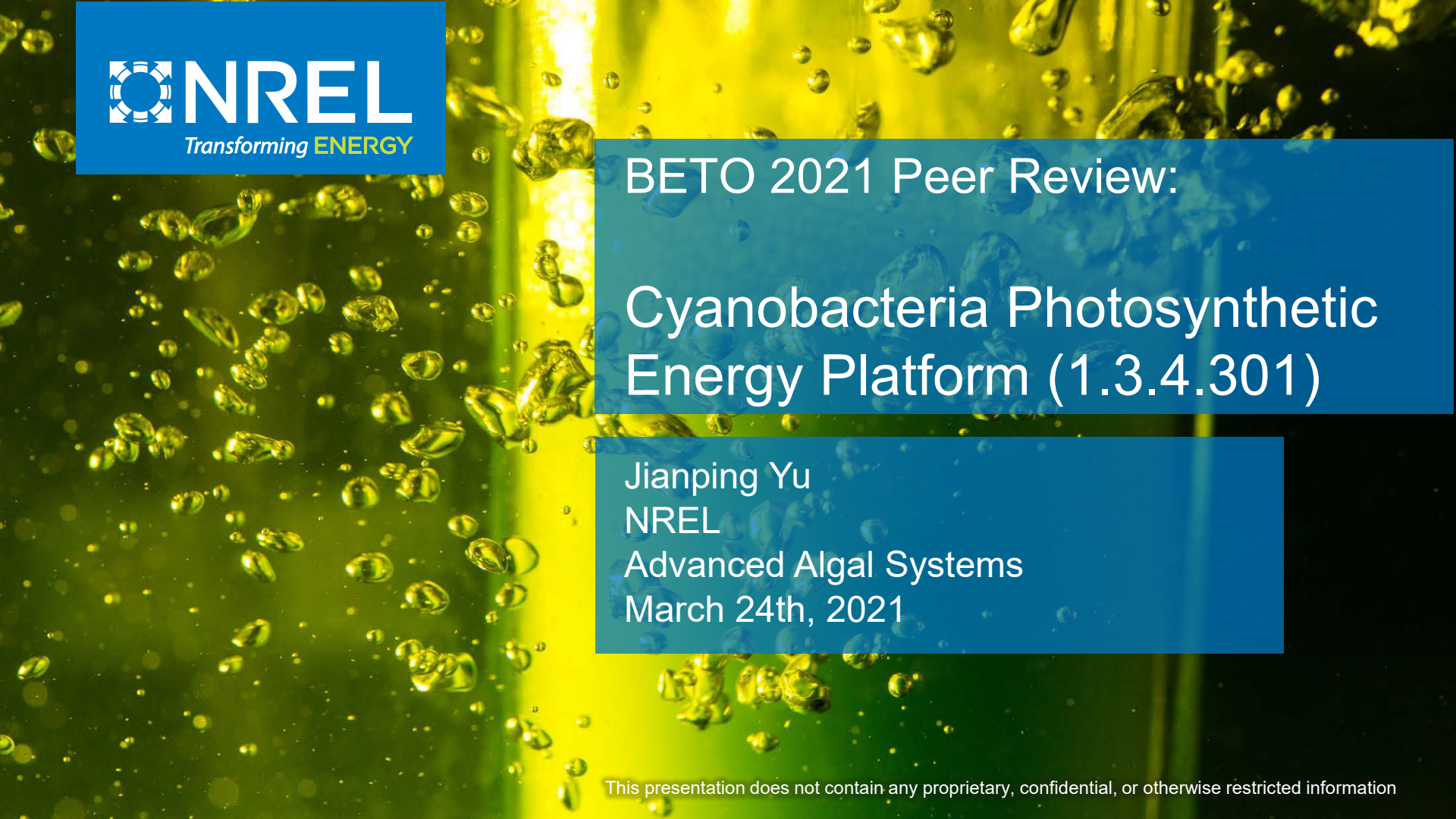


NREL
Transforming ENERGY

# BETO 2021 Peer Review:

# Cyanobacteria Photosynthetic Energy Platform (1.3.4.301)

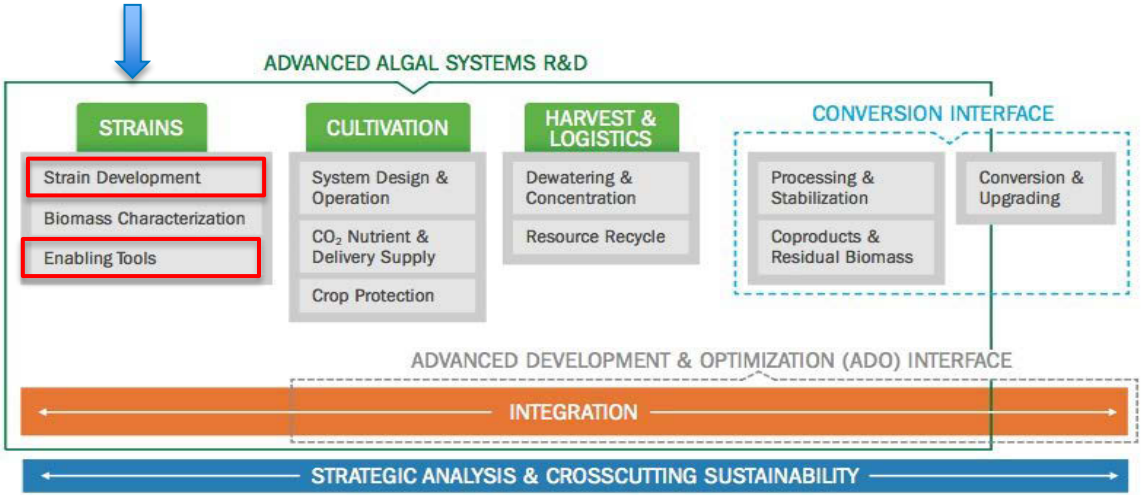
Jianping Yu
NREL
Advanced Algal Systems
March 24th, 2021

# Project Overview

**Goals**: Develop cyanobacteria genetic tools to improve photosynthetic efficiency and biomass productivity through manipulation of