Oral Presentations

Oral Presentation 1

Kinetics and targeting of Vipp1 aggregation in cyanobacteria

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Vesicle-inducing protein in plasmids 1 (Vipp1) is an essential protein for photoautotrophic growth across the tree of life. Vipp1 forms aggregates which the cell uses to remodel thylakoid membranes, repair damage, and relieve membrane stress. Herein we spatiotemporally resolve exchange between inactive cytoplasmic Vipp1 and its active functionalities and address the mechanism by which the cell recruits and allocates Vipp1 to sites of damage. We demonstrate that Vipp1 can be aggregated to a specific site in the thylakoid membrane by (blue) laser damage, but cytoplasmic Vipp1 is rendered unresponsive to nearby damage on a time scale three orders of magnitude faster than the aggregation process itself. Recruitment is initiated by physical damage to the thylakoid membrane rather than disruption of photosynthetic electron transport. Vipp1 aggregation requires the existence of a proton gradient across the thylakoid membrane, favorable osmotic pressure for proton release, and adequate mechanical stress on the thylakoids to force protons out through sites of damage. Vipp1 aggregates acting near the thylakoid organizing center may act on the cell membrane rather than the thylakoids. We posit that scale of Vipp1 aggregation to a particular site on the thylakoid membrane is directly proportional to local availability of protons and that rate of aggregation is diffusion-based.

Oral Presentation 2

Thermodynamics plays an important role in enhancing terpene productivity in cyanobacteria

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The terpene family is very diverse with more than 50,000 unique chemical structures. Terpenes are condensed from two C5 precursor isomers, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Limonene, a C10 monoterpene, has been tested to use as a drop-in fuel additive for combustion engines and as the kerosene substitute for aviation fuels. Photosynthesis-driven CO_2 conversion to terpenes is particularly attractive for biofuel applications due to its low carbon footprint but is challenged with far lower productivities compared to heterotrophic hosts. We hypothesize that the low terpene yield in phototrophs is due to the low driving force determined by thermodynamics as it readily does not favor efficient photosynthetic CO_2 conversion. To bypass this limitation, we used the sigma factor engineering approach. An essential sigma factor, RpoD, encoding was overexpressed in photosynthetic cyanobacteria and showed a 1.7-fold improvement in limonene productivity. Further investigations on the engineered strains revealed a higher photosynthetic rate stimulating enhanced carbon fixation. Computational modeling and wet lab analyses showed an increased flux toward both native carbon sink glycogen synthesis and the non-native limonene synthesis from photosynthate output. Interestingly, our proteomics result showed decreased abundance of terpene biosynthesis enzymes indicating their limited role in determining terpene flux. Taken together, increased abundance of RpoD illustrates interesting metabolic rewiring that supports robust direct CO_2 to limonene conversion. This study reveals thermodynamics as the key determinant to boost low flux metabolites like terpene and demonstrates the merits of sigma factor engineering for efficient chemical productions.

Oral Presentation 3

Multi-layer Regulation of Rubisco in Response to Altered Carbon Status in *Synechococcus elongatus* PCC 7942

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Abstract

Photosynthetic organisms possess various balancing mechanisms between absorbed light (source) and the capacity to metabolically utilize or dissipate this energy (sink). Unlike plant, source-sink balance is poorly studied in photosynthetic microbes. Herein, we explore systemic changes that result from alterations in carbon availability in the model cyanobacterium *Synechococcus elongatus* PCC 7942 by taking advantage of an engineered strain where influx/efflux of a central carbon metabolite, sucrose, can be regulated experimentally. We observe that induction of a high-flux sucrose export pathway leads to depletion of internal carbon storage pools (glycogen), and concurrent increases in photosynthetic parameters. Further, a proteome-wide analysis and fluorescence reporter-based analysis revealed that upregulated factors following the activation of the metabolic sink (sucrose) are strongly concentrated on ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) and axillary modules involved in Rubisco maturation. Carboxysome number and Rubisco activity also increase following engagement of sucrose secretion. Conversely, by feeding exogenous sucrose (influx) results in increased glycogen pools, decreased Rubisco abundance, decreased photosystem II quantum efficiency, and carboxysome reorganization. By analysis of system level proteomic responses and physiological responses, we show that Rubisco activity and organization is a key target related to metabolic balancing in cyanobacteria. Our findings have implications for microbial source-sink regulatory process and fine tuning of cellular metabolism toward improved production of bio-based materials.

