Engineering S. elongatus PCC 7942 chassis for interrogating plant-like photorespiration

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Photorespiration occurs when Rubisco fixes an O_2 molecule instead of a CO_2, producing the toxic intermediate 2-phosphoglycolate (2-PG), which must be recycled into 3-phosphoglycerate (3-PGA) for reintegration into the Calvin-Benson-Bassham (CBB) cycle. In most plants, the oxygenation reaction occurs once for every four to five carboxylation reactions, making it the second-highest flux pathway on Earth, right after the CBB cycle itself. Photorespiration is thought to have originated in ancient cyanobacteria and was subsequently passed onto plants and algae via endosymbiosis. While certain enzymes in the pathway are highly conserved throughout the photosynthetic lineage, others have been adapted to meet the specific needs of different organisms. Nonetheless, the essential function of photorespiration remains consistent: recycling the inhibitory 2-PG into a useful carbon skeleton. In cyanobacteria, photorespiration seems to be less impactful than in plants, primarily due to the efficiency of the carboxysome carbon concentrating mechanism (CCM), which elevates CO_2 levels around rubisco and minimizes oxygenation reactions. Therefore, the role of photorespiration in cyanobacteria is relatively understudied. However, with recent advancements in understanding carboxysome dynamics and permeability, there is growing interest in elucidating the contributions of photorespiration to cyanobacterial metabolism and photosynthesis. This study seeks to characterize cyanobacterial photorespiration and its impact on photosynthetic performance in parallel with the construction of plant-like photorespiratory strains, which will serve as platforms for protein engineering by providing a fast-growing, robust system to test enzymes identified in our engineering pipeline. The pipeline entails using molecular dynamics to identify enzymatic domains that can be stitched into plant photorespiratory enzymes to improve performance under a range of stress conditions. To achieve this, peripheral pathways in cyanobacterial photorespiration will be modified to mimic a more plant-like pathway, allowing for the optimization of engineered enzymes. The performance of strains and photorespiration generally will be assessed using several core techniques such as membrane inlet mass spectrometry (MIMS), chlorophyll fluorescence, and gas exchange.