Poster Presentations

Poster 1

Pigment Hunter: An Online Graphical Computational Tool for Excitonic Calculations on Chlorophyll Proteins

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We report the development of an online application called PigmentHunter that enables "point-and-click" simulation of excitonic spectra of chlorophyll proteins based on PDB structures. PigmentHunter uses the nanoHUB.org science gateway to link a graphical user interface to a high-performance computing backend. The application automates the process of:

- Importing structural data from PDB files and classifying recognized photosynthetic pigments (chlorophylls, bacteriochlorophylls, and pheophytins)
- Calculating inter-pigment coupling values using the Transition Electrostatic Potential method [J. Phys. Chem. B 2006, 110, 17268]
- Introducing point mutations into the protein sequence
- Preparing and running molecular dynamics (MD) simulations
- Predicting pigment site energies using the charge density coupling (CDC) method [Photosythesis Research. 2008, 95, 197]
- Simulating absorption and circular dichroism (CD) spectra of the protein complex.

The application was tested against an experimental series of point mutations at the glutamine 57 site in water-soluble chlorophyll protein from *Lepidium virginicum*. PigmentHunter's calculated frequency shifts were shown to match the experimental shifts with an average error of 1.6 nm over a 9 nm spread in wavelengths.

Poster 2

Probing the roles of the endogenous clock in synchronizing N_2-fixation and photosynthesis in the unicellular diazotrophic cyanobacterium *Cyanothece* sp. ATCC 51142

Anindita Bandyopadhyay, Michelle Liberton, Annesha Sengupta and Himadri B. Pakrasi

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The implementation of daily rhythms by a circadian clock is an essential component of cyanobacterial metabolism that impacts fundamental physiological processes. Cyanobacteria are the only prokaryotes known to have a circadian clock defined by the *kaiABC* genes. The clock function is hypothesized to be critical in unicellular diazotrophic cyanobacteria, where circadian segregation of incompatible processes like photosynthesis and nitrogen fixation is imperative for cell survival under nitrogen-fixing condition. However, the role of the circadian clock in this ubiquitous and widely recognized group of cyanobacteria remains largely unexplored. *Cyanothece* 51142 is a well-studied model unicellular diazotrophic cyanobacterium that provides a platform to explore the function of the clock in orchestrating various cellular processes. There are two *kai* gene clusters in *Cyanothece* 51142 – one comprised of *kaiA, B1* and *C1* and the other comprised of *KaiB2* and *C2* genes. In addition, there are two more *kaiB* genes – *kaiB3* and *B4.* We are exploring the function of the clock at the molecular level by applying mutagenesis studies in *Cyanothece* 51142. We have deleted multiple clock genes in this strain by using the CRISPR/Cpf1 technology, creating markerless gene mutations and generating strains with deletions in multiple genes. These mutants are being characterized under different growth regimes and various physiological parameters are being analyzed to dissect the function of the different groups of *kai* genes in this strain. Analysis of the two main *kai* gene clusters suggests a distinct regulatory role for each. Characterization of these mutants will be presented.

Acknowledgements: This work is supported by funding from the National Science Foundation through Grant (MCB 1933660) to HBP.

Poster 3

Introducing Cysteine-Mediated Quenching in CP43 Builds Resilience to High-Light Stress in the Cyanobacterium *Synechocystis* sp. PCC 6803

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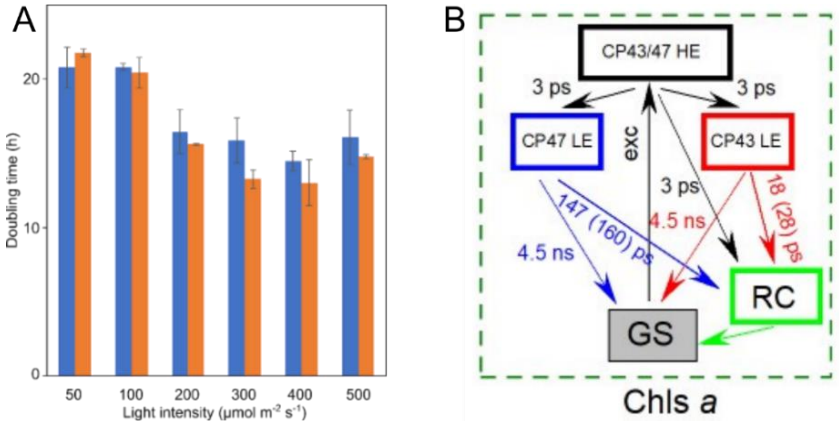
The vulnerability of photosystem (PS) II to light and reactive oxygen species is well documented and studied. The oxygenic photoautotrophs have developed processes like PSII repair and non-photochemical quenching to prevent and protect PSII from damage. In cyanobacteria, the CP43 protein homolog IsiA (CP43') is a specialized protein that dissipates excess light energy under oxidative stress conditions. We recently identified the role of conserved cysteine (C) 260 residue of IsiA from *Synechocystis* sp. PCC 6803 in light-energy dissipation. The CP43 and CP43' show similar structure, cofactor and pigment arrangement. Structural alignment of the two proteins aligns the C260 of CP43' with valine (V) 277 of CP43. We hypothesized that introducing C at the 277th position in CP43 would reduce light assimilation and thus be photoprotective under high light conditions. Physiological assessment of the mutant cell line showed slightly better growth under high light. Spectroscopic observations made with the isolated PSII suggested delayed excitation energy transfer to the reaction center. The mutant was also tolerant to photodamaging conditions compared to control. In addition, the mutation does not change the photosynthetic electron transport. Our results suggest that modifying the light-harvesting properties of PSII core antenna can be used as a successful strategy towards developing high light tolerance.
This study was supported by U.S. Department of Energy (DOE), Office of Basic Energy Sciences, grant DE-FG02-99ER20350 to H.B.P.



(A) Doubling time under different light intensities. Control (blue), Mutant (orange). (B) Kinetic model depicting target analysis of transient absorption datasets of Control-and Mutant-PSII, excited at 665 nm and recorded at 77 K. Time constants in parentheses are distinct for the PSII mutant. RC-reaction center; GS-ground state; HE-high energy; LE-low energy.

Poster 4

Mapping the energy and vibrational landscape of the photosynthetic complexes using temperature-dependent spectroscopy

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The mechanism of spectral tuning that photosynthetic pigment-protein systems utilize to increase light absorption remains unclear. Water soluble chlorophyll binding proteins (WSCPs) stand out as a model system for pigment-protein studies due to their relative simplicity. Hydrogen bonds involving the chlorophyll keto group and nearby charges are known to influence the absorption spectrum of Chlorophyll a (Chl-a). We introduced a set of point mutations into the Q57 site of the Lepidium virginicum (Lv) WSCP to investigate the role of charges and electrostatic interactions. Temperature-dependent photoluminescence spectroscopy was employed to investigate electron-phonon coupling strength within the pigment – protein complexes. A clear trend between the Q_y peak shift and vibronic coupling and charge of the aminoacid residue was observed. Introduction of a negative sidechain in the Q57 site of the Lv WSCP resulted in higher relative intensity and a blue shift of the Q_y peak while for the positive sidechain the trend was reversed. Observed pattern indicates that mutations in the Q57 site of Lv WCSP change vibrational coupling strength of Chl-a.

Poster 5

Exploring Cyanobacterial Carboxysome Activity as a Function of Carboxysome Lifecycle

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Cyanobacteria are notable for both their invention of oxygenic photosynthesis and their capability to fix carbon dioxide (CO_2) at high efficiency compared to other photosynthetic organisms. Their CO_2-concentrating mechanism features bicarbonate (HCO_3^-) transporters and a protein-encapsulated, selectively permeable bacterial microcompartment called a carboxysome. The carboxysome houses the carbon- and oxygen-fixing enzyme Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and the CO_2/HCO_3^- equibulator, carbonic anhydrase (CA). Recent work by *Hill et al. 2020* tracking single carboxysome activity over several generations revealed position-based activity levels during the carboxysome lifecycle. The life cycle can be broken down into three stages: the liquid-phase aggregate of RuBisCO and CA pro-carboxysome at the cell pole, the fully shelled active carboxysome in the cell center, and the broken open and degrading carboxysome back at the cell pole. These stages correspond to the theory of three pools of RuBisCO: a pre-carbamylated inactive form in the pro-carboxysome, carboxylase in the active carboxysome, and an oxygenase in the degrading carboxysome. In this poster we are proposing ways to probe RuBisCO activity in relation to the carboxysome life cycle stage. This work will provide foundational insight into carboxysome capabilities and RuBisCO function in the cell.

Poster 6

STRUCTURE FUNCTION STUDIES OF THE NDHD4 PROTEIN OF THE CO_2 UPTAKE MECHANISM IN SYNECHOCOCCUS ELONGATUS (PCC 7942)

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Carbon dioxide (CO_2) uptake and fixation by cyanobacteria, algae and plants is one of the unique and distinctive features of photosynthetic organisms. The CO_2 fixation allows these organisms to create sugars, create biomass and effectively store nutrition and energy in the form of complex organic molecules. Unlike plants and algae, cyanobacteria evolved a unique way of concentrating inorganic carbon (C_i) from the atmosphere for efficient fixation via the Calvin-Benson (CB) cycle. Facilitated by various transporters and anhydrases in the plasma/thylakoid membrane, cyanobacteria are able to concentrate high levels of bicarbonate (HCO_3^-) within the cytoplasm. Once in the cytoplasm, HCO_3^- can diffuse into the carboxysome, which contains Rubisco and carbonic anhydrase, with the latter converting it into CO_2 and producing a high local concentration around Rubisco. This allows Rubisco to overcome its notoriously poor affinity for CO_2 and competitive inhibition with molecular oxygen. This mechanism referred to as the CO_2 concentrating mechanism (CCM) also depends upon poorly understood CO_2 uptake enzymes which function as powered carbonic anhydrases. The CO_2 uptake activity uses specialized forms of the NDH-1 dehydrogenase complex, which convert CO_2 to HCO_3^- using the redox energy.