energy regulation mechanisms, leading ultimately to lower cost for fuels and chemicals

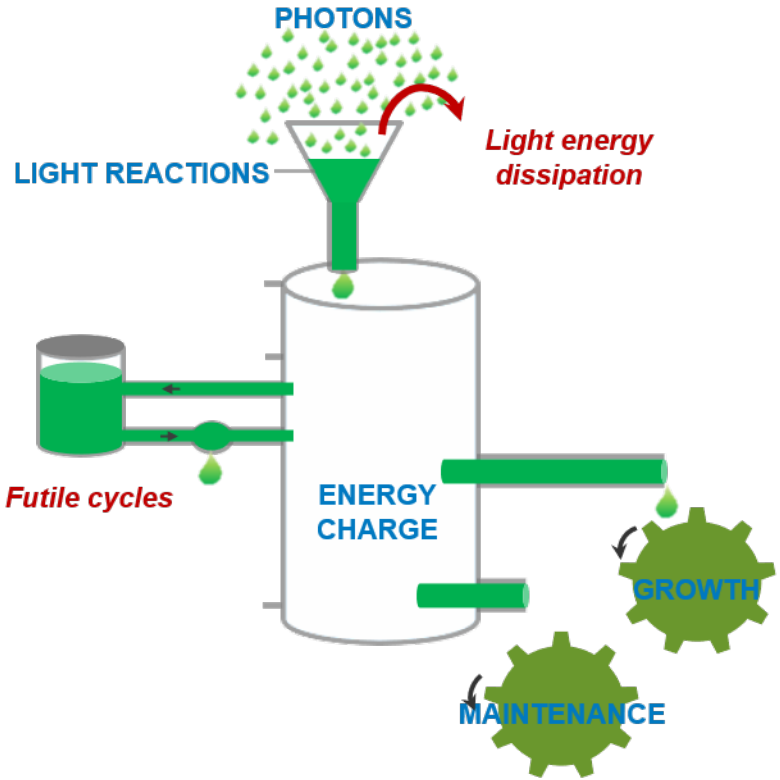
**Outcome:** New concept of Energome, new discoveries of energy management mechanisms, novel strategies to improve photosynthetic productivity

**Relevance:** Model cyanobacteria allow for rapid hypothesis testing and transfer of lessons to other cyanobacteria and eukaryotic algae