Oral Presentation 4

Engineering natural competence into the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973

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Cyanobacteria are photosynthetic prokaryotes that are widely studied as model systems for understanding photosynthesis, circadian oscillation, and carbon neutral product synthesis. One of the important characteristics of an efficient model bacterial system is its amenability to genetic manipulation and natural transformability makes the system more appealing. Natural transformation is the process by which a bacterial system actively takes up extracellular DNA and integrates it. Though a few cyanobacterial species are known to be naturally competent, natural transformation in cyanobacteria has not been characterized to the extent that it has been in other gram-negative bacteria. *Synechococcus elongatus* UTEX 2973 is the fastest growing cyanobacterium known, with an optimal doubling time of 1.5 hours, and is a strong candidate for serving as a model organism. Although the genes involved in natural transformation are present in *Synechococcus* 2973, the strain lacks natural transformability. Genetic analysis has shown that *Synechococcus* 2973 is 99.8% genetically identical to a relatively slower growing, naturally competent, model strain *Synechococcus elongatus* PCC 7942. The comparison of these two strains provides a unique opportunity to better understand the nuances of natural transformation in cyanobacteria. By substituting polymorphic alleles from *Synechococcus* 7942 for native *Synechococcus* 2973 alleles, two loci were identified as the key players for natural competency, transformation pilus component *pilN* and circadian transcriptional master regulator *rpaA*. Further through targeted genome editing and enrichment outgrowth, a strain was generated that exhibited both natural transformability and fast-growth. This new *Synechococcus* 2973-T strain will serve as a valuable resource to the cyanobacterial research community.
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Oral Presentation 5

High-resolution cryo-EM structure of photosystem II from the mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803

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Photosystem II (PSII) enables global-scale light-driven water oxidation. Genetic manipulation of PSII from the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 has provided insights into the mechanism of water oxidation; however, the lack of a high-resolution structure of oxygen-evolving PSII from this organism has limited the interpretation of biophysical data to models based on structures of thermophilic cyanobacterial PSII. Here, we report the cryo-EM structure of PSII from *Synechocystis* sp. PCC 6803 at 1.93-Å resolution (**Fig. 1**). A number of differences are observed relative to thermophilic PSII structures, including: the extrinsic subunit CyanoQ is maintained, the C-terminus of the D1 subunit is flexible, some waters near the active site are partially occupied, and differences in the PsbV subunit block the Large water channel. These features strongly influence the structural picture of PSII, especially as it pertains to the mechanism of water oxidation.