The purpose of this study is to investigate the NdhD4 protein, which is part of the NDH-1_4 complex, and is positioned in the thylakoid membrane. Upon the hydration of CO_2 into HCO_3^- via CupB, a proton is released on the cytosol side of the thylakoid membrane. It is hypothesized that the NdhD4 protein functions to remove these protons by pumping them across to the luminal side of the membrane away from the anhydrase active site and avoiding the back reaction. This hypothesized 'product removal mechanism' allows the reaction in CupB to maintain energetic favorability in the direction of HCO_3^- production. Provided the Ndhd4 protein is unable to function properly, it is also hypothesized that the accumulation of protons in the cytosol will hinder the ability of the CO_2 hydration reaction to occur as the reversible reaction proceeds towards equilibrium.

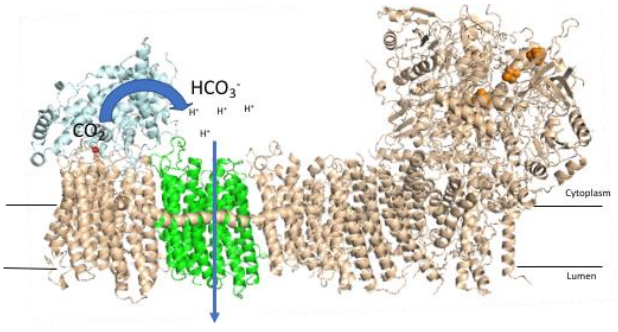

Figure 1: Hypothesized structure of Ndh-1_4 dehydrogenase complex. Featured in Blue is CupB and in Green is NdhD4. The cartoon shows an energized CO_2 hydration reaction with hypnotized proton pumping through NdhD4 across the thylakoid membrane.

Poster 7

Communicating Global Cyanobacterial Impact Through Film

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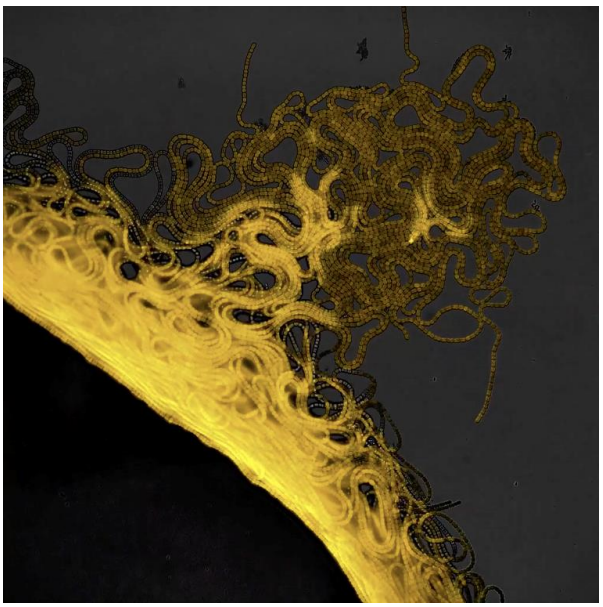
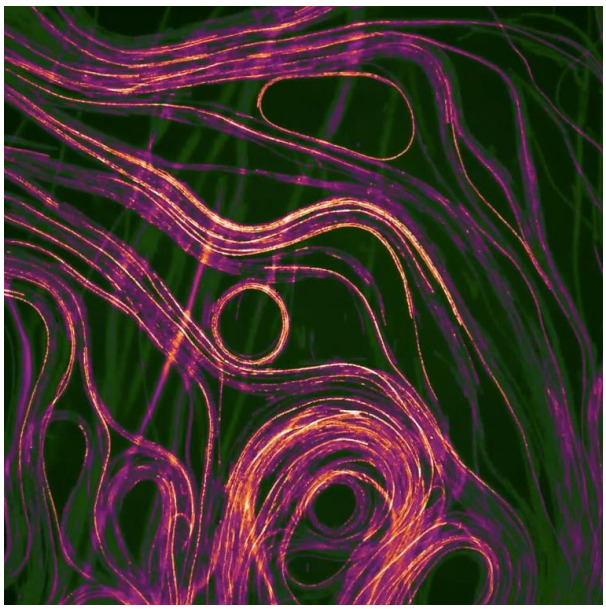
Cyanobacteria are key players in ecosystems and nutrient cycles worldwide. In order to communicate this importance to a wider audience, a feature-length art film is in production as well as a shorter version for an exhibit at the Denver Museum of Nature and Science. A custom Nikon TiE microscope was used to capture time-lapse images of growth and motility of various cyanobacteria. These images allow laypeople to directly see the effects that cyanobacteria have on the elements around them. Nitrogen fixation can be visualized as heterocysts forming along diazotrophic filaments. Oxygen evolution can be seen as bubbles overtaking the field of view. Phosphorous is visible as phosphate bodies within cells, and calcium is visualized as biogenic calcite crystals growing alongside their parent cells. The audience will gain a new understanding of carbon fixation as they watch GFP-tagged carboxysomes support exponential growth. Connecting with scientific concepts through art brings new relevance to environmental issues.





Poster 8

Psb27, a Photosystem II assembly protein, enables quenching of excess light energy during its participation in the PSII lifecycle

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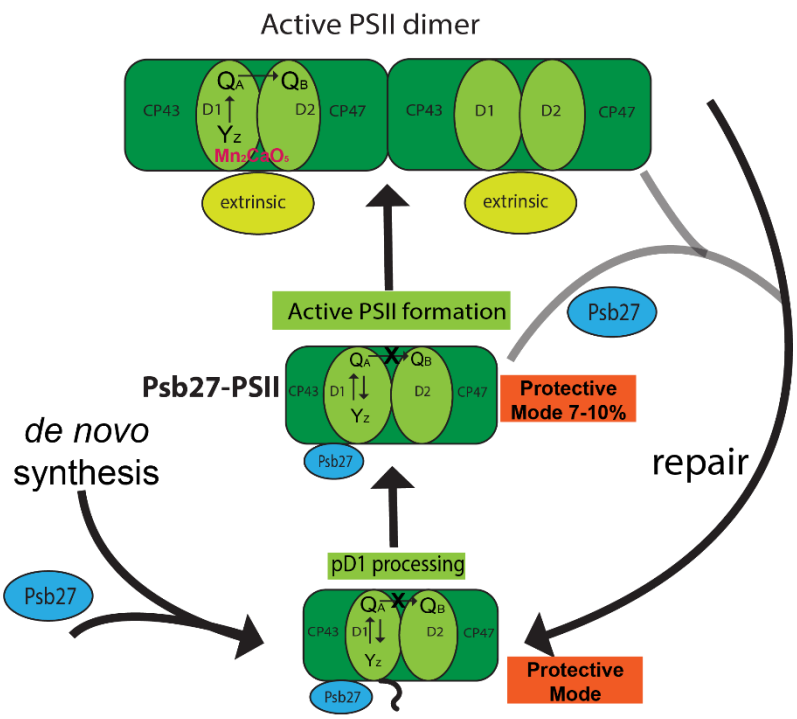
Photosystem II (PSII) is a multi-subunit chlorophyll-protein complex. It undergoes a complex lifecycle involving numerous protein chaperones. One such protein, Psb27, associates with the CP43 chlorophyll-binding subunit of PSII to form a Psb27-PSII sub-complex but dissociates prior to the formation of fully functional PSII. In this study, we analyzed a series of Psb27 mutant strains in the cyanobacterium *Synechocystis* sp. PCC 6803. Wild type (WT); *psb27* genetic deletion (Del27), genetically complemented *psb27* (Com27); and over-expressed Psb27 (OE27) were assayed for non-photochemical quenching and other photosynthetic parameters. The Del27 strain demonstrated decreased non-photochemical fluorescence quenching, while the OE27 strain had both increased non-photochemical quenching and increased tolerance to fluctuating light conditions. Fluorescence decay analysis showed that OE27 is the most resistant to change over multiple flashes. It had the least affected maximum PSII quantum yield of the strains, and least affected charge-transfer kinetics. We propose that the close association between Psb27 and CP43 and the absence of a fully functional manganese cluster allow for excitation energy dissipation in Psb27-PSII. Efficient energy dissipation prevents damage to this pre-PSII pool and allows for efficient PSII repair and maturation. Participation of Psb27 in the PSII life cycle ensures high PSII function and enhances environmental adaptability.



This study was supported by U.S. Department of Energy (DOE), Office of Basic Energy Sciences, grant DE-FG02-99ER20350 to H.B.P. H.L. was partially supported by DOE grant (DE-FG02-07ER15902).