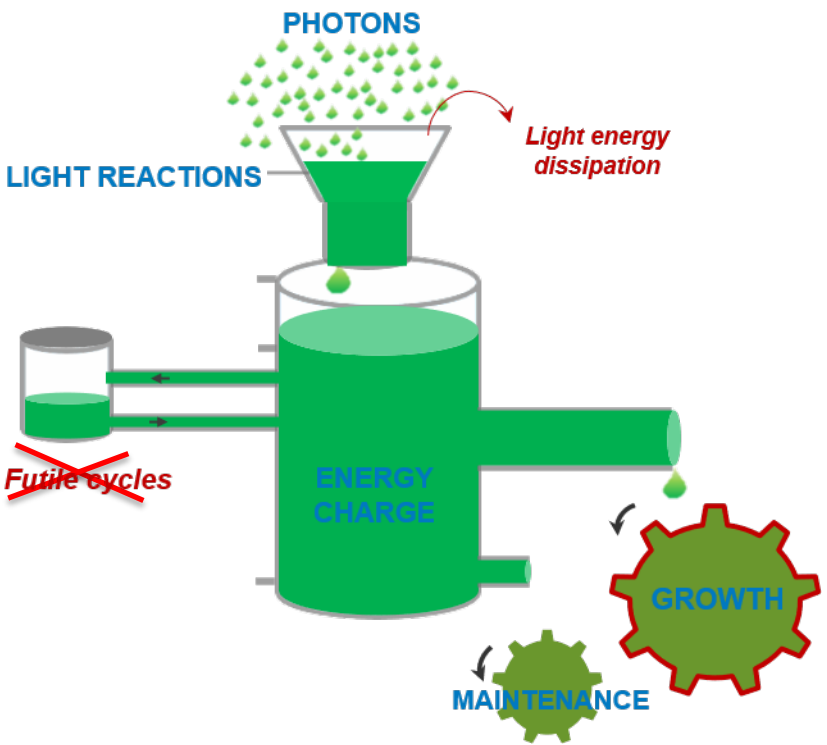


# Photosynthetic Energy Management Models

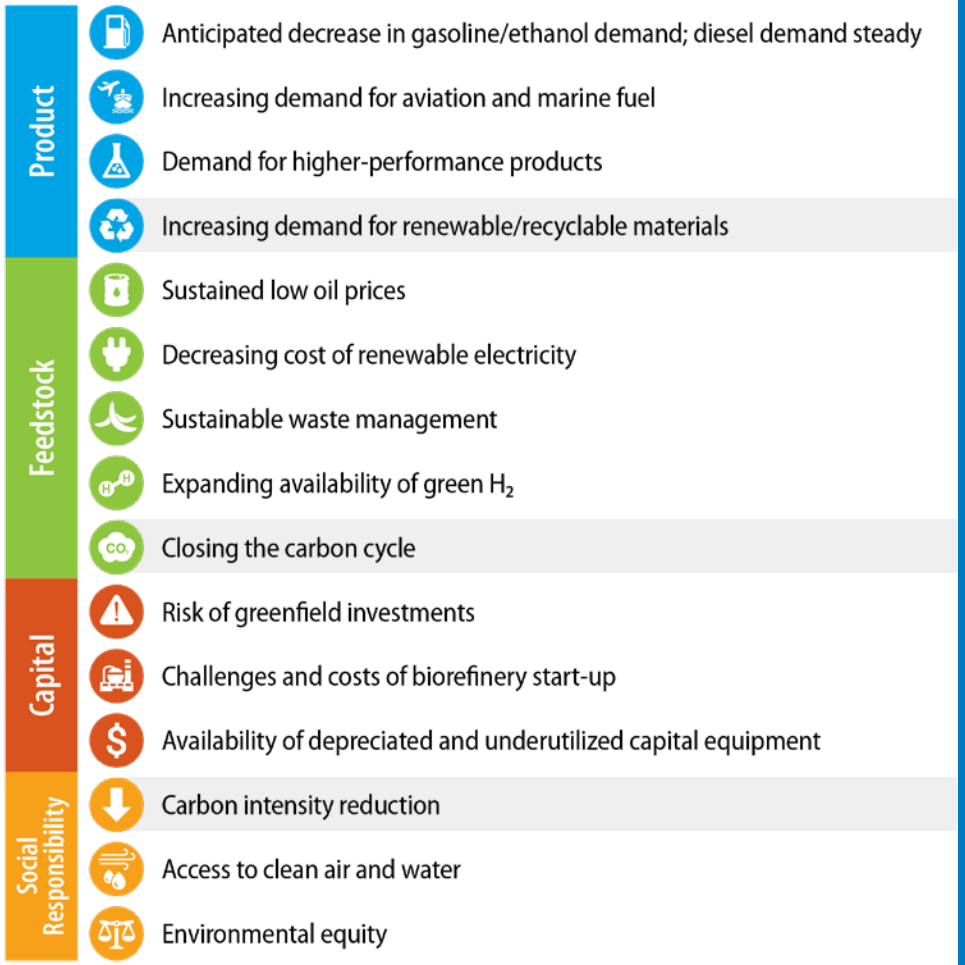
## Evolved System




## Engineered System



# Market Trends




## NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

### Value Proposition

- Increasing algal biomass productivity by 20% from current SOT would translate to about $0.90 per GGE MFSP reduction

### Key Differentiators

- We developed Energome (energy-ome) concept to guide novel engineering strategies to unleash unused potential in photosynthesis
- In-house mutant library and genetic engineering toolboxes in model cyanobacteria enable rapid hypothesis testing.

