Conference Support, 33rd Western Photosynthesis Conference 2024

This award (DE-SC0024880) was used to support the attendance of 19 early career scientists to the 33rd Western Photosynthesis Conference in Oracle, AZ. The conference took place at the Biosphere 2 from January 5-7, and all 19 scientists supported by this award gave presentations in the form of talks or posters.

Full list of supported early career scientists:

Assistant Professors (2)

Dr. Po-Lin Chiu – Arizona State University

Dr. Robert Jinkerson – U. California, Riverside

Postdoctoral researchers (4)

Dr. Rachael DeTar – Colorado State University

Dr. Edwin Gonzalez - Arizona State University

Dr. Malgorzata Krysiak - Washington State U.

Dr. Alex Siegel - California Institute of Technology

PhD students (13)

Fidaa Ali – U. Tennessee

Tyler Chapman - Washington State University

Mohammad Azim – Donald Danforth Center

Halima Khatun - Arizona State University

Alaina LaPanse - Colorado School of Mines

Alexandria M. Layton – Arizona State University

Michelle Meagher - Colorado School of Mines

Kuenzang Om – Washington State University

Mahipal Rao - U. Tennessee

Aline Rodrigues de Queiroz – U. Nebraska

Matthew Runyon – U. Illinois

Calvin Rusley - California Institute of Technology

Haley Schrader – Washington State University

Presentation titles and abstracts

Po-Lin Chiu – Arizona State University

"Molecular regulatory mechanism of light transfer in *Chlorobaculum tepidum"*

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Green sulfur bacteria (GSB) are photoautotrophs that thrive in low-light environments, employing a remarkably efficient light-harvesting and transfer molecular system. Their photochemical reaction center (RC) features a dimeric architecture for charge separation across the membrane, presenting a primitive form of photosynthesis. Central to this molecular organization are the RC and trimeric Fenna-Matthews-Olson (FMO) protein complexes attached to the RC cytoplasmic surface, mediating light energy transfer from the light-harvesting chlorosomes. To investigate the overall molecular assembly of the photosynthetic apparatus, we employed a mild extraction of the supercomplexes from the inner membrane of the GSB *Chlorobaculum tepidum* and determined its high-resolution structures using single-particle cryogenic electron microscopy (cryo-EM). Our structure presents an overall view of two FMO trimers, two cytochrome *c* subunits (PscC), and small subunits, PscB, PscD, PscE, and PscF, that are assembled on the symmetric RC core (PscA). One linker bacteriochlorophyll (BChl) was identified located in one of the two FMO-PscA interfaces. The spatial arrangement of the protein subunits and pigments is asymmetric, implicating a bias in light transfer along the two FMO-PscA branches. Our structure of the GSB photosynthetic supercomplex provides mechanistic insights into the routes for light excitation energy transfer and a possible evolutionary transition intermediate of the bacterial photosynthetic supercomplex from the primitive homodimeric RC.

To further investigate the assembly impacted by environmental light intensities, we use cryogenic electron tomography (cryo-ET) and structural mass spectrometry (MS) to probe the distribution and assembly of the photosynthetic supercomplex. The outcome promises a comprehensive understanding of how primitive photosynthetic bacteria adapt to changing environmental conditions.

Robert Jinkerson – U. California, Riverside

An electrochemical-biological system for the energy-efficient production of food

Artificial photosynthesis seeks to overcome the limitations of biological photosynthesis, including low efficiency of solar energy capture and poor carbon dioxide reduction, and could provide an alternative route for food production. However, most electrochemically derived substrates cannot support the growth of the widely consumed food-producing organisms like plants, fungi, or animals. In this talk I will describe our work to decouple food production from biological photosynthesis through the development of a hybrid inorganic–biological system for the cultivation of food producing organisms. A two-step electrochemical process converts CO2 to acetate, which serves as a carbon and energy source for the heterotrophic cultivation of food producing organisms. We have demonstrated the cultivation of yeast, mushroom-producing fungus, and a photosynthetic green alga, all in the dark without inputs from biological photosynthesis. An evaluation of nine crop plants found that carbon from exogenously supplied acetate incorporated into biomass through major metabolic pathways but could not natively sustain the growth of these plants. Metabolic engineering efforts are underway to enable heterotrophic growth on acetate as the sole carbon and energy source. Coupling this artificial photosynthesis approach to existing photovoltaic systems could increase solar-to-food energy conversion efficiency by about fourfold over biological photosynthesis, reducing the solar footprint required. This technology allows for a reimagination of how food can be produced in controlled environments.