Poster 9

Mapping ultrafast energy transfer in engineered chlorophyll *f* containing photosystem I complexes via two-dimensional electronic spectroscopy

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Naturally occurring and engineered photosystem I (PSI) complexes containing chlorophyll *f* (Chl *f*) molecules have an extended absorption spectrum with transitions in the 700-800 nm spectral region associated with the Chl *f* molecules. The Chl *f* molecules enable the PSI complexes to harvest more red-shifted photons. In this work we applied two-dimensional electronic spectroscopy (2DES) to engineered PSI complexes containing Chl *f* molecules (Chlf-PSI) and the corresponding wild-type (WT) PSI complexes isolated from *Synechococcus* 7002. The engineered Chlf-PSI contains 3.8 Chl *f* molecules per PSI monomer and shows an expanded absorption spectrum up to 740 nm. The 2DES spectra show evidence of downhill energy transfer to the red shifted Chls as a growth in the crosspeak region. Additional insight is gained by applying a global analysis to the 2DES spectra of Chlf-PSI and WT-PSI where we extract the two-dimensional decay-associated spectrum (2D-DAS). We compare the 2DES and resulting 2D-DAS for the two different PSI complexes to gain further insight into how Chl *f* molecules impact energy transfer in the engineered Chlf-PSI.

Poster 10

Exploring new functions for the Ferric Uptake Regulator FurA in the cyanobacterium *Anabaena* sp. PCC 7120

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The Ferric Uptake Regulator FurA from the cyanobacterium *Anabaena* sp. PCC 7120 was initially described as a transcriptional regulator responsible for the control of iron homeostasis. However, further studies revealed that it was involved in the regulation of genes belonging to many other processes such as nitrogen metabolism or photosynthesis. Besides, comparative transcriptomics studies of a misregulation strain of *furA* unveiled significant alterations in the transcription of around 40 transcriptional regulators. This suggests that FurA could also modulate a large number of regulators not yet characterized that will enlarge its regulon. Consequently, a deeper study of the function of these regulators would cast new light on the role of FurA in the physiology of this cyanobacterium.

Moving on to molecular aspects, FurA contains five cysteines, four of them arranged into two CXXC motifs. *In vivo,* FurA displays several redox isoforms, and the oxidation state of its cysteines determines its activity as a regulator and its ability to bind to metabolites such as heme. However, the precise mechanism underlying the reduction of FurA, as well as its functional electron donor, remains still unknown. Since thioredoxins are essential players in thiol-based redox regulation and are involved, among many other processes, in the regulation of the activity of many transcription factors, we sought to investigate the potential role of type-m thioredoxin A (TrxA) in the redox modulation of FurA. Additionally, as the photosynthetic electron transporters flavodoxin (Fld) and ferredoxin (Fdx) are able to transfer electrons to hemoproteins and previous studies showed that FurA binds to heme, we also analyzed the interaction of these proteins with FurA.

In this work, we have identified several genes with regulatory functions that are direct targets of FurA. Besides, as the role of the vast majority of these genes is unknown, CRISPR-Cpf1 based genome editing strategy is being used to create markerless deletion mutants of these regulators. On the other hand, both *in vitro* cross-linking assays and *in vivo* two-hybrid studies confirmed that FurA was able to interact with TrxA, Fld and Fdx. Reconstitution of an *in vitro* electron transport chain evidenced the ability of TrxA to reduce FurA and light to dark transitions resulted in reversible changes in the redox states of the regulator. On the contrary, in the case of Fld and Fdx, the interaction does not appear to have a redox implication, but seems to modulate the transport of electrons from FNR to Fld and Fdx.

Taken together, these results show that FurA functions go beyond the control of iron homeostasis. On the one hand, it has been found that FurA is connected to photosynthesis by modulating photosynthetic electron transport and by having photosynthetically reduced TrxA as one of its main redox partners. On the other hand, FurA presents itself as a cornerstone in transcriptional networks of *Anabaena.*

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Poster 11

Adaptation and stress acclimation in Antarctic alga, *Chlamydomonas priscuii* alters short-term light stress response

Devon Popson and Rachael Morgan-Kiss

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Permanently ice-covered lakes located in the McMurdo Dry Valleys (Antarctica) represent harsh yet stable aquatic habitats for diverse phytoplankton adapted to permanent stress conditions. One such organism is *Chlamydomonas priscuii* (formerly, *C.* sp UWO 241) which has evolved under the extreme conditions of Lake Bonney, including permanent low temperatures and hypersalinity. Recent publications have reported on major mechanisms associated with long-term adaptation to high salt and low temperatures, including i) assembly of a PSI supercomplex, ii) constitutively high rates of cyclic electron flow, and iii) rewired downstream carbon metabolism (Kalra et al. 2020). Notably, the native light environment of *C. priscuii* is extreme shade and blue-green wavelengths. Climate-related change in Antarctica is causing thinning ice covers and lake level rise, which may alter the light environment of *C. priscuii*. Previous studies in other, nonextremophilic photosynthetic organisms have shown that acclimation to other stresses, including low temperature, can confer higher resistance to photoinhibition. Recently, our lab reported that *C. priscuii* can acclimate to long-term high light stress (Stahl-Rommel et al. 2021); however, relatively little is known about its ability to tolerate short-term high light stress. To better understand how *C. priscuii* might respond to perturbations in light intensity, we asked two questions: i) What is the susceptibility of *C. priscuii* to short-term high light stress, and ii) Does acclimation to long-term stress (low temperature, high light, or high salt) confer increase resistance to photoinhibition? Surprisingly, despite adaptation to extreme shade and loss of some short-term mechanisms (for e.g. state transitions), *C. priscuii* exhibited resistance to photoinhibition that was comparable with the model strain *C. reinhardtii*. Furthermore, pre-acclimation to long-term stress resulted in a further decline in susceptibility to photoinhibition in low temperature- and high light-acclimated cultures. On the other hand, high salt-acclimation exacerbated sensitivity to photoinhibition. Our results suggest that *C. priscuii* mediates short-term light stress through alternative mechanisms compared to traditional algal models.

References:

Kalra, I., Wang, X., Cvetkovska, M., Jeong, J., McHargue, W., Zhang, R., Hüner, N., Yuan, J.S. and Morgan-Kiss, R., 2020. *Chlamydomonas* sp. UWO 241 exhibits high cyclic electron flow and rewired metabolism under high salinity. *Plant physiology*, *183*(2), pp.588-601.

Stahl-Rommel, S., Kalra, I., D'Silva, S., Hahn, M.M., Popson, D., Cvetkovska, M. and Morgan-Kiss, R.M., 2021. Cyclic electron flow (CEF) and ascorbate pathway activity provide constitutive photoprotection for the photopsychrophile, *Chlamydomonas* sp. UWO 241 (renamed *Chlamydomonas priscuii*). *Photosynthesis Research*, pp.1-16.

Poster 12

Vibrational Properties of Semi-Phylloquinone in the Q_A Binding Site of Purple Photosynthetic Bacteria

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The electron acceptor Q_A operates as the neutral and one-electron reduced quinone (semiquinone) in purple bacterial reaction centers (PBRCs). FTIR difference spectra obtained for the Q_A in PBRCs reconstituted with a series of non-native, unlabeled/isotope edited quinones are available; the experimental data for the substituted quinones reveal the binding interactions in the protein environment in terms of the change in vibrational bands.

We investigated vibrational frequencies of one-electron reduced phylloquinone (semi-phylloquinone, PhQ^-) in the Q_A binding site in PBRCs from Rhodobacter sphaeroides. Calculated frequencies for PhQ^-, ^{18}O-, and ^{13}C-PhQ^- in the protein, using the hybrid QM/MM computational technique were undertaken. At the next step, calculated spectra for unlabeled and isotope edited PhQ^- in a relatively polar and aprotic solvent were also performed.

Our results indicate that both sets of calculated spectra, in the protein binding site and the solution, agree with the available FTIR spectra for the Q_A binding site. The similarity could suggest that the overall binding interactions between PhQ^- and protein in the Q_A binding site are similar to the interaction with the solution. Moreover, vibrational frequencies of substituted PhQ in the Q_A binding site were compared to the A_1 binding site of photosystem I (PSI), where PhQ is the native electron acceptor. In contrast to the Q_A site, the vibrational bands of PhQ^- in the A_1 binding site are not comparable to the solution spectra. The noticeable interactions with the surrounding environment were discussed to address the origin of vibrational frequencies of PhQ^- in two surrounding protein environments.

Poster 13

Streamlining the genome of a fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973 for enhanced bioproduction.

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Cyanobacteria are oxygenic photosynthetic prokaryotes that have gained significant recognition as sustainable carbon-neutral bio-producers. Recently, a high-light tolerant cyanobacterium *Synechococcus elongatus* UTEX 2973 was isolated which exhibits photoautotrophic growth rates comparable to yeast. Unlike heterotrophic producers, cyanobacteria-mediated production is not economically viable even with the fast-growing strain and this is being largely attributed to the genetic complexities of these organisms. Genome streamlining in heterotrophs have shown to confer unique benefits. Similar benefits can be expected in cyanobacteria where the genome harbors more complex regulatory networks required for adaptation to adverse conditions. Eliminating such regulation could improve the performance of cyanobacteria as a cell-factory and yield a more readily understood and modeled genome. To that end, we are aiming to reduce the genome of *Synechococcus* 2973 iteratively by using state-of-the-art genome-editing technology, guided by experimental analysis and computational tools . To identify the gene set essential for rapid growth and high CO_2 fixation, a high-throughput Tn-Seq analysis was performed. High transposition efficiency was achieved and sequencing of 10 individual colonies showed unbiased, single transposition event. Dispensable regions identified using MinGenome algorithm are being targeted for deletion exploring both CRISPR/Cas3 and CRISPR/Cas12a methods. Both tools have been successfully used to delete a 30kb region and further attempts of tiered deletions are in progress.