# 1. Management

**Single task:** Cyanobacterial genetics and physiology

**Team members:** *Michael Cantrell* – postdoc on physiology and engineering in cyanobacteria; weekly updates. *Nick Sweeny* assisted with SAGE reactors.

**BETO:** Regular meetings, quarterly and milestone reports

## Related Projects

Lessons may be transferred to algae in DISCOVR and other projects; interaction with SAGE reactor work.

## Risks

Energy regulation processes in algal systems are not well understood. Lack of precedents in improving biomass productivity by targeting energy levels.

## Risk mitigation strategies

Screen multiple mutant strains in a model cyanobacterium for rapid hypothesis testing.

# 2. Approach

1. Why cyanobacteria? Good genetic engineering tools. Some commercial strains such as spirulina.
2. Manipulate cellular energy management system which controls growth and carbon partition, based on studies in carbon storage mutants.
3. Building on a wealth of tools, data and strains in the genetic model Synechocystis 6803. Evaluate impact of gene knockout and knockdown on growth rate, energy levels, and photosynthesis under various light conditions.
4. Extend the lessons to other cyanobacteria and test for fast growth.

Go/No-go Decision 3/31/2020: Demonstrate successful 20% increase in biomass productivity under simulated outdoor light conditions using Synechocystis strains, and to deliver a strategy to initiate transfer of the technology to other strains of industrial interest.

# 3. Impact

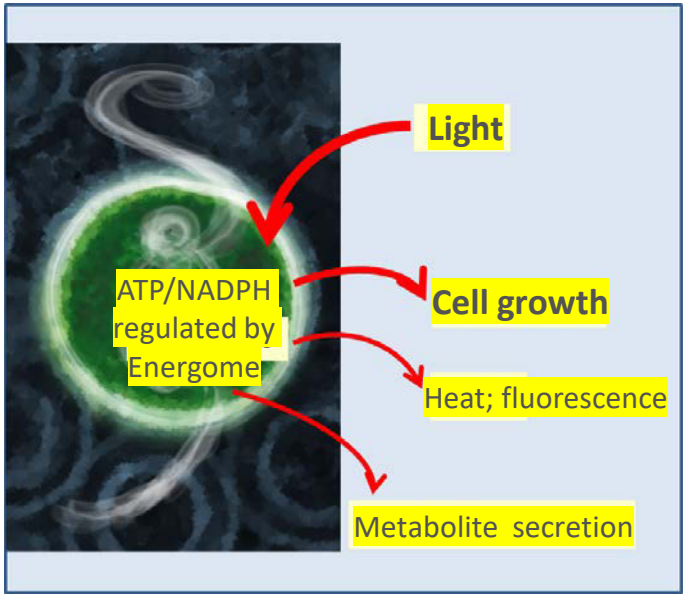
- Improved biomass productivity in cyanobacteria by >20%.
- Published papers FY19-FY21 and Impact Factors
  1. Metabolic Engineering 8.7
  2. Green Chemistry 8.6
  3. Biotechnology for Biofuels 6.4
  4. Frontiers in Microbiology 4.2
  5. Book chapter in Cyanobacteria Biotechnology


- One provisional US patent application and a Record of Invention in FY21
- Mutant library contributed to new Office of Science BER SFA on BioSecurity, and new EERE TCF project on wastewater treatment

