appearance, political views, military service, health status or religion. We welcome the opportunity to bring photosynthesis research to all people regardless of their identity or background.

As a program that aims to share ideas and freedom of thought and expression, it is essential that the interaction between participants take place in an environment that recognizes the inherent worth of every person by being respectful of all. All participants strive to be empathetic, respectful, welcoming, friendly, and patient. We strive to be collaborative and use language that reflects our values.

The conference does not tolerate harassment in any form. Harassment is any form of behavior intended to exclude, intimidate or cause discomfort. Harassment includes, but is not limited to, the use of abusive or degrading language, intimidation, stalking, harassing photography or recording, inappropriate physical contact, and unwelcome sexual attention.

Examples of unacceptable behavior

All participants are committed to making participation in this community a harassment-free experience.

We will not accept harassment or other exclusionary behaviors or actions that are illegal, such as:

- The use of sexualized language or imagery
- Excessive profanity (please avoid curse words; people differ greatly in their sensitivity to swearing)
- Posting sexually explicit or violent material
- Violent or intimidating threats or language directed against another person or group
- Inappropriate physical contact and/or unwelcome sexual attention or sexual comments

- Sexist, racist, or otherwise discriminatory jokes and language
- Trolling or insulting and derogatory comments
- Written or verbal comments which have the effect of excluding people on the basis of membership in a specific group, including level of experience, gender, gender identity and expression, sexual orientation, disability, neurotype, personal appearance, body size, race, ethnicity, age, religion, or nationality
- Public or private harassment
- Continuing to initiate interaction (such as photography, recording, messaging, or conversation) with someone after being asked to stop
- Sustained disruption of talks, events, or communications, such as heckling of a speaker
- Publishing (or threatening to post) other people's personally identifying information ("doxing"), such as physical or electronic addresses, without explicit permission
- Other unethical or unprofessional conduct
- Advocating for, or encouraging, any of the above behaviors All participants are governed by local laws, including Turkey Run State Park rules and their organization's code of conduct and policies.

How to Submit a Report

If you feel your safety is in jeopardy or the situation is an emergency, contact local law enforcement before making a report to the conference organizers. (In the U.S., dial 911.)

Anyone who experiences, observes or has knowledge of threatening behavior is expected to immediately report the incident to a member of the event organizing committee, state park staff, or a trusted friend or colleague. The Midwest/Southeast photosynthesis conference reserves the right to take appropriate action.

Take care of each other. Alert the organizers if you notice a dangerous situation, someone in distress, or violations of this code of conduct, even if they seem inconsequential. For possibly unintentional breaches of the code of conduct, you may want to respond to the person and point out this code of conduct (either in public or in private, whatever is most appropriate). If you would prefer not to do that, please report the issue to the conference chair or co-chair.

What Happens Next

All complaints will be reviewed and investigated and will result in a response that is deemed necessary and appropriate to the circumstances. All reports will be kept confidential, with the exception of cases where the organizers or state park staff determines the report should be shared with law enforcement. In those cases, the report will be shared with the proper legal authorities.

In some cases the conference organizers may determine that a public statement will need to be made. If that's the case, the identities of all involved parties and reporters will remain confidential unless those individuals instruct us otherwise.