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Poster 14

MECHANISM OF PROTON-POWERED CARBONIC ANHYDRASE ACTIVITY IN PHOTOSYNTHESIS

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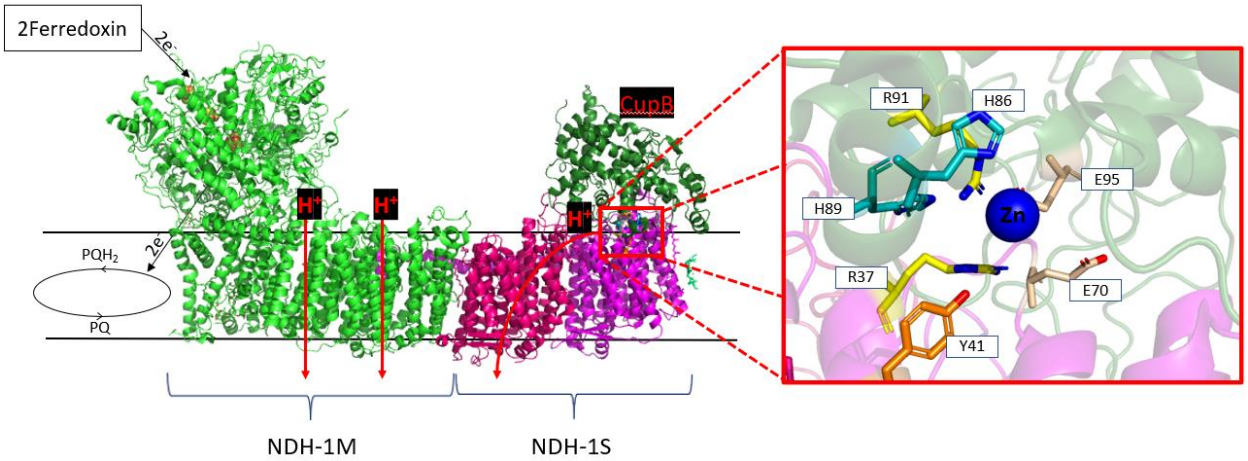
The NADPH Dehydrogenase Type 1 (NDH-1) complexes in cyanobacteria are involved in the CO_2 concentrating mechanism (CCM), a process which allows cells to provide sufficient concentrations of inorganic carbon (C_i) in the form of CO_2 to Rubisco in low C_i conditions. These complexes possess subunits specially designed for high or low affinity CO_2 uptake: CupA and CupB. Deletion of either gene impairs cell growth and CO_2 uptake with respect to C_i. While a structure for CupA and the associated NDH-1 complex has recently been resolved, the mechanism by which this protein functions remains unknown. To elucidate this mechanism, an expression system in *Synechococcus elongatus* PCC 7942 has been developed. Conserved histidine, arginine, and glutamate residues in CupB have been hypothesized to participate in a unidirectional, powered CO_2 hydration reaction taking place near the interface between CupB and the proton-pumping subunits of the NDH-1 complex. In a constructed homology model, the residues are in relative proximity to a Zn ion modeled to form the catalytic site of CO_2 hydration. Physiological assays show mutation of these conserved residues results in severe defects in C_i fixation activity. Proteomic analysis showed that some site-directed mutations in CupB caused the upregulation of bicarbonate uptake proteins to a greater extent than complete deletion of CupB. These results lead us to hypothesize that the mutations at these residues disrupt the energy coupling activity of the NDH-1 complex which normally drives CO_2 hydration forward, thereby introducing a fully reversible carbonic anhydrase into the cytoplasm and eliminating the HCO_3^- gradient.



NDH-1 is an energy conversion complex the subunits of which can be classified in one of three ways. Electron transfer associated proteins, proton pumping associated proteins, and CO_2 uptake associated proteins. Using these classifications, we can see that NDH-1 is primarily an energy conversion complex that uses energy from redox reactions to pump protons across the thylakoid membrane. In addition, cyanobacteria have evolved a version of this complex which may utilize the power of NDH-1 to concentration HCO_3^- within the cytoplasm.

Poster 15

SINGLE-CELL MICROSCOPY REVEALS ASYMMETRIC SURVIVAL TO PHOTODAMAGE IN CYANOBACTERIA

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As photosynthetic microbes, cyanobacteria harvest energy from light, using it to convert carbon dioxide and water into biomass. However, these reactions are strongly energetic, resulting in damage to photosystem II (PSII), which is a reaction complex responsible for harvesting light energy to drive the photosynthetic reactions. Consequently, PSII undergoes continuous repair and turnover to ensure a functional population. The rate of photodamage is proportional to the incident light intensity; When a cell is exposed to intense light such that the rate of damage exceeds the cell's ability to repair, that cell is no longer able to grow. Thus, understanding the repair pathway is important in understanding how cells maintain optimal growth under changing environmental conditions. Here, we report on a method which uses a microscope platform to induce photodamage using UV-A light, then films the subsequent recovery of cells. This platform allows varying intensities and durations of UV-A radiation to be applied. In addition to imaging, we also report on the development of computational image analysis software to analyze the resulting image datasets. We show that this platform allows multiple strains to be grown simultaneously during the same imaging experiment, then identified computationally in subsequent analysis, allowing direct comparison between control and experimental strains. Our analysis reveals an asymmetric cellular response to photodamage in cyanobacteria colonies, where one daughter cell survives but its siblings do not. This work has implications for understanding how cells achieve maximum growth under varying light conditions, as well as the evolution of the photosynthetic apparatus in phototrophic microbes. This study was financially supported in part by the U.S. Department of Energy (DOE) DE-SC0019306 (to J.C.C.).

Poster 16

Chloride facilitates Mn(III) formation during photo-assembly of the Photosystem II oxygen-evolving complex

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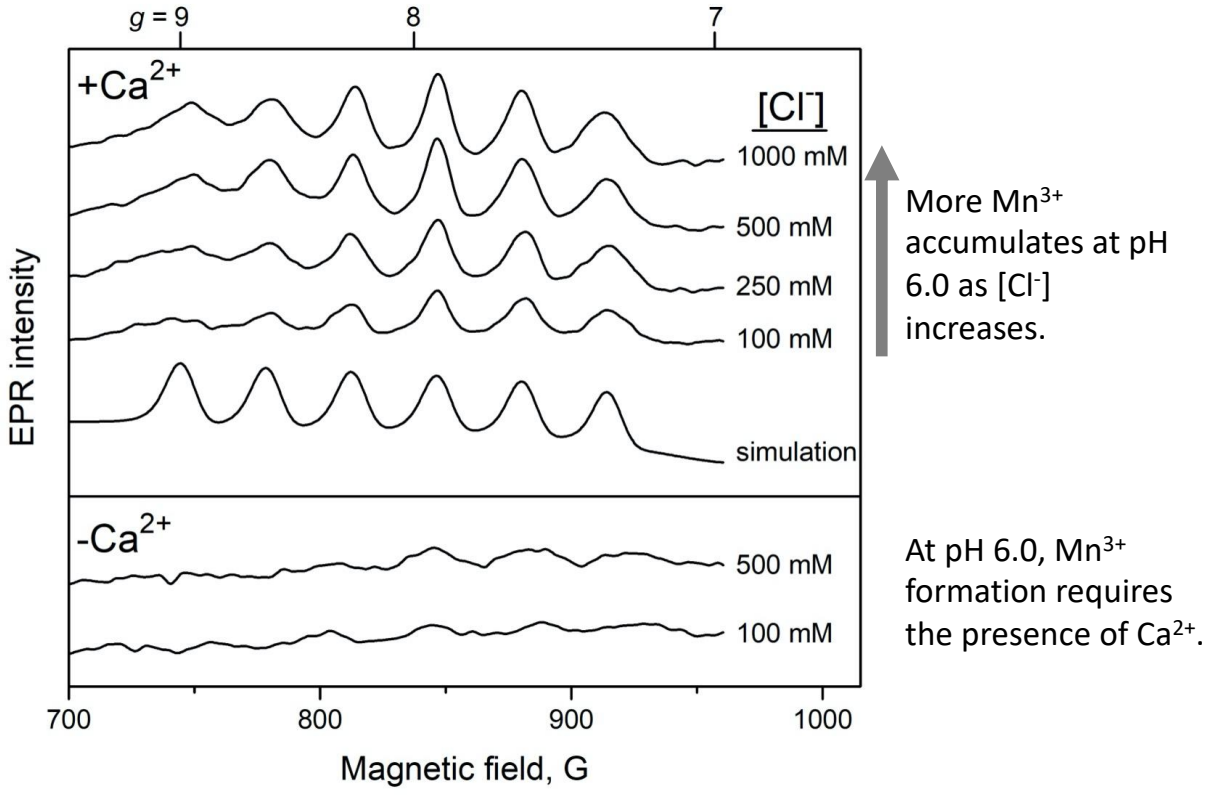
The Mn_4Ca oxygen-evolving complex (OEC) in Photosystem II (PSII) is assembled in situ from free Mn^{2+}, Ca^{2+}, and water. In an early light-driven step, Mn^{2+} in a protein high-affinity site is oxidized to Mn^{3+}. Using dual-mode electron paramagnetic resonance spectroscopy, we observed that Mn^{3+} accumulation increases as chloride concentration increases in spinach PSII membranes depleted of all extrinsic subunits at physiologically relevant pH values. This effect requires the presence of calcium. When combined with pH studies, we conclude that the first Mn^{2+} oxidation event in OEC assembly requires a deprotonation that is facilitated by chloride.