# 4. Progress and Outcomes

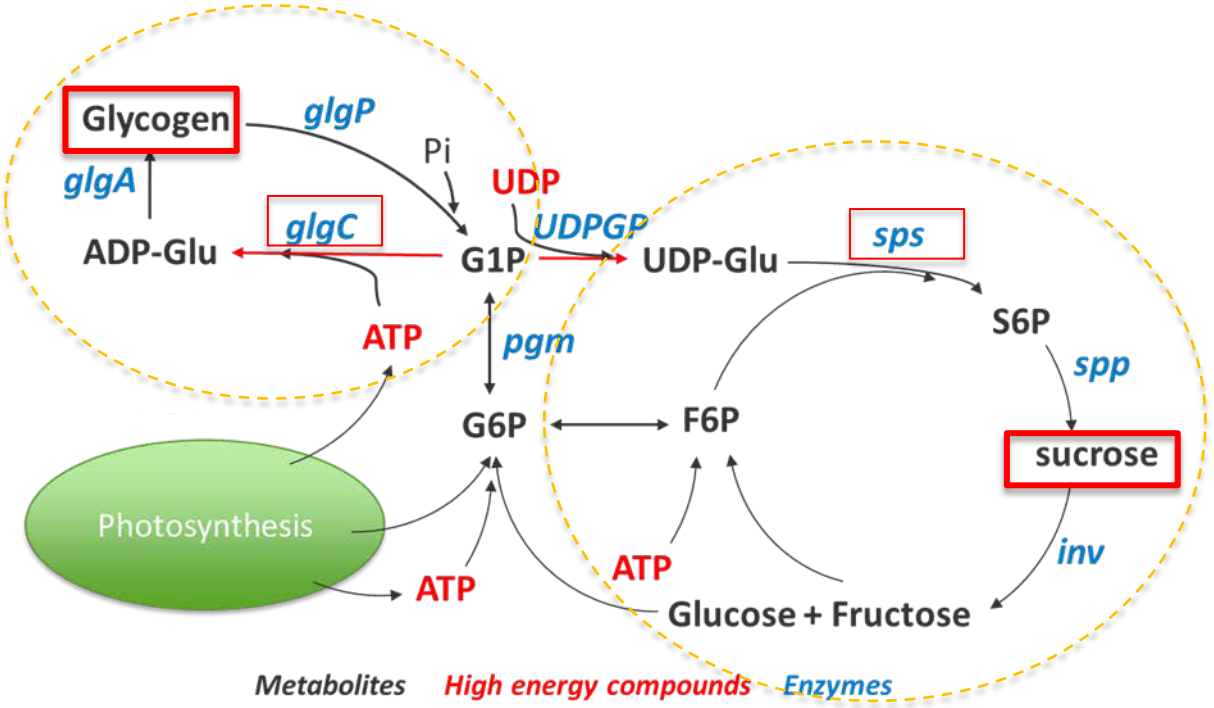
- The primary challenge in algal technology is energy conversion efficiency, currently up to a few percentages from photon energy to biomass energy.
- Most incoming solar energy is lost in photosynthesis, through mechanisms that are not fully understood. This work shows large room for improvement via the identification and manipulation of mechanisms in the cellular energy regulation system - **Energome**.

- We generated and analyzed multiple mutants, and discovered that cyanobacteria use futile cycles to dissipate ATP
- We demonstrated that by reducing energy dissipation, more energy is directed to cell growth.



# Two futile cycles in energy management

- Glycogen cycle: key enzyme ADP Glucose Pyrophosphorylase (*glgC* gene)
- Sucrose cycle: key enzyme Sucrose Phosphate Synthase (*sps* gene)



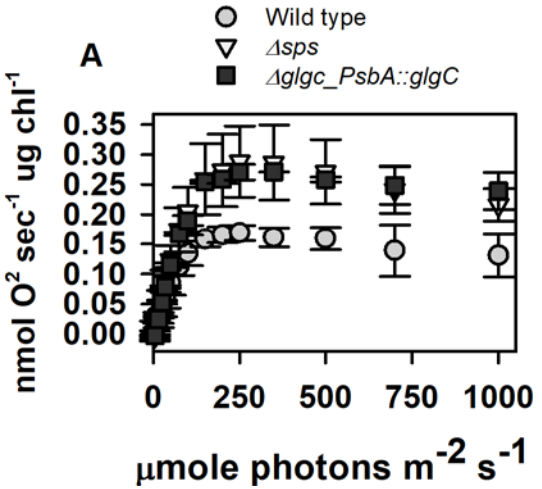
# Energy engineering: Reducing futile cycle flux increased ATP levels, photosynthetic and growth rates

- When glycogen cycle was knocked out in Synechocystis 6803, the mutant showed higher Energy Charge (ATP / ATP+ADP), but poor growth under outdoor light conditions. Here we knocked it down instead.
- We have eliminated sucrose cycle flux by knocking out *sps* gene.

Both the glycogen knock-down strain and the Δsps strain showed in flasks

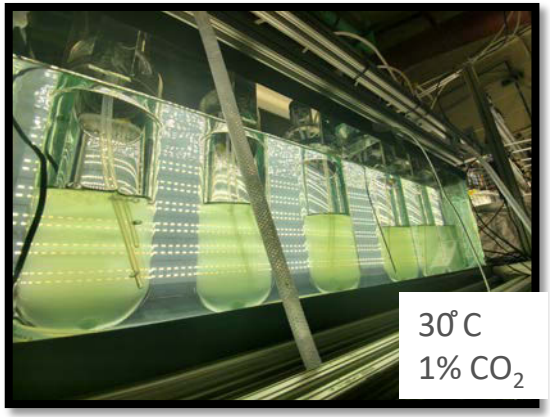
1. increased photosynthesis ($O_2$ evolution)
2. higher energy charge
3. faster (>20%) growth

in continuous light of moderate or high intensities or simulated outdoor light.