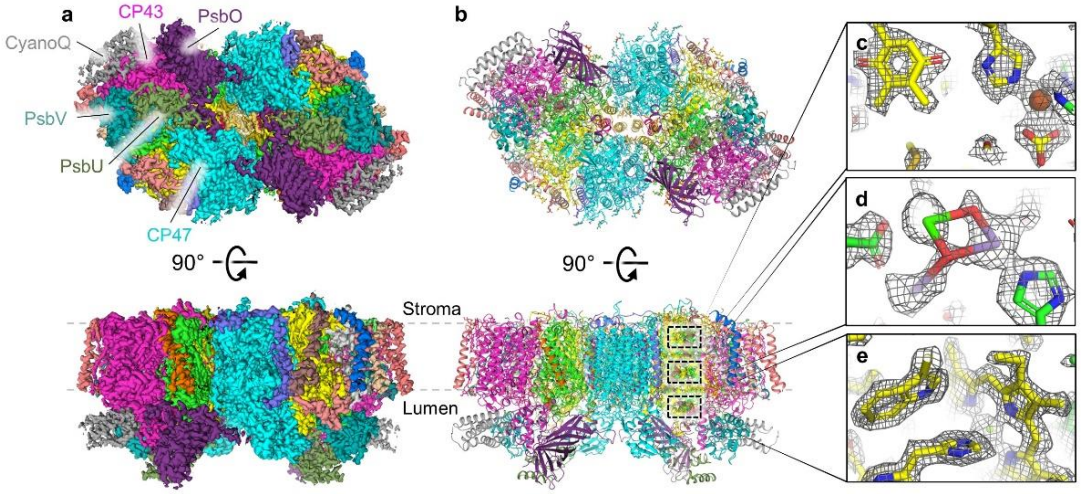

Fig. 1. Cryo-EM structure of PSII from *Synechocystis* 6803 at 1.93-Å resolution. a The unsharpened map (4σ) where ESP map regions assigned to subunits are colored individually. Top shows a lumenal view where the lumenal domains of CP47 and CP43, and the extrinsic subunits of one monomer are labeled. The bottom shows a membrane plane view. **b** The structural model derived from the cryo-EM map in the same views as *A*. In the membrane plane view, three regions are boxed which correspond to panels *C-E* from top to bottom. **c** Map and model of Q_A and the non-heme Fe coordinated by D2-His214 and bicarbonate (13σ). **d** Map and model of the OEC and nearby D1-Asp170 and D1-His332 residues (12σ). **e** Map and model of the axial ligation of P_B in P_{680} by D2-His197 and the nearby D2-Trp191 (13σ). In *C-E*, the sharpened map is shown.

Oral Presentation 6

Functional role of D1-E189 ligand during the light driven assembly of nature's water oxidizing complex

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The assembly of the Mn_4O_5Ca cluster of the photosystem II (PSII) starts from the initial binding and photooxidation of the first Mn^{2+} at a high affinity site (HAS). Recent cryo-EM apo-PSII structures reveal an altered geometry of amino ligands in this region and suggest the involvement of D1-Glu189 ligand in the formation of the HAS (1). We now find that Gln and Lys substitution mutants photoactivate with reduced quantum efficiency compared to the wild-type. However, the affinity of Mn^{2+} at the HAS in D1-E189K was indistinguishable from the wild-type (~1 μM), whereas the previously examine D1-D170A mutant showed the predicted lower affinity (~30 μM) in the same fluorescence assays. Thus, we conclude that D1-E189 does not form the HAS and that the reduced quantum efficiency of photoactivation in D1-E189K cannot be ascribed to the initial photooxidation of Mn^{2+} at the HAS. Besides reduced quantum efficiency, the D1-E189K mutant exhibits a large fraction of centers that fail to recover activity during photoactivation starting early in the assembly phase, becoming recalcitrant to further assembly. Fluorescence relaxation kinetics indicate on the presence of an alternative route for the charge recombination in Mn-depleted samples in all studied mutants and exclude damage to the photochemical reaction center as the cause for the recalcitrant centers failing to assemble and show that dark incubation of cells reverses some of the inactivation. This reversibility would explain the ability of these mutants to accumulate a significant fraction of active PSII during extended periods of cell growth. The failed recovery in the fraction of inactive centers appears to a reversible mis-assembly involving the accumulation of photooxidized, but non-catalytic high valence Mn at the donor side of photosystem II, and that a reductive mechanism exists for restoration of assembly capacity at sites incurring mis-assembly. Given the established role of Ca^{2+} in preventing misassembled Mn, we conclude that D1-E189K mutant fails to ligate Ca^{2+} at the correct binding site in all PSII centers that consequently leads to the mis-assembly resulting in accumulation of non-catalytic Mn at the donor side of PSII. Our data indicate that D1-E189 is not functionally involved in Mn^{2+} oxidation\binding at the HAS but rather involved in steps following the initial Mn^{2+} photooxidation e.g. dark rearrangement or/and Ca^{2+} binding.