Poster 17

Isothermal Titration Calorimetry of Membrane Protein Interactions: FNR and the Cytochrome b6f Complex

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Ferredoxin-NADP+ reductase (FNR) was previously inferred to bind to the cytochrome b6f complex. In the present study, this inference has been examined through analysis of the interaction thermodynamics between FNR and the b6f complex. Isothermal titration calorimetry (ITC) was used to characterize the physical interaction of FNR with b6f complex derived from two plant sources (spinach, Zea maize). ITC did not detect significant interaction of FNR with b6f complex in detergent solution nor with the complex reconstituted in liposomes. A previous report on a weak but defined FNR-b6f interaction is explained by FNR interaction with micelles of the UDM detergent used to purify b6f. Circular dichroism (CD) analysis, employed to analyze the effect of detergent and pH on the FNR structure, did not reveal significant changes in secondary and tertiary structures in the presence of UDM detergent, which could be responsible for the small free energy changes detected by ITC. Thermal melting analysis implied a structure change caused by detergent, attributed to a significant weakening of interaction between the FAD and NADP+ binding domains of FNR. In addition to the conclusion regarding the absence of a binding interaction of significant amplitude between FNR and the b6f complex, these studies provide a precedent for consideration of significant background protein-detergent interactions in ITC studies involving an integral membrane protein (Study supported by grants from the DOE to W. A. Cramer and S. Savikhin).

Poster 18

Normal-coordinate Structural Decomposition Calculations in the PigmentHunter App: Comparing Chlorophyll Ring-deformation Variability across PSII Structures

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We have implemented the normal-coordinate structural decomposition (NSD) site-energy prediction method [1,2] in the PigmentHunter app and used it to compare chlorophyll site energies in various photosystem II crystal structures. The NSD method uses the magnitude of a chlorophyll's distortion with respect to out-of-plane normal deformations to predict site energies. The site energies of chlorophyll a from 26 different photosystem II crystal structures from a variety of species (*Th. elongatus*, *Th. vulcanas*, *Pisum sativum*, and *Ch. Gracili*) were calculated and compared. Predicted site energies of different crystal structures from the same organism were compared to determine the sensitivity of the NSD method to uncertainty in the crystal structure. The site energies are also compared between photosystem II's of different organisms to determine statistical differences between organisms. The results clarify the usefulness of the NSD method for predicting site energies based on experimental crystal structures.

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2. Zucchelli, Giuseppe; Brogioli, Doriano; Casazza, Anna Paola; Garlaschi, Flavio M.; Jennings, Robert C.; "Chlorophyll Ring Deformation Modulates Qy Electronic Energy in Chlorophyll-Protein Complexes and Generates Spectral Forms". *Biophys. J.*, 93, 2240-2254 (2007).

Poster 19

Designing a CRISPR System for Gene Knockout in Cyanobacteria

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NdhF4 is a component of the cyanobacterial CO_2 concentrating mechanism that may be involved in the CO_2 hydration mechanism of CO_2 uptake proteins. To elucidate the function of NdhF4, site-directed mutations need to be made in conserved residues of *ndhF4*. Mutant *ndhF4* will be introduced to a strain lacking *ndhF4*. CRISPR has been used to make mutations in cyanobacteria. A CRISPR system could be used to knock out *ndhF4* to allow the introduction of mutant *ndhF4* into a markerless background. Using a CRISPR system developed by the Pakrasi lab [1] and modified by the Zhang lab [2], a markerless knockout of *ndhF4* in *Synechococcus elongatus* PCC 7942 can be made. Cas12a can bind to and cut a DNA sequence that matches both the crRNA and the PAM site. To maximize efficiency, a piece of DNA containing a green fluorescent protein (GFP) or a pink colored protein can be inserted into the plasmid and transform E. Coli can be transformed with the plasmid. Cells containing a plasmid encoding for GFP will fluoresce when exposed to UV light, and cells containing the plasmid encoding for the pink protein will turn pink. GFP and the pink protein will provide a phenotypic marker to easily identify cells containing the plasmid. Then, the plasmid will be extracted, and a crRNA sequence will be inserted into a plasmid, interrupting a *lacZ*. Cells with plasmids containing disrupted lacZ genes (and thus containing the crRNA insert) will appear white under a blue/white selection. The final step for preparing a plasmid capable of creating a markerless deletion of *ndhF4* is to add a repair template. To this end, a repair template consisting of chromosomal DNA upstream and downstream to n*dhF4* will be inserted into the plasmid, replacing GFP or the pink protein. The plasmid will be harvested from E. coli and introduced to *Synechococcus elongatus* PCC 7942. When the Cas12a protein cuts *ndhF4*, the cell will repair it via homologous recombination with the repair template provided in the plasmid.

1. Ungerer, J. and Pakrasi, H. B. Cpf1 Is A Versatile Tool for CRISPR Genome Editing Across Diverse Species of Cyanobacteria. *Sci. Rep.* **6**, 39681; doi: 10.1038/srep39681 (2016).
2. Tian-Cai Niu, Gui-Ming Lin, Li-Rui Xie, Zi-Qian Wang, Wei-Yue Xing, Ju-Yuan Zhang, and Cheng-Cai Zhang. Expanding the Potential of CRISPR-Cpf1-Based Genome Editing Technology in the Cyanobacterium Anabaena PCC 7120. ACS Synthetic Biology 2019 8 (1), 170-180; DOI: 10.1021/acssynbio.8b00437

Poster 20

COMBINING BULK 77K FLUORESCENCE SPECTROSCOPY AND SINGLE CELL CONFOCAL MICROSCOPY TO UNCOVER HETEROCYST PHOTOSYSTEM BEHAVIORS

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The filamentous cyanobacteria genus *Anabaena* achieves both carbon fixing and nitrogen fixing simultaneously by separating the two processes spatially in different cells along a single filament and transferring nutrients between cells. Cyanobacteria serve as a major component in the global nitrogen cycle and present an intriguing potential as a biofertilizer. Studying the nitrogen fixing cells in the filaments is not a trivial matter, as the nitrogen fixing heterocysts exist as a small population among the vegetative cells, making bulk culture analysis difficult. Photosystem balancing has been previously measured using 77K fluorescence spectroscopy, an ultra-low temperature fluorescence technique that allows for separation of different photosystem and pigment fluorescence signals in bulk cultures. This technique can then be used in parallel to confocal microscopy with tunable laser excitation and specific emission wavelengths to gain a window into photosystem balancing of individual cell types in a culture with differentiation into multiple cell types. Use of carefully aligned bulk and microscopy samples has allowed for further understanding as to the photosystem balancing of any remaining chlorophyll and phycobilisomes in heterocysts at different stages of differentiation, and of the vegetative cells surrounding them.

This study was financially supported, in part, by the NIH/CU Molecular Biophysics Program, by the U.S. Department of Energy (DOE) DE-SC0018368 and DE-SC0020361.

Poster 21

Spectroscopic and Computational Analysis of the Oxygen Evolving Complex Transformations in Photosystem II

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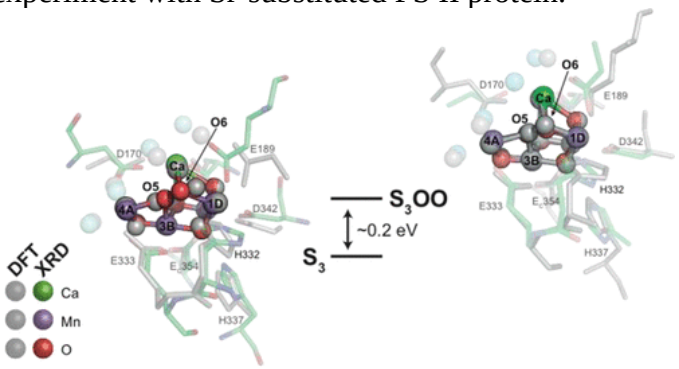
The light-driven water oxidation processes inherent to Photosystem II (PS II) depends on the oxygen evolving complex (OEC), the $CaMn_4O_5$ cluster which cycles through five redox states, S_0-S_4, finally evolving molecular oxygen (O_2). Despite being the focus of recent study, the precise timing and mechanisms of O-O bond formation and O_2 remains unknown. It has been established that substitution of the OEC's Ca^{2+} with Sr^{2+} results in a decrease in the rate of oxygen evolution; we present DFT calculations on intermediates of the Kok cycle, yielding insight into energetic and structural differences on ~600 electron models of the OEC (BP86 level of theory, def2tzvp basis set for all atoms). Though DFT and cryogenic XAS are integral for reveling possible structures, models, and energetics of the S_0-S_4 transformations in PS II, they are insufficient for deciphering kinetics; use of a pump probe system with ns laser flashes advances the Kok cycle reveals rapid changes in the Mn K-beta emission spectra associated with progression through the Kok cycle. Comparison of Mn K-beta emission spectra of 2 flash (2F) intermediate (associated with advancement to the S_3 intermediate) with 3F intermediates probed at different us delays indicate rapid (<50 us) changes to the Mn K-beta emission spectra. XANES spectra indicate that once the all-Mn^{IV} state is achieved, no further oxidation of the $CaMn_4O_5$ cluster is observed prior to O-O bond formation. A high energy – yet accessible – peroxide isoform of the S_3 state may be oxidized by Tyr^{ox} during final electron transfer prior to O_2 evolution, possibly explaining kinetics of O_2 evolution and an evolutionary adaption to avoid release of harmful peroxides. Based off the XANES data, DFT-optimized models analogous to the previous ~600 electron OEC models are produced considering this S_3 state peroxo isoform. Models are computed both with native Ca-based clusters as well as with Sr-substituted clusters, and their geometric and energetic differences are explored. Future work may include similar pump probe XANES experiment with Sr-substituted PS II protein.