Rachael DeTar – Colorado State University

"Photosynthesis drives retention of bacterial-like tRNA metabolism in plant organelles"

Rachael Ann DeTar[1,], Joanna Chustecki[2], Anna Martinez-Hottovy[2], Luis Federico Cerrioti[3,4], Amanda K. Broz[1], M. Virginia Sanchez-Puerta[3,4], Christian Elowsky[5], Alan C. Christensen[2], Daniel B. Sloan[1]*

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Eukaryotic nuclear genomes often encode distinct sets of protein translation machinery for function in the cytosol vs. organelles (mitochondria and plastids). This phenomenon raises questions about why multiple translation systems are maintained even though they are capable of comparable functions, and whether they evolve differently depending on the compartment where they operate. These questions are particularly interesting in land plants because translation machinery, including aminoacyl-tRNA synthetases (aaRS), is often dual-targeted to both the plastids and mitochondria. These two organelles have quite different metabolisms, with much higher rates of translation in plastids to supply the abundant, rapid-turnover proteins required for photosynthesis. Previous studies have indicated that plant organellar aaRS evolve more slowly compared to mitochondrial aaRS in other eukaryotes that lack plastids. Thus, we investigated the evolution of nuclear-encoded organellar and cytosolic translation machinery across a broad sampling of angiosperms, including non-photosynthetic (heterotrophic) plant species with reduced rates of plastid gene expression to test the hypothesis that translational demands associated with photosynthesis constrain the evolution of bacterial-like enzymes involved in organellar tRNA metabolism. Remarkably, heterotrophic plants exhibited wholesale loss of many organelle-targeted aaRS and other enzymes, even though translation still occurs in their mitochondria and plastids. These losses were often accompanied by apparent retargeting of cytosolic enzymes and tRNAs to the organelles, sometimes preserving aaRS-tRNA charging relationships but other times creating surprising mismatches between cytosolic aaRS and mitochondrial tRNA substrates. Our findings indicate that the presence of a photosynthetic plastid drives the retention of specialized systems for organellar tRNA metabolism.

Edwin Gonzalez - Arizona State University

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The flow of electrons and protons, coupled together by proton-coupled electron transfer (PCET), plays a crucial role in the photosynthetic process. Aiming to explore the thermodynamics and dynamics of PCET, the benzimidazole-phenol (BIP) moiety has been employed to mimic the role of the Tyr_Z-His190 pair of photosystem II (PSII) [1]. Electrochemical studies demonstrated that modifying the His190 mimic by incorporation of polybenzimidazoles to extend the "proton wire" is accompanied by a decrease in the redox potential of the phenoxyl radical/phenol couple by 60 mV per benzimidazole unit [2]. Coupling this proton wire to a high-potential porphyrin having an excited-singlet state that can oxidize the phenol and initiate the associated translocation of protons is a relevant photosynthetic mimic of proton management in water oxidation by PSII. We will describe the synthetic path for forming three *beta*-fluorinated porphyrins with BIP ligands. With only one BIP, an E1PT process occurs (one proton transfer associated with a one-electron oxidation event). Electrochemical characterization shows that by incorporating a second proton acceptor, an E2PT process, i.e., translocation of two protons, and a decrease of 160 mV for the phenoxyl radical/phenol couple was observed. Adding a third proton acceptor resulted in a total decrease of 250 mV for the phenoxyl radical/phenol couple in an E3PT process. In each case, infrared spectroelectrochemistry was used to confirm the arrival of the proton on the terminal proton acceptor after the oxidation event. This result illustrates that managing and maintaining proton activity in proton wires is an important design parameter in artificial photosynthesis.

Malgorzata Krysiak - Washington State U.

"Light-induced changes in the cytochrome b_6f turnover rate"

Malgorzata Krysiak and Helmut Kirchhoff

The intricate structural interplay of proteins within the photosynthetic machinery in thylakoid membranes is essential for efficient energy conversion in plants and algae. The cytochrome b6f complex stands as a linchpin in photosynthetic electron transport chain, orchestrating the flow of electrons between the two photosystems. One recent research idea is that the stacking of thylakoids to grana is a means to define domains for linear (stacked grana) and cyclic electron transport (unstacked thylakoid membranes), where cytochrome b6f complexes localized in stacked thylakoid domains are involved in linear electron transport whereas their counterparts in unstacked domains support cyclic electron transport. However, experimental proof for this idea is missing.

Here we present the functional electron transport measurements to probe light-induced changes in the activity of cytochrome b6f complex. Our data suggest significant acceleration of cytochrome b6f turnover rate during dark to light transition. This acceleration is likely not caused

by higher activity of individual cytochrome b6f complexes but with (i) its thylakoid sub-localization, (ii) more efficient diffusion of its substrates, plastoquinol from PSII and oxidized plastocyanin from PSI, (iii) increase in cyclic electron transport. Independent experimental approaches will be presented that test these three different possibilities. Furthermore, mutant studies will be presented revealing how protein phosphorylation and mesoscopic protein arrangements determine the accelerated cytochrome b6f turnover rate. Our results indicate that the stacked/unstacked cytochrome b6f domain concept for regulating linear/cyclic electron transport is too simple and require substantial revision.

Alex Siegel - California Institute of Technology

"Regulated capture and delivery of light-harvesting complex proteins by chloroplast SRP"

Assembly of the light-harvesting complex (LHC) depends on the coordinated insertion of both chlorophyll a,b-binding proteins (LHCPs) and chlorophyll. The abundant membrane-protein LHCPs are synthesized in the cytosol and transported through the stroma to the thylakoid membrane. To prevent their hydrophobic transmembrane domains (TMDs) from aggregating outside the membrane, plants employ the chloroplast signal recognition particle (cpSRP), a heterodimer consisting of a chaperone subunit, cpSRP43, and regulatory subunit, cpSRP54. Chlorophyll is synthesized through a multi-step tetrapyrrole biosynthesis (TBS) pathway in the stroma. When the metabolic flux of TBS is not properly maintained, a buildup of reactive oxygen species (ROS) intermediates leads to proteostatic dysfunction. In addition to its canonical role delivering LHCPs, we find that cpSRP also maintains that levels and activity of TBS enzymes and is potent chaperone during thermal stress to prevent their aggregation. To act on both types of clients, cpSRP43 exchanges between two conformations: a well-folded 'closed' state with high binding affinity towards a conserved motif on LHCP but no protection towards TBS enzymes, and a partially disordered 'open' state that protects TBS enzymes from heat-induced aggregation but cannot bind or protect LHCPs. cpSRP54 binding shifts the conformation towards the closed state and enhances the protection and delivery of LHCPs, while elevated temperature drives dissociation of cpSRP54 and the primarily open state apo cpSRP43 becomes a potent chaperone towards TBS enzymes exactly when they most require thermo-protection. The thermostatic behavior of cpSRP43 coordinates protein and pigment delivery to adapt LHC biogenesis to environmental changes.

Fidaa Ali – U. Tennessee

"Characterization of Oligomeric PSI in Their Native Lipid Environment Using Experimental and Computational Methods"

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In oxygenic photosynthesis, organisms like plants and cyanobacteria use Photosystem I (PSI) and II (PSII). Cyanobacteria are unique in having PSI in monomeric, trimeric, and tetrameric forms. The tetrameric PSI shows enhanced excitation-energy quenching, offering better protection against intense light, and its concentration increases in bright environments. Recent advances involve using styrene-maleic acid copolymers (SMAs) to create nanodiscs that preserve the native thylakoid membrane environment of PSI, enhancing its photochemistry. The focus is on characterizing PSI from thermophilic cyanobacteria, using techniques like Blue Native PAGE and lipidomic analysis. These studies reveal that PSI is enriched with various lipids, potentially affecting its electron and exciton transfer properties. We are interested in characterizing the oligomeric form of PSI from two thermophilic cyanobacteria, *Chroococcidiopsis* sp TS-821 and *Thermosynechococcus elongatus* in their native membrane environment using styrene maleic acid and other alkane-containing copolymers using a variety of biochemical and biophysical analyses, including Blue Native PAGE, SDGC, 77 K fluorescence, single-particle analysis, and lipidomic analysis. Understanding membrane protein organization and lipid-protein interactions is crucial. Experimental methods provide some insights but lack spatial resolution. Coarse-grained molecular dynamics (CG-MD) simulations offer a more detailed view, exploring lipid-protein interactions at a molecular level and the dynamics of PSI-lipid membrane interactions over extensive time scales. Therefore, by using the CG-MD approach, we are capable of investigating the dynamics of the PSI-lipid membrane at temporal scales in the range of tens of microseconds (60µs).

Tyler Chapman - Washington State University

"Understanding the Protective Mechanism of Small Molecules for Photosystem II Functionality"

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The splitting of water into protons, electrons, and oxygen occurs within the oxygen evolving complex (OEC) of photosystem II (PSII). However, PSII is quite fragile, and is highly susceptible to abiotic damage (photodamage, temperature, salt stress, or combinations of those). The main site of damage on PSII is the OEC, shielded by the extrinsic subunits PsbO, PsbP, and PsbQ in higher plants, all of which can dissociate from the OEC under abiotic stress leading to loss of function. It was suggested that dissociation of the extrinsic subunits can be mitigated by the amino acid derivative glycine betaine (GB), a small zwitterionic kosmotrope which has been shown to help stabilize protein quaternary structure as well as osmotic pressure (Huang et al., 2020). Our experiments investigating the effect of heat stress on dissociation of PsbO indicate that GB indeed protects against dissociation. Measurement of the rate of oxygen evolution under heat stress further provides evidence that GB protects the OEC from high temperature stress. Other small molecules that are structurally or functionally analogous to GB were also tested, yet GB remained the most protective, leading us to further examine the mechanism by which GB interacts with the OEC. To investigate this unknown mechanism, the protein-ligand modelling software AutoDock Vina and SwissDock were used to characterize interactions between PSII and GB. Current preliminary evidence suggests that GB interacts directly with the OEC and PSII core subunits, acting as a sort of “molecular glue”, which may hold together and stabilize PSII structure and functionality.

Mohammad Azim – Donald Danforth Center

“Investigating the chloroplast retrograde signals involved in intercellular trafficking through plasmodesmata”

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Plasmodesmata (PD) are membrane-lined cytoplasmic continuities between adjacent plant cells that allow intercellular trafficking of metabolites, protein, RNA, and ribonucleoprotein complexes. The regulation of PD-mediated trafficking is essential for plant growth, development, and defense. We proposed the Organelle-nucleus-plasmodesmata-signaling (ONPS) hypothesis to explain how specific changes in organellar (plastid or mitochondrial) gene expression led to signaling to the nucleus (chloroplast-to-nucleus retrograde signaling, CRS) to tune the expression

of nucleus-encoded genes associated with plasmodesmal regulation. Studies with Arabidopsis *gun4* and *gun5* mutants have revealed the role of tetrapyrrole intermediates in CRS. In *Nicotiana benthamiana*, virus-induced gene silencing (VIGS) of *GUN4* caused mild chlorosis, while severe chlorosis occurred in *GUN5*-silenced plants. Silencing of *GUN5* led to decreased PD-mediated intercellular trafficking. Surprisingly, when both *GUN4* and the chloroplast RNA helicase *ISE2* were silenced, intercellular trafficking increased, similar to the effect of silencing *ISE2* alone. This revealed that *ISE2* was epistatic to *GUN4*. In contrast, silencing both *ISE2* and *GUN5* reduced PD-mediated trafficking, phenocopying silencing of *GUN5* alone. This suggests that ISE2 regulates PD-mediated trafficking in a GUN5-dependent signaling pathway. We measured reactive oxygen species levels, the redox status of chloroplasts, and the redox state of the plastoquinone pool but did not find any correlation between these redox signaling parameters and PD-mediated trafficking. In contrast, we found that the level of heme, a known retrograde signal, was correlated with decreased PD-mediated trafficking. Overall, our results suggest that ISE2 may need heme to regulate PD-mediated trafficking.

Halima Khatun - Arizona State University

"The role of photosystem I core antenna red chlorophylls in Photoinhibition in cyanobacterial photosynthesis"

The school of molecular sciences, Arizona State University; Center for applied structural Discovery, Biodesign Institute, Arizona State University

[1]Halima Khatun, [1]Zhen da and [1]Yuval Mazor.

The photosystem I core antenna in cyanobacteria is composed of approximately ninety chlorophyll a molecules. A small number of these chlorophylls absorb longer wavelengths than 700 nm and are known as "red chlorophylls." These red chlorophylls are believed to function by harvesting far-red light, affecting the rate of excitation energy transfer, and possibly photoprotection. While red chlorophylls in light-harvesting complexes have been found to play a role in photoprotection in plants, their role in photoprotection within the core photosystem I antenna in cyanobacteria is still unexplored. Herein, we report a study to investigate the roles of red chlorophyll in core antenna in photoinhibition of photosystem I in cyanobacteria. We isolated and purified four distinct photosystem I trimers from wild-type *Synechocystis* sp. PCC 6803 and three mutant strains: Red a, Red b, and Red ab. In order to assess the impact of red chlorophylls on these mutants, four purified PSI complexes from both wild-type and mutant strains were exposed to high light. Our initial findings from our experiments do not indicate a significant difference in total chlorophyll damage resulting from the presence of additional red chlorophylls. However, we did observe different mutants respond to high light differently and, that the Red ab

mutant maintained higher efficiencies following high light treatment. Future work will focus on further characterizing the effect of the red mutations on photoinhibition to determine the roles of red chlorophyll in PSI photoinhibition.

Alaina LaPanse - Colorado School of Mines

"Understanding carbonic anhydrases in the high productivity marine microalga *Picochlorum celery*"

Photosynthetic carbon utilization has been studied for decades as the main driver behind atmospheric oxygen production[1]. Many algal species use a carbon concentration mechanism (CCM) to enable uptake of inorganic carbon and sequestration of CO_2 adjacent to the active site of RuBisCO[2]. The presence of several carbonic anhydrase enzymes enables the functionality of the algal CCM through the catalysis of bicarbonate and CO_2 conversion. However, carbonic anhydrases play additional roles in cellular pH control, respiration, and inorganic carbon speciation in systems outside of the CCM[3]. Here, an investigation of carbonic anhydrases in the fast-growing green algae *Picochlorum celeri* reveals a unique role for carbonic anhydrases in a microalgal system apparently lacking a traditional CCM[4]. Through the targeted knock out of carbonic anhydrase 7 using CRISPR/Cas9 ribonucleoprotein complexes (RNPs), unique stationary-phase growth phenotypes have been uncovered[5]. Localization of carbonic anhydrase 7 outside of the chloroplast and mitochondria is established through confocal microscopy, indicative of functions independent from a CCM. Furthermore, genome gazing reveals insights into the functionality of carbonic anhydrase 7 through nearby gene representation and orientation. These insights into carbonic anhydrase 7 provide a roadmap for understanding, and engineering, greater carbon use efficiency in industrial algal strains like *Picochlorum celeri.*

Alexandria M. Layton – Arizona State University

"Exploration and Alteration of Carbon Metabolism in *Heliomicrobium modesticaldum*"

Alexandria M. Layton, Kevin E. Redding

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Heliomicrobium modesticaldum, a moderately thermophilic phototrophic member of the Firmicutes, utilize a split forward and reverse tricarboxylic acid (TCA) cycle to process its provided carbon. In this study, we analyze mechanisms of carbon metabolism, its limitations, and the ability to change *H. modesticaldum*'s metabolism from heterotrophy to autotrophy through minimal gene changes in a laboratory set-up mimicry of evolution through lateral gene transfer. We aim

to create various metabolic mutants of *H. modesticaldum* that can mimic potential evolutionary intermediates on the path from heterotrophy to autotrophy, or vice versa. Here, we deleted the gene for the native Re-Citrate Synthase, eliminating the cell's ability to access the forward TCA cycle. This mutant is unable to grow in minimal media using acetate as a carbon source, and they are limited for both electrons and carbon. Growth was restored when the mutants were provided an exogenous electron donor, such as formate or ascorbate. The cells required glutamine when grown in minimal media, suggesting that the flux through the rTCA cycle to 2-oxoglutarate was insufficient to produce glutamate and amino acids originating from it (Gln, Pro, Arg, His).

In addition, we inserted a clostridial citrate lyase to assess if the supplemented citrate lyase is enough to enable the cells to grow autotrophically through use of the full rTCA cycle. The enzyme itself displayed activity, and we aim to continue pushing the growth of this mutant to see if the insertion of this enzyme is sufficient to complete the cycle and enable autotrophic growth.

Michelle Meagher - Colorado School of Mines

"A Custom Continuous Flow Apparatus for Hydroponic Growth of *Arabidopsis thaliana"*

Michelle Meagher[1]* and Nanette Boyle[1]

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Arabidopsis thaliana is used as a model organism to study photosynthesis in plants, and is currently being studied in a hybrid inorganic-biological artificial photosynthesis system [1]. In order to overcome the inefficiencies associated with photosynthesis, acetate produced by a CO_2 electrolysis process is fed to the plants to supplement growth. *A. thaliana* is capable of using acetate to a small degree, but toxicity is encountered at higher concentrations of this substrate. To overcome this problem, we developed a continuous flow hydroponics growth system that provides constant flow of media containing lower concentrations of acetate, maximizing the plants' ability to uptake and assimilate acetate. I designed this system using CAD software and 3D printed it using HTPLA which was then thermally annealed to improve material strength and temperature resistance of the material during autoclaving. Liquid media from this hydroponics system is more easily sampled for metabolomics analysis than the agar media used in traditional *A. thaliana* cultivation, allowing the acetate uptake rate in this plant to be measured. In this system, roots extend down into the liquid media, while the shoots grow up out of the well plate. This physical separation of roots and shoots facilitates quick harvesting and quenching of these different tissue types allowing for internal metabolomics analysis of these plants. Future studies

will employ this cultivation system for isotopically assisted metabolic flux analysis in *A. thaliana* strains grown on acetate.

Kuenzang Om – Washington State University

"Characterization of *Zea mays* C_4 phototsynthetic phospho*enol*pyruvate carboxylase kinetics expressed in *Oryza sativa* cv. Kitaake"

Global climate change and the rapid rise in population are intensifying the issue of global food security. Currently, rice, a C_3 plant, is the primary carbohydrate source for two-thirds of the global population. In C_3 plants, the enzyme Rubisco fixes CO_2 into three-carbon organic molecules. However, Rubisco's efficiency is often reduced due to its tendency to interact with O_2, leading to photorespiration. In contrast, C_4 plants have developed a carbon concentrating mechanism (CCM) that overcomes this inefficiency. C_4 plants are more resource-efficient, requiring less nitrogen, water, and light to produce the same amount of biomass as C_3 plants.

Converting rice into a C_4 plant could increase its yield significantly bolstering global food security. This transformation requires integrating the C_4 photosynthetic biochemical pathway, which includes introducing numerous enzymes, into rice. A key step in this pathway is the conversion of bicarbonate into a four-carbon organic compound by PEPC (phospho*enol*pyruvate carboxylase), regulated by L-malate (inhibition) and glucose 6-phosphate (activation), and undergoes diurnal phosphorylation changes.

Our research focuses on expressing maize's C_4 photosynthetic PEPC in rice (PEPC-OE rice) and examining its kinetic properties and allosteric regulation. We observed that the PEPC-OE rice lines exhibit a similar affinity for substrates PEP (phosphoenolpyruvate) and HCO_{3^-} (bicarbonate) as seen in maize. Additionally, *in planta* ^{13}C labeling data from these PEPC-OE lines indicate successful operation of the initial carboxylation step in the CCM pathway. This study is a crucial step toward engineering C_4 photosynthesis in rice, potentially increasing agricultural productivity and global food security.

Mahipal Rao - U. Tennessee

"Effects of Ethylene-mediated Metabolic Priming on Photosynthesis and Plant Growth"

Enhancing plant growth and crop yield as we face global climate change is a critical challenge that must be addressed to ensure food security for the increasing human population. A recent report showed dramatic improvements in photosynthesis in soybeans and other crops to ameliorate crop yield (1). At UTK, a similar recent report has shown a significant increase in

plant growth and biomass after treatment with ethylene, a non-toxic gaseous plant hormone. This pretreatment or priming of Arabidopsis seedlings increases photosynthesis and carbohydrate utilization, resulting in a 20% increase in plant growth (2). It was shown that the ethylene priming increases stress tolerance and adds an apparent growth enhancement. Despite a clear effect on roots, aerial tissue, and increased stress tolerance, the primary mode of action from ethylene priming is unclear. In my work, I will investigate the direct effect of ethylene priming on early changes to photosynthesis and thylakoid development. Minor changes to chloroplast development may result in higher rates of PS and/or resistance to photoinhibition or NPQ that lowers photosynthetic efficiency. I will utilize PAM fluorometry (Pulse Amplitude Modulated) to check energy conversion efficiency and electron transport in ethylene-primed versus non-primed plants. I further attempt to record the developmental status of the photosynthetic apparatus over time post-priming using TEM, LSCM, and immunoblotting. The significance of this work is that it helps us understand the regulation of photosynthesis and related proteins in higher biomass-generating ethylene-primed plants. This work will also be instrumental in generating more resilient, high-yielding crop plants without genetically modifying them.

Aline Rodrigues de Queiroz – U. Nebraska

"Redox Regulation of Non-photochemical Quenching: Insights from Antioxidant Treatments"

Aline Rodrigues de Queiroz[1,2], Katarzyna Glowacka[1,2,3], Rebecca Roston[1,2]

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The exogenous application of antioxidants can lead to significant improvements in plant growth and stress resilience. These benefits are often accompanied by enhancements in photosynthetic capacity. However, variations in methods of application, timing, and concentrations pose challenges in comparing their effects, limiting the understanding of molecular mechanisms behind these improvements. In this study, we systematically tested the effects of six commonly applied antioxidants on the light reactions of photosynthesis in a high-throughput Arabidopsis seedling system. Our focus was on understanding antioxidant impact on non-photochemical quenching (NPQ), a heavily redox-regulated process. Our results indicate that the effects of exogenous antioxidants are not general and are unique to each compound. Their influence on NPQ rates and magnitude depends on factors such as chemical composition, dosage, incubation timing, and pre-illumination conditions. Notably, we identified an archaeal antioxidant that can reduce NPQ magnitude by over 40%. The application of this compound correlated with a decrease in the biosynthesis of zeaxanthin, the downregulation of the expression of violaxanthin

de-epoxidase and Photosystem II Subunit S. Furthermore, we observed contrasting effects between native and non-native antioxidants on native antioxidant systems and core metabolism. Our systematic study of exogenous antioxidant application revealed that in spite of their similar effects on plant growth, they have differing effects on redox regulation of photosynthesis that may prove useful for further dissection of photosynthetic regulation.

Matthew Runyon – U. Illinois

"Smaller Leaves for Bigger Yields: Investigating the utility of a novel maize canopy architecture modifier at elevated planting density"

Runyon, Matthew J.[1]; Sible, Connor N.[1]; Below, Fred E.[1]; Moose, Stephen P.[1]; Studer, Anthony J.[1]

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Historical increases in planting density are a key driver of maize yield response under intensified management. Selection for canopy architecture traits that optimize light interception at high density has typically focused on the cumulative effect of quantitative loci. A recessive, qualitative locus denoted as *reduced leaf area* (*rdla*) was previously shown to reduce leaf area in the maize inbred Oh43 by 33%. A 250kb region on Chromosome 4 containing this locus was introgressed into a panel of 28 expired Plant Variety Protection (ExPVP) inbred lines representing a diversity of germplasm sources, breeding eras, and native leaf areas. A subset of 16 *rdla* introgression lines phenotyped in the 2023 field season showed leaf area reductions ranging from 15 – 50%. A screen of 15 hybrids created from *rdla* introgression lines had similar ear leaf area reductions of 15 – 45%. Notably, reductions in hybrid grain yield were less severe than reductions in ear leaf area. Hybrids grown at variable planting densities showed the advantage of the *rdla* trait relative to wild type under high plant populations. While a per-plant yield penalty from *rdla* was observed, area-based yield was compensated and can potentially be improved due to reduced interplant competition. While no differences in photosynthetic assimilation have been observed, *rdla* hybrids are more efficient at producing grain per unit ear leaf area. These findings suggest that there is further opportunity for improving light interception efficiency in maize that can translate to increased grain yield. Attempts to fine-map *rdla* are ongoing.

Calvin Rusley - California Institute of Technology

"Distribution and evolution of phototrophy in the bacterial phylum Gemmatimonadota—rules for acquisition via horizontal gene transfer"

Calvin Rusley and Woodward W. Fischer

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The evolutionary history of chlorophototophy is rife with examples of horizontal gene transfer, but the trait is only sparsely distributed within eight exclusively bacterial phyla. First isolated in 2014, phototrophic Gemmatimonadota have since been found in metagenomes from freshwater, soda lake, and wastewater environments. Phylogenetic comparison of reaction centers suggests the phylum acquired its photosynthetic gene clusters from the Proteobacteria. However, preliminary analysis of Gemmatimonadota gene content found the redox metabolism necessary for phototrophic growth surprisingly uncommon. This work analyses 348 Gemmatimonadota genomes, including that of a phototrophic member newly recovered from a Mono Lake metagenome. Using a suite of metagenomic and comparative biological approaches, we evaluate how the phylum came to acquire phototrophy. Gemmatimonadota show evidence of vertical inheritance of a core bioenergetic scheme built around Alternative Complex III. Additional genomic analyses reveal numerous modular additions to the core Gemmatimonadota electron transport pathway, including high potential aerobic and anaerobic respiration, carbon fixation, and potential lithotrophy using hydrogen, sulfide, and ferrous iron. We propose that the presence of this modular high energy metabolism, in addition to the acquisition of a reaction center and (bacterio)chlorophyll synthesis genes, are the primary controls on the distribution of phototrophy.

Haley Schrader – Washington State University

"Leveraging the extremophile *Tidestromia oblongifolia* to characterize the kinetic thermal tolerance of the key C_4 photosynthetic enzyme PEP carboxylase"

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Optimizing photosynthesis is critical for sustaining a growing population under changing climate conditions. Most crops are not adapted to extreme environments, necessitating exploration of new model species to understand the boundaries of photosynthetic organisms living at environmental thresholds. One such extremophile plant, *Tidestromia oblongifolia*, has a photosynthetic temperature optimum of 47°C and utilizes the C_4 photosynthetic pathway, similar to many agronomically relevant crop species like maize and *Sorghum*. C_4 photosynthesis is initiated by phospho*enol*pyruvate carboxylase (PEPC) using substrates PEP and bicarbonate

(HCO_3^-). Due to PEPC's often rate-limiting role in this pathway, its activity and kinetic properties are key to determining rates of photosynthesis. Prior research has characterized the thermal kinetic response of PEPCs native to temperate environments, however the extremes of this thermal tolerance have not been investigated. I hypothesize that the thermal kinetic properties of PEPC will differ between hot and temperate adapted C_4 plants, and that these differences are due to specific changes in amino acid composition. To test this hypothesis, spectrophotometry and membrane inlet mass spectrometry were used to measure PEPC kinetics from 15-50°C in photosynthetic and non-photosynthetic isoforms from *T. oblongifolia* and the photosynthetic isoform from the temperate C_4 plant *Setaria viridis*. The thermal response between PEP and HCO_3^- affinity differed significantly between *T. oblongifolia* and *S. viridis* PEPC, particularly at high temperatures. These *in vitro* data provide the foundation for future work modeling structural changes in PEPC to investigate amino acid residues that confer thermal stability.