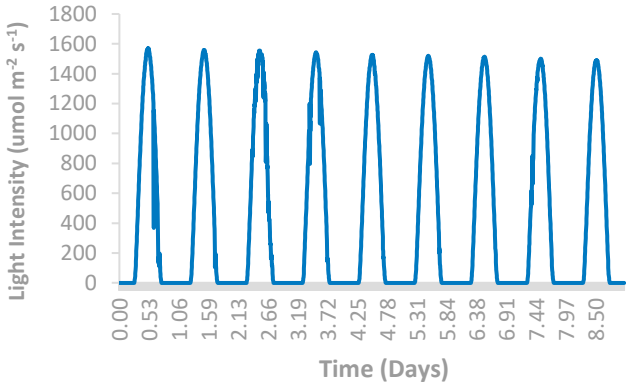


# SAGE reactors mimic outdoor light cycles

**A** Simulated Algae Growth Environment

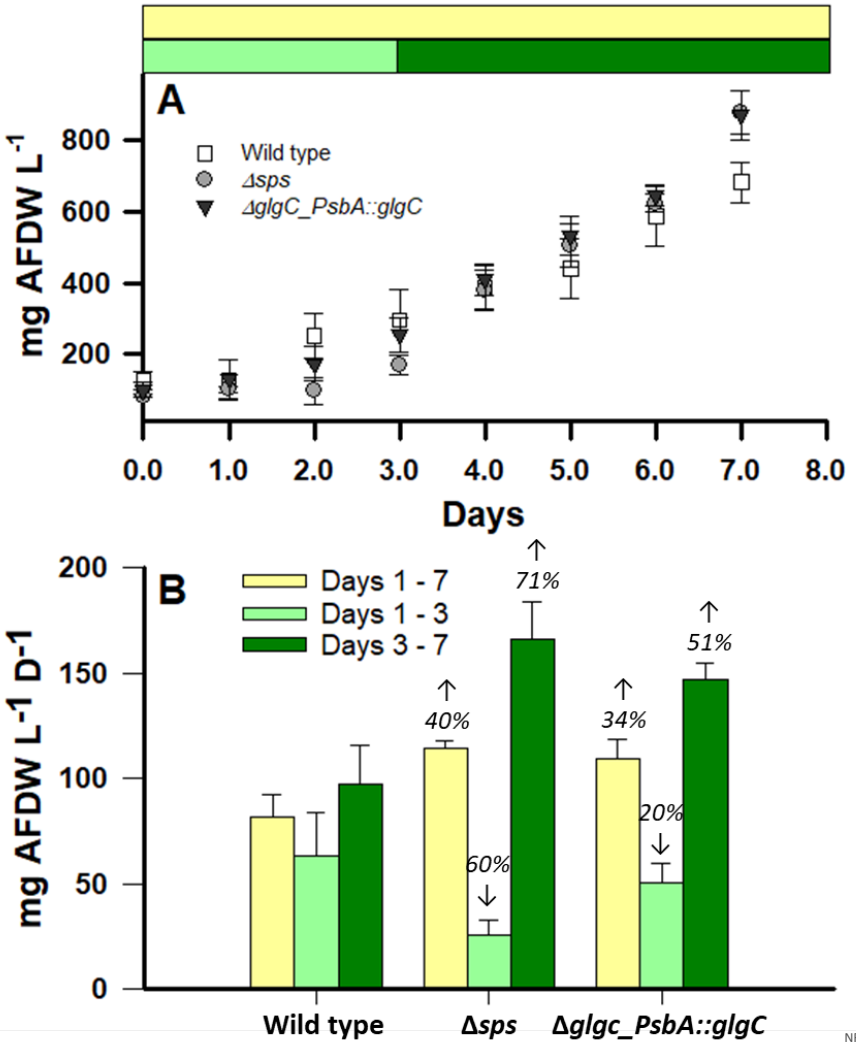



**B** Light regime: AzCATI site

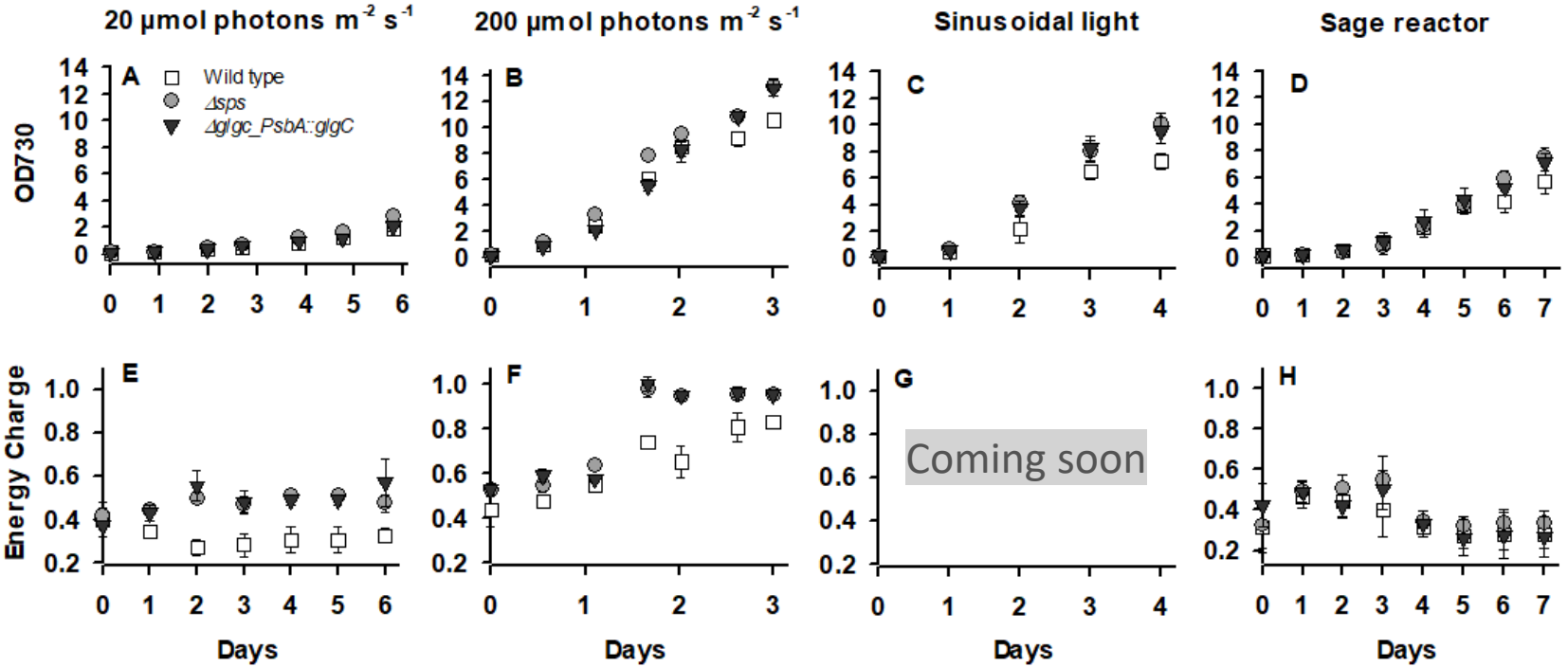


# Increased growth rates in SAGE reactors

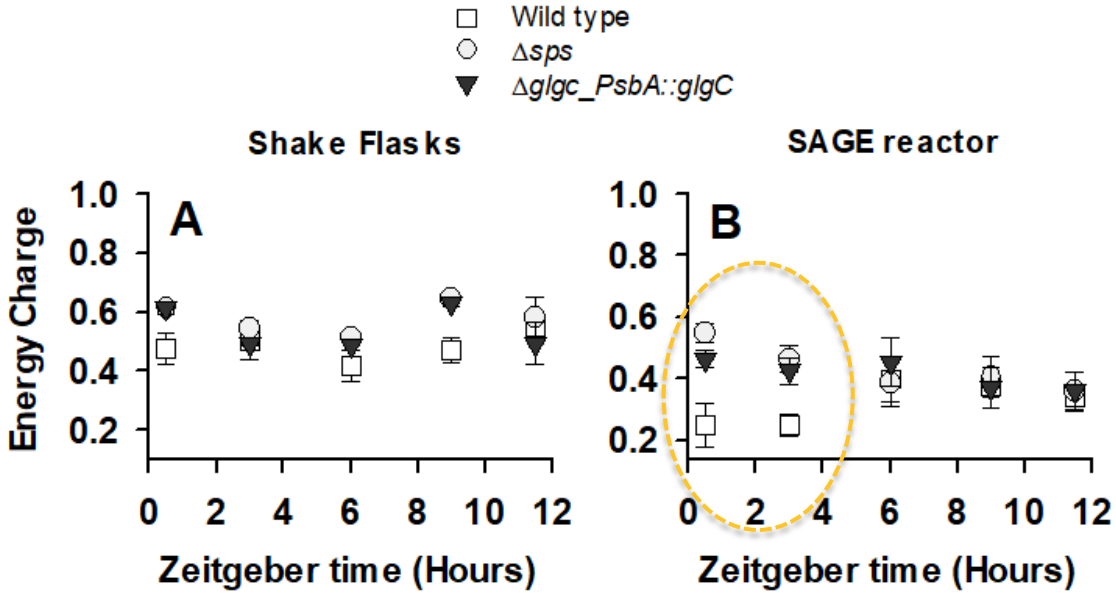
- Mutants showed 34-40% faster growth over WT in 7 days
- In the first 3 days, mutants grew more slowly than WT, likely due to change in growth conditions from flasks to SAGE where the mutations may slow down the adaptation process as metabolic penalty
- After day 3, mutants grew 51-71% faster than WT
- Longer growth periods and pre-adapted cells are being tested.