Poster 22

Generation and characterization of OCP-PSII complex for structural and functional analysis in cyanobacteria

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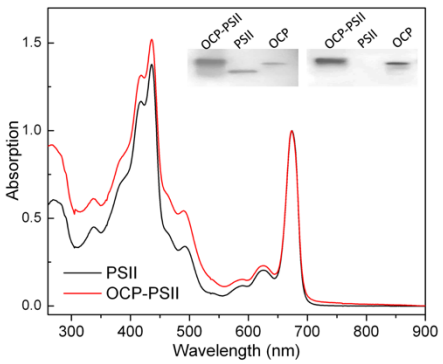
Orange carotenoid protein (OCP) dissipates the extra energy from phycobilisome (PBS), relieving the over excitation energetic pressure to reaction centers (RCs), Photosystem II (PSII) and Photosystem I (PSI), protecting the integrity of the electron transport chain in the thylakoid membrane, particularly the components on the stromal side of RCs. To probe the molecular mechanism of OCP during its photocycle and the interactions between PBS, OCP, and PSII, we have generated several strains for structural studies. In this report, we show that a genetically OCP-tagged PsbM, one small subunit peptide located in the interface of dimeric PSII, allows for forming a chimeric OCP-PSII complex. C-terminally polyhistidine modified OCP further facilitates biochemical purification of a functional OCP-PSII complex. Genetic analysis indicates complete segregation. Biochemical analysis shows the chimeric form of PsbM-OCP in the isolated OCP-PSII complex. We used molecular spectroscopy and HPLC analysis to characterize the pigment(s) that is loaded onto the OCP portion. We also characterized the excitation energy transfer landscape using time resolved fluorescence in whole cells and isolated OCP-PSII complex. Mass spectrometry analysis promises the residue level protection of PSII on its reduced side.



This study was supported by U.S. Department of Energy (DOE), Office of Basic Energy Sciences, grant DE-FG02-07ER15902 to HL. DMN is supported by EECE.

Poster 23

Probing copper binding in orange carotenoid protein by using H/DX and native mass spectrometry

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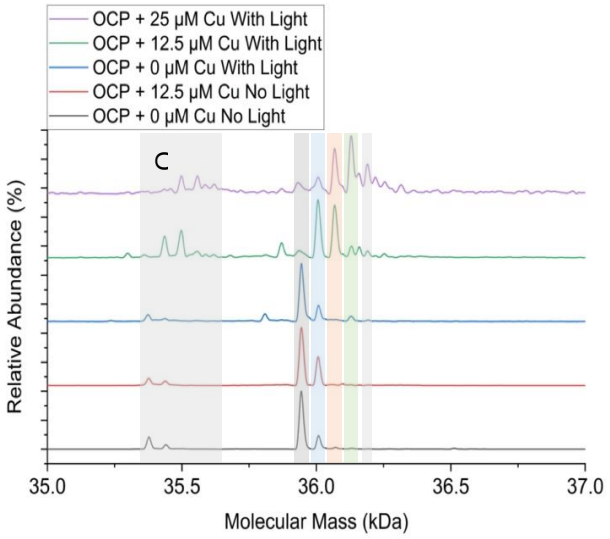
Orange carotenoid protein (OCP) is a light-responsive regulatory protein found in cyanobacteria. OCP changes from its "orange form" (OCP^O) to a "red form" (OCP^R) upon light illumination. The pigment color changes are accompanied by dramatic protein conformational changes. We recently discovered that copper ion has no effect on the photoactivation kinetics. However, the relaxation process from OCP^R to OCP^O is largely delayed or completely blocked by the presence of copper ion. To probe the binding stoichiometry of copper ion on OCP^R, we adopted native electrospray mass spectrometry, a technique that has the capability of studying protein with its intact pigment or cofactors. Additionally, we describe hydrogen deuterium exchange (H/DX) coupled with mass spectrometry for probing copper binding site(s) of OCP. Our native MS using low concentration of OCP (3 µM) indicated that monomeric OCP (mOCP) is the dominant species with charge states of +9, +10, +11 on SNAPT G2 versus charge state of +10, +11, +12, +13 on EMR. We also observed that copper-bound OCP is sprayed to show similar charge states, indicating tight copper binding in OCP^R. OCP has two structural domains: N-terminal domain (NTD) and C-terminal domain (CTD), where the pigment is buried. To probe further the copper binding domains, we generated NTD and CTD by using low temperature partial proteolysis. It was observed that at least one copper ion can bind with NTD and one with CTD.



This study was supported by U.S. Department of Energy (DOE), Office of Basic Energy Sciences, grant DE-FG02-07ER15902 to HL. We thank NIH for support (NIH NIGMS 5P41GM103422 to MLG).

Poster 24

Exploring the relationship between the proteomes of the periplasm and thylakoid lumen

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Cyanobacteria possess unique intracellular organization, which includes the thylakoid membranes, an additional set of membranes enclosing the thylakoid lumen. Recently, the thylakoid lumen proteome in cyanobacteria has been elucidated. Unexpectedly, many proteins that have previously been localized to the cyanobacterial periplasm were found within the thylakoid lumen, including PratA, CtpB, and AmiC. To better understand the relationship between the thylakoid lumen and the periplasm, an experiment to compare the proteome of the two compartments has been devised. This experiment utilizes APEX2 for proximity-based proteomics combined with tandem mass tag peptide labeling (TMT) to determine the proteome of the periplasm, thylakoid lumen, and cytoplasm. The proteomic data will also be used to identify factors important for localizing proteins to the thylakoid lumen or the periplasm. In the future, a similar technique could be used to investigate the localization of photosystem II (PSII) assembly, a cellular process that occurs in both the plasma membrane and the thylakoid membrane with assembly factors located in the cytoplasm, periplasm, and thylakoid lumen.

Poster 25

Are Conserved Cysteines in ApcE Important for Redox Regulation of Phycobilisomes?

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Phycobilisomes are photosynthetic antennas associated with the thylakoid membrane in red algae and cyanobacteria. Light in cyanobacteria is primarily collected by chromophores in the phycobilisome evolved to provide directional energy migration to chlorophyll in photosystems I and II. The phycobilisome is a large complex that consists of two domains: the core and rods both consisting of disc-shaped phycobiliprotein aggregates.

Located in the core is ApcE, the anchor protein, that engages in stabilizing the core and anchoring phycobilisomes to the photosynthetic membranes where it can transfer energy. In 2015, a proteomic study in cyanobacteria identified multiple cysteines in ApcE, one being involved in chromophore binding in Synechocystis sp. PCC 6803, and another as a potential site of glutathionylation. Gluathionylation, the formation of a mixed disulfide bond between glutathione and a cysteine residue, occurs frequently under oxidative stress and indicates a potential mechanism for the uncoupling and subsequent dissociation of the phycobilisome. A bioinformatics study revealed the glutathionylated cysteines to be conserved across multiple species of cyanobacteria. As the mechanism by which phycobilisomes disassociate under excess illumination and redox stress is not known, we hypothesize that these conserved cysteines could play a critical role in this acclimation process.

The goal of this project is to understand the characteristics of ApcE and the mechanism of antenna binding. This can be accomplished in two steps: knocking out gene coding for ApcE in Synechococcus sp. PCC 7002 and constructing cysteine-to-serine mutations in the conserved, potential glutathionylation cysteine candidates identified in ApcE. I have previously constructed the knockout mutant and successfully complemented the mutant by expressing the wild-type allele at a second site in the genome. The ApcE knockout mutant reveals a remarkable phenotype. The absorbance spectra of the ApcE knockout are modified. It also exhibits a high fluorescence phenotype. Using fluorescence microscopy, we visualized a change in the quantity and localization of thylakoid membranes. The mutant displays a modified localization of
thylakoid membranes and asymmetric partitioning of thylakoid membranes during division.

Poster 26

Using Single Molecule Spectroscopy to identify the sites involved in energy quenching inside the phycobilisome core

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Phycobilisomes (PBs) are light harvesting complexes of many cyanobacteria and some algae. These complexes covalently bind linear tetrapyrrole pigments and form large multisubunit structures located on the surface of thylakoid membranes – in proximity to photosynthetic reaction centres. The physicochemical properties of pigments in PBs are tuned to transfer the energy of absorbed light to the reaction centres. However, we have recently shown that pigments in PBs can reversibly enter states in which they dissipate excitation energy instead of transferring it. Switching between the states is light induced, and with increasing intensity of the incoming light, the equilibrium between the states shifts towards the energy-quenching ones. We have also shown that while each pigment in the PBs structure is capable of entering these energy-dissipative states, the pigments in the PBs core are more likely to undergo a switch. Here, we are reporting a single molecule spectroscopy study in which we examined the PBs isolated from a mutant and compared them with the WT-PBs of *Synechocystis* PCC6803. In this mutant – the ApcE-C190S-PBs – one of the most red-shifted pigments in PBs is missing (394 pigments *vs* 396 in WT-PB), but the structure of the complex remains unchanged. Our characterization revealed that the overall light-induced dynamics of the ApcE-C190S-PB complexes are similar to WT-PBs. However, importantly, we also showed that the pigment of ApcE is a vital player in energy dissipation in PBs. Lack of this pigment makes PBs less likely to enter energy dissipative states in response to illumination. Detailed studies of the involvement of PBs subunits in energy dissipation will help identify structural arrangements responsible for the dynamic response of pigments in PBs to the changes in quality and quantity of light.