1. C. J. Gisriel *et al.*, Cryo-EM structure of monomeric photosystem II from *Synechocystis sp* PCC 6803 lacking the water-oxidation complex. *Joule* **4**, 2131-2148 (2020).

Oral Presentation 7

A Simple Electrostatic Model for Accurate Prediction of Mutation-Induced Frequency Shifts in Water-Soluble Chlorophyll Proteins

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Photosynthetic pigment-protein complexes (PPCs) are an essential component of the light-harvesting machinery of photosynthetic organisms, enabling the transduction of absorbed light energy into chemical energy. The local chlorophyll (Chl) transition frequencies in these PPCs are controlled by a variety of electrostatic and steric factors. To reveal the important details about native photosynthetic activity and thus to rationally design Chl proteins with tailored optical properties, site-directed mutagenesis is an efficient approach. Unfortunately, no reliable methods exist at this time that can quantitatively predict the mutation-induced frequency shifts in advance, limiting the applicability of this approach. We tackle this problem by creating a series of point mutants (glutamine at position 57) in water-soluble chlorophyll protein (WSCP) of *Lepidium virginicum* and using them to test a simple computational procedure for estimating mutation-induced site energy changes. To create mutant protein structures, the method employs molecular dynamics (MD) and a Charge Density Coupling (CDC) model for site energy prediction. The procedure is implemented in PigmentHunter, an automated, online graphical interface hosted at nanoHUB.org. We find that the estimated frequency shifts match the experiment rather well, with an average error of 1.6 nm throughout a 9 nm wavelength range, with the exception of a single outlier owing to unanticipated structural changes. This work is a significant step toward the quantitative prediction of mutation-induced frequency shifts in Chl proteins and the rational customization of PPC optical characteristics. With more refined sampling of mutant protein structures, we believe the accuracy of the method can be enhanced in the future.

Oral Presentation 8

Interplay between LHCSR proteins and state transitions govern the NPQ response in intact cells of *Chlamydomonas* during light fluctuations.

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Photosynthetic organisms use light energy as the primary energy source to fix CO_2. However, in the environment, light energy fluctuates rapidly and often exceeds saturating levels for periods ranging from seconds to hours, which can lead to detrimental effects for cells. Safe dissipation of excess light energy occurs primarily by non-photochemical quenching (NPQ) processes. In *Chlamydomonas reinhardtii*, photoprotective NPQ is mostly mediated by pH-sensing light-harvesting complex stress related (LHCSR) proteins and redistribution of light-harvesting complexes between the photosystems (state transitions). Although each mechanism underlying NPQ has been documented, their relative contribution to the dynamic functioning of NPQ under fluctuating light remains unknown. Here, by monitoring NPQ throughout multiple high light-dark cycles with fluctuation periods ranging from 1 to 10 minutes, we show that the dynamics of NPQ depend on the frequency of light fluctuations. Mutants impaired in the accumulation of LHCSRs (*npq4*, *lhcsr1*, and *npq4 lhcsr1*) showed significantly less quenching during light phases, demonstrating that LHCSR proteins are responsible for the majority of the NPQ during light fluctuations. Surprisingly, activation of NPQ was also observed in the darkness and this was exacerbated in mutants lacking LHCSRs. By analyzing 77K chlorophyll fluorescence spectra and chlorophyll fluorescence lifetimes and yields in a mutant impaired in state transitions, we show that this phenomenon arises from state transitions. Finally, we quantified the contributions of LHCSRs and state transitions to the overall NPQ compared those with cell growth under various frequencies of fluctuating light. These results highlight the dynamic functioning of photoprotection under fluctuating light and open a new way to systematically characterize the photosynthetic response to an ever-changing light environment.