# Mutants show faster growth and higher energy charge under all tested light conditions



# Mutants show higher energy charge especially in early morning hours




Measurements were made during exponential growth for each culture – day 2 for shake flasks and day 4 for SAGE reactors

# Key accomplishments

- Discovered futile cycles as energy management mechanisms
- Demonstrated novel strategies to increase biomass productivity

**Future work**

- Transfer lessons from Synechocystis 6803 to two Synechococcus strains and test for higher biomass productivity.
- Use Energome concept to identify new engineering targets, such as an enzyme that “hides” ATP, towards higher biomass productivity.

# Quad Chart Overview

## Timeline

- Project start date: October 1, 2018
- Project end date: September 30, 2021

| | FY20 | Active Project |
|---|---|---|
| **DOE Funding** | 400K | 1200K |

## Barriers addressed

Atf-C: Biomass genetics and development

## Project Goal

Manipulate energy regulation in photosynthesis to improve energy conversion efficiency which currently stands at up to a few percentages

## End of Project Milestone

Demonstration of a successful platform for rapid testing of photosynthesis and biomass improvement hypotheses in cyanobacteria, including strains of industrial relevance, as well as a route to implement these strategies in selected eukaryotic species, selecting at least 3 translatable engineering targets with a projected biomass productivity improvement of at least 20%.

## Funding Mechanism

AOP.

# Thank you

**www.nrel.gov**

NREL/PR-2700-79126

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office . The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

# Additional Slides

# Responses to Previous Reviewers' Comments

- **FY19 Peer Review comment:** Not clear how the findings in Synechocystis will transfer to other algae
- **Response:** Currently strain development in other algae is often limited by lack of easy genetic engineering tools. Development of such tools is a priority in the field. Meanwhile we are testing the lessons in other algae, starting with other cyanobacteria

- **Go/No Go Milestone (3/31/2020):** Demonstrate successful 20% increase in biomass productivity under outdoor conditions using stacked Synechocystis strains, and to deliver a strategy to initiate transfer of the technology to other strains of industrial interest.
- **BETO Executive Summary of Results:** NREL demonstrated successful 23% increase in biomass productivity under outdoor conditions using a mutant Synechocystis strains. NREL developed a strategy to initiate transfer of the technology to other strains of industrial interest, Synechococcus PCC 7002 and Synechococcus UTEX 2973.

# Publications, Patents, Presentations, Awards, and Commercialization

1. Comparative analysis of cyanobacteria species reveals a novel guanidine-degrading enzyme that controls genomic stability of ethylene-producing strains. Bo Wang, Yao Xu, Xin Wang, Joshua S. Yuan, Carl H. Johnson, Jamey D. Young, Jianping Yu. Submitted.

2. Xiang Gao, Chao Wu, Michael Cantrell, Melissa Cano, Jianping Yu, Wei Xiong (2021) What We Can Learn from Measuring Metabolic Fluxes in Cyanobacteria. In: Cyanobacteria Biotechnology (Paul Hudson, ed). Wiley. In press.

3. Claudia Durall, Pia Lindberg, Jianping Yu and Peter Lindblad (2020) Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the cyanobacterium Synechocystis PCC 6803. Biotechnology for Biofuels 13:16. DOI: 10.1186/s13068-020-1653-y

4. Chao Wu, Huaiguang