Poster 27

Understanding the function of CupA in the CO2 Concentrating Mechanism of Synechococcus sp.PCC7942

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The CO_2 concentrating mechanism (CCM) in cyanobacteria is one of the most unique and efficient systems used to optimize photosynthetic output on the planet. An important part of the CCM is the use of CO_2 uptake proteins (CupA and CupB) in cooperation with the NDH-1 complexes embedded in the thylakoid membrane. Together, they convert CO_2 to bicarbonate (HCO_3), which creates high concentrations of HCO_3^- in the cytoplasm. When inorganic carbon in the environment is limited, CupA is induced, alongside its constitutively expressed paralog, (CupB). CupA and CupB possess remarkably similar functionality, differing slightly in their expression and kinetic ability. While CupB is constitutively expressed in the cell, it is shown to have substantially lower affinity for CO_2. CupA's increased affinity for CO_2, compared to CupB, is particularly useful when CO_2 is limited in the environment. However, these two Cup proteins work together is largely unknown. Previous work suggests that CupA may only work with CupB present, while the reverse is not true as CupB works efficiently with or without CupA. This research is to test this hypothesis, and to better determine the relationship between these proteins. This was done by genetically modifying strains of *Synechococcus sp.* PCC7942 for comparison by physiological assays.

Poster 28

Photoprotective dynamics in the microalga *Nannochloropsis oceanica* under periodic and sporadic light

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8 Howard Hughes Medical Institute, University of California, Berkeley, CA

In aquatic environments, water can act as a lens, altering the amount and type of wavelengths at varying depths. Additionally, currents can cause algae to rise from low light conditions to high light (HL) conditions rapidly. Photosynthetic organisms have adapted to these altering light levels by creating photoprotective pathways when exposed to saturating light levels which outpace the photosynthetic rate, leading to the formation of damaging reactive oxygen species. Non-photochemical quenching (NPQ) pathways dissipate excess energy as heat. Organisms that are able to rapidly respond to changes in light levels have higher efficiency in carbon fixation. Previous work in this lab (Park et al. PNAS, 2019) has shown that *Nannochloropsis oceanica*, a microalga, known for its small size (~2-3 μm) and ability to accumulate large amounts of lipids, responds quickly to changes in light levels. *N. oceanica* is thought to exhibit only two components of NPQ: energy-dependent (qE) and zeaxanthin (Zea)-dependent quenching (qZ). Lhcx1 is the main pH-sensing protein associated with qE in this species. Lhcx1 is thought to bind Zea as well as being the quenching location. By using time-correlated single photon counting (TCSPC), along with both periodic actinic light sequences (1 min HL-1 min dark, etc.) and variable light sequences, we were able to determine the kinetics of *N. oceanica* NPQ. Based on this data, we propose a model in which protonated Zea-Lhcx1 complex is needed for a rapid and strong response to high light. In dark adapted alga with no Zea, the initial exposure to light induces a gradual increase in the protonated Zea-Lhcx1 complex as Zea is accumulated. Once the organism is 'pre-primed' with Zea, the response to subsequent HL tracks with the light sequence. This theory was tested in HL grown plants and was consistent with the proposed mechanism. In the dark, Zea-Lhcx1 complex is de-protonated, which may induce some conformational change resulting in nearly complete relaxation. A quantitative model was created based on the interactions of the fast (qE) and slow (qZ) processes seen in *N. oceanica*.

Poster 29

Comparative FTIR Spectroscopy of *T. elongatus* PSI isolated in Detergent Micelles and SMALP Nanoparticles.

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Microsecond time-resolved step scan (μs-TRSS) FTIR difference spectroscopy at 77 K has been used to study photosystem I (PSI) particles from *Thermosynechococcus elongatus (T. elongatus)*. PSI particles were prepared in two different ways, one is using the detergent dodecyl maltoside (β-DDM), the other using a styrene maleic copolymer (SMA-1440). To aid in interpretation and assignment of the bands in the spectra, comparisons were made with μs-TRSS FTIR DS obtained using PSI particles from *Synechocystis sp.* PCC 6803 WT (*S6803*) and from PCC 6803 *menB*$^-$ PSI mutant with PhQ incorporated into the A_1 binding site.

Photo-accumulated ($P700^+$–P700) FTIR DS obtained at 298 and 77 K for the two differently prepared *T. elongatus* PSI samples are essentially identical, indicating that the polymer treatment has no impact on P700, even at the molecular level. Similarly, time-resolved ($P700^+A_1^-$–$P700A_1$) and (A_1^-–A_1) FTIR DS (at 77 K) are also very similar in the differently prepared PSI samples, indicating that the polymer treatment also has no impact on A_1.

FTIR absorption spectra at 298 K were obtained for PSI prepared with copolymer and with β-DDM (Figure 1). These spectra exhibit bands that are due to the polymer and the protein, and allow an assessment of the polymer and protein concentration ratios in the samples. Bands in the spectra and how they relate to the polymer and the protein will be discussed.

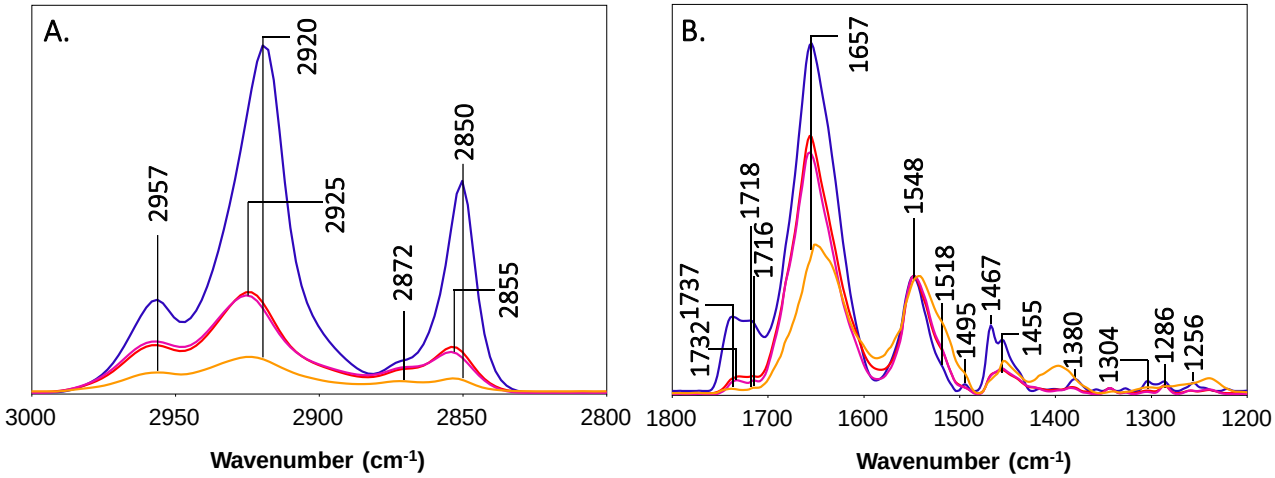

Figure 1. FTIR absorption spectra at 298 K for PSI from *S*6803 (*pink*), β-DDM prepared (*red*) and SMA copolymer prepared *T. elongatus* PSI (*blue*). Spectra of the SMA copolymer after drying is also shown (*orange*).

Acknowledgements: This work was supported in part by a U.S. Department of Energy through Grant (DE-SC-0017937) to GH, and support from the Gibson Family Foundation to BDB.

Poster 30

Circular Dichroism and First Principles Simulations of Triplet Dynamics in FMO

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High fidelity time resolved spectroscopy is a powerful tool for inspecting the excitonic states that arise as a result of interactions between strongly coupled pigments. Excitonic states due to the 8 interacting pigments in FMO play a central role in the complex energy transfer dynamics within the Fenna-Matthews-Olson (FMO) complex. Many Hamiltonians have been proposed to empirically describe the electronic interactions between these 8 pigments, but none have satisfactorily reproduced experimentally observed spectra. Recent efforts have focused on computing a Hamiltonian from first principles. We report here preliminary results comparing first-principles modeling efforts and spectra from wild-type (WT) FMO. Furthermore, we compare the WT spectra and dynamics to those of FMO with mutations near pigment 3 (Y16F) and pigment 4 (Y345F). It is well understood that pigment 3 is the lowest energy of the 8 pigments and therefore is thought to be the quencher for triplet states in FMO. However, we report here that, contrary to expectations, the pigment 3 mutant does not show dramatically slower triplet state decay compared to WT.

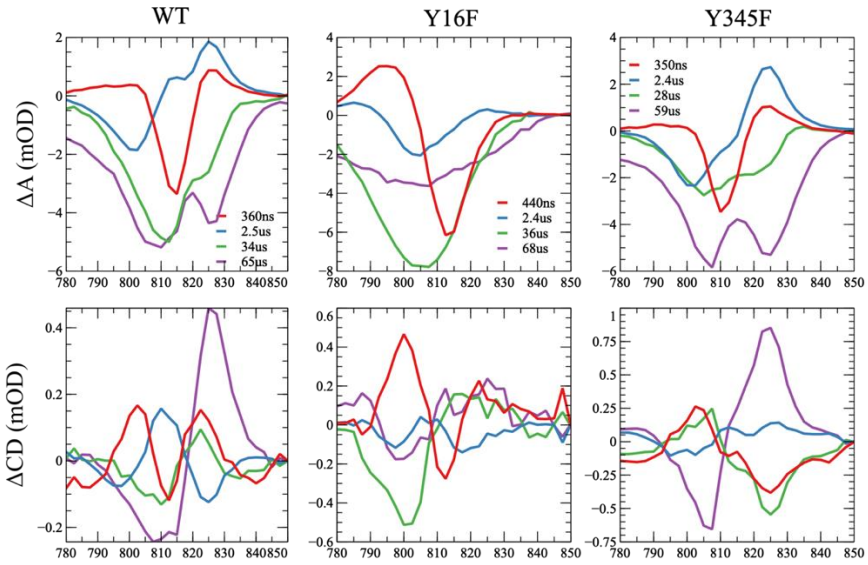

Fig 1: Absorption and circular dichroism (CD) decay associated spectra for WT FMO along with two mutants: Y16F (pigment 3 mutant) and Y345F (pigment 4 mutant).

