Use of synthetic biology to probe molecular machines in photosynthesis

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Sunlight is the ultimate source of energy for most of the biosphere. The primary producers on our planet are photosynthetic organisms that harvest solar energy and use CO_2 as a feedstock to generate many valuable products that are essential for the survival of all nonphotosynthetic (heterotrophic) organisms, including humans. Evolution has provided a profound diversity in overall architectures and molecular designs among photosynthetic microbes and fostered adaptations to many different habitats and life styles.

Cyanobacteria are able to carry out "plant-like" photosynthesis and are the only photosynthetic bacteria that possess phycobilisomes for highly efficient light capture, as well as carboxysomes, specialized microcompartments for CO_2 fixation. The thylakoid membrane accommodates photosystem II and photosystem I, which are organized in series and connected by a third multi-protein complex, the cytochrome b_6/f complex, to transfer electrons from H_2O to $NADP^+$, with concomitant evolution of O_2 and translocation of H^+ across the membrane to ultimately generate ATP (Figure 1). The key role of cyanobacteria in the formation of the present day oxygen-rich environment has been recognized long ago. In addition, recently cyanobacteria have attracted significant attention as potential biocatalysts for the production of clean energy and green chemicals from sunlight and atmospheric CO_2.

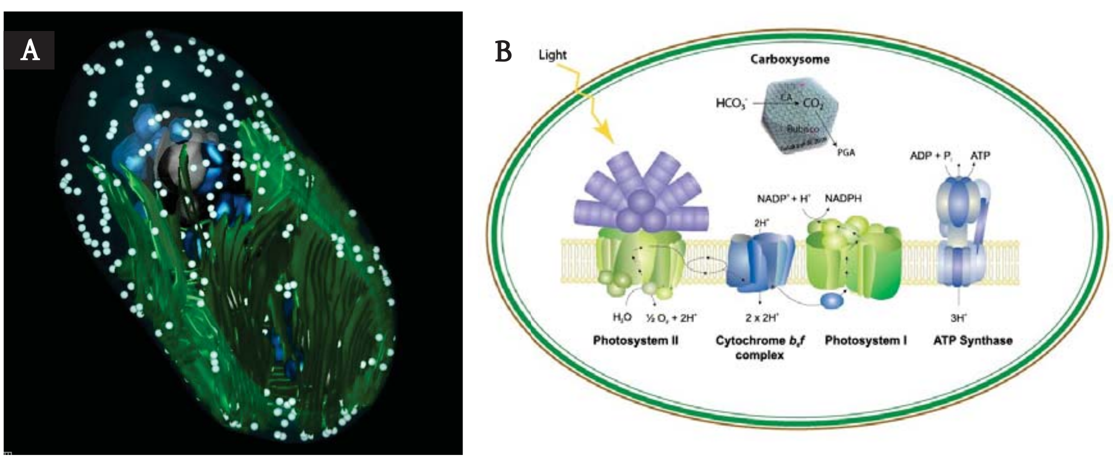

Figure 1. (A) Tomographic model of a *Cyanothece* sp. ATCC 51142 cell. An extensive system of thylakoid membranes (green) harbors the photosynthetic apparatus. Cyanobacteria contain numerous intracellular bodies, including carboxysomes (blue), polyphosphate bodies (gray), and lipid bodies (white) [adapted from (2)]. (B) Organization of the molecular machines of photosynthesis in cyanobacterial cells. In the thylakoid membranes, light energy harvested by phycobilisome antenna complexes (purple) powers photochemistry performed by the photosynthetic complexes. Carboxysomes are carbon-concentrating microcompartments.

Cyanobacteria provide distinct advantages over other prospective candidates for bioenergy production because they grow at a much faster rate, are readily amenable to genetic modifications and possess high photosynthetic efficiencies. The structural designs that enable these organisms to harvest energy, reducing power and carbon have been elucidated in Angstrom resolution (3-7) and thus set the stage to guide further man-made improvements using synthetic biology approaches. Different methods, such as metabolic engineering, have long been feasible and have resulted in a large collection of cyanobacterial mutants with modified light capture capabilities, photosynthetic efficiencies and CO_2 fixation rates. Decoding the cellular language that controls these specific metabolic operations will provide the framework to elucidate the design principles that drive the production of renewable energy sources in a more efficient way.

OBJECTIVE
Characterize and optimize the cellular machines and molecular components that drive biofuel production in the well-established cyanobacterial chassis strain *Synechocystis* 6803.

We have shown in our previous EMSL collaborations our ability to develop systems-level models of cyanobacterial processes. In the present proposal, our objective is to characterize and improve the molecular machines in photosynthesis that govern bioenergy production in cyanobacteria. This proposal results from an EMSL workshop (MBGC 2.0) held in spring 2011. We propose to apply sets of experiments and analysis to achieve this goal using sophisticated imaging instrumentation available at EMSL. The profound expertise of the External Project Team in the areas of cyanobacterial and systems biology will be leveraged to attain the goals under this objective. The PIs Pakrasi, Sherman and Aurora have had extensive experience in collaborating with key investigators at EMSL/PNNL over the past 5 years, resulting in numerous publications (8-15, as examples). The experiments outlined in this proposal will strive to investigate the products of a synthetic biology approach targeted in the following important areas of cyanobacterial biology; the goal of each is designed to provide new approaches to increasing photosynthetic productivity.