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Poster 31

The Effects of Quinone Concentration on Potentiostatic Measurements of *Rhodobacter sphaeroides* Reaction Centers Immobilized onto Graphene Oxide Electrodes.

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Rhodobacter sphaeroides reaction centers (RCs) have been previously coupled to electrodes to generate light-dependent current. Several approaches have been reported in the literature, including Langmuir Blodgett (LB) films that orient RCs on reduced graphene oxide promising higher current outputs. However, reported values are only 200-400 nA cm^{-2}, which is at least two orders of magnitude lower compared to similar values observed using membranes (chromatophores) or purified RCs on silver electrodes. Previously, we examined graphene oxide as a substrate amenable to crosslinking as well as non-covalent interactions of his-tagged purified reaction centers. Here we report data showing that covalently crosslinked RCs attached to graphene oxide electrodes outperform his-tag oriented ones utilizing nickel-immobilized on similar graphene oxide electrodes. Interestingly, the current observed is oxygen sensitive and is directly proportional to the concentration of quinone present, reaching 2.1 μA cm^{-2} at a concentration similar to previously published (300μM vs 250μM). These initial data will be further evaluated with various concentrations of the donor electrolyte, cytochrome c to infer the orientation of the cross-linked RCs as judged by direct vs diffusion limited electron transport into the graphene electrodes.

Poster 32

REVEALING THE DIMERIC NATURE OF THE PRIMARY ACCEPTOR IN PHOTOSYSTEM I

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The Type I photosynthetic reaction center (RC), Photosystem I (PS I), is an exquisitely tuned pigment-protein complex that is comprised of multiple polypeptide subunits and protein-bound electron-transfer cofactors. Recent work in PS I has suggested that the six core chlorophyll (Chl) a molecules are highly coupled, allowing for efficient creation and stabilization of the charge-separated state. Involved in this coupled complex is the primary acceptor, A_0. While the properties of the intermediate and terminal acceptors are fairly well characterized, the role of A_0 and the factors that contribute to its ultrafast processes and redox properties remain unclear. We employed a combination of 2D-HYSCORE spectroscopy and DFT calculations to explore the nature the $A_0^{\cdot-}$ state. Analysis of the hyperfine coupling constants revealed that A_0, once thought to be a Chl *a* monomer, instead functions as a dimer of Chl_2/Chl_3 with an asymmetric distribution of electron density favoring Chl_3.[1] Interestingly, this dimerization occurs independently of the axial ligand, as seen when the Met that ligates Chl_3 is changed to a His, with the only noticeable change being a shift in the asymmetry.[2] This dimerization of the primary acceptor likely serves to ensure charge separation is energetically downhill, and that subsequent recombination is slowed. Structural comparisons of A_0 in PS I with the primary acceptor of other Type I and Type II RCs reveals that dimerization of A_0 is predicted to occur in other Type I RCs, and unlikely to occur in Type II RCs. This change in the mechanism of primary charge separation may shed light on the evolutionary divergence of Type I and Type II RCs.

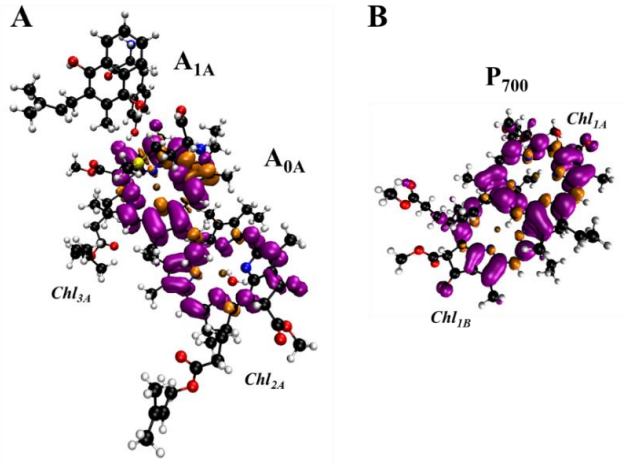

Figure 1: Computational model of: (**A**) The electron spin density distribution in the singly occupied molecular orbital (SOMO) of the $A_0^{\cdot-}$ state. The model includes the cofactors: A_{1A}, Chl_{3A}, and Chl_{2A} as well as the A-Met688 and A-Tyr696 residues that are axially ligated and hydrogen bonded to the Chl_{3A} cofactor, respectively. (**B**) The $P_{700}^{\cdot+}$ state, containing the Chl_{1A} and Chl_{1B} cofactors and their axial ligands.

1. M. Gorka, P. Charles, V. Kalendra, K. V. Lakshmi and J. H. Golbeck "A Dimeric Chlorophyll Electron Acceptor Differentiates Type I from Type II Photosynthetic Reaction Centers" (2021) *iScience* (Cell Press), 24, 102719.

2. M. Gorka, E. Gruszecki, P. Charles, V. Kalendra, K. V. Lakshmi and J. H. Golbeck "Two-dimensional HYSCORE Spectroscopy Reveals Histidine as the Axial Ligand to Chl_{3A} in the $M688H_{PsaA}$ Genetic Variant of Photosystem I" (2021) *Biochim. Biophys. Acta*- Bioenergetics, 1862, 148424.

Poster 33

Monomeric and Trimeric PSI Particles Studied Using Time-Resolved Step Scan Fourier Transform Infrared Difference Spectroscopy

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Time-resolved step-scan Fourier transform infrared (FTIR) difference spectroscopy (DS) has been used to study trimeric and monomeric photosystem I (PSI) particles from *Synechocystis* sp. PCC 6803. Time resolved $P700^+A_1^-/P700A_1$ and photoaccumulated $P700^+/P700$ FTIR difference spectra were produced for both trimeric and monomeric PSI. Native PSI has a phylloquinone (PhQ) (2-methyl-3-phytyl-1,4-naphthoquinone) molecule in the A_1 binding site. Trimeric $menB^-$ PSI was also studied. $MenB^-$ PSI has a plastoquinone molecule (PQ) (2,3-dimethyl-1,4-benzoquinone) in the A_1 binding site.

By subtracting $P700^+A_1^-/P700A_1$ DS from $P700^+/P700$ DS, an A_1^-/A_1 FTIR DS was constructed. For PSI with PhQ incorporated the A_1^-/A_1 FTIR DS display bands at 1668 and 1627 cm^{-1} that are likely due to C_1=O and C_4=O stretching vibrations of neutral PhQ, respectively. For $menB^-$ PSI corresponding bands are found at 1666 cm^{-1} and 1635 cm^{-1}.

Bands are also observed at 1495 and 1417 cm^{-1} for monomeric or trimeric PSI with PhQ incorporated. These bands are due to C_1=O and C_4=O modes of PhQ^-, respectively. For menB $menB^-$ PSI with PQ in the A_1 binding site, corresponding semiquinone bands are observed at 1489 and 1415 cm^{-1}.

Poster 34

Photoswitching of terahertz structural dynamics in the photosynthesis photo protector orange carotenoid protein

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Computational studies have shown picosecond protein structural dynamics enable efficient conformational transitions during biological function. These structural dynamics necessarily change with each conformational change, providing overall steering throughout the functional cycle. Here we examine the switching of these long range collective structural vibrations of the photoprotective protein orange carotenoid protein (OCP) using anisotropic terahertz microspectroscopy, which provides dynamical fingerprinting for biomacromolecules[1, 2]. OCP controls efficiency of the phycobilisome (PBS), the light harvesting antenna in cyanobacteria, to prevent oxidative damage. The 35 kDa structure of OCP consists of two globular domains, connected by an unstructured loop, that form a hydrophobic pocket for the carotenoid. In low light, carotenoid bound OCP appears orange (OCP^o). Absorption of blue-green light results in a 12Å translocation of the carotenoid into the N-terminal domain and conversion to an active red state (OCP^R) which interacts with the light harvesting antenna, the phycobilisome (PBS), to induce fluorescent quenching. We examine the changes in the collective vibrations with photoexcitation under two conditions. Under excitation similar to the solution phase switching we detect reversible photoswitching of the THz vibrational bands through double difference analysis. However, as there has been no report of photoswitching of the optical spectrum for OCP crystals, we determined conditions for photoswitching in the crystal for 450 nm narrowband excitation to confirm that the changes observed in the collective vibrations arise from the transition to the photoprotective state. Under these conditions, we again find that the photoswitching is accompanied by intramolecular vibrational changes. Comparison with normal mode ensemble analysis calculations of the anisotropic absorption for chromophore bound and chromophore free OCP indicates that the observed changes coincide with the breaking of the H-bonds between residues tyrosine 201 and tryptophan 288 and the keto group of the carotenoid in the C terminal domain. The protein's apparent reorganization of the internal dynamics with the initial photoexcitation may then provide the dynamical bias towards the subsequent reorganization of the N-terminal domain and the final opening between the N-terminal domain and the C- terminal domain necessary for the interaction with the phycobilisome.

1. Deng, Y.T., et al., *Near-Field Stationary Sample Terahertz Spectroscopic Polarimetry for Biomolecular Structural Dynamics Determination*, Acs Photonics, 2021. 10.1021/acsphotonics.0c01876.
2. Niessen, K.A., et al., *Protein and RNA dynamical fingerprinting*, Nat. Comm., 2019. https://dx.doi.org/10.1038/s41467-019-08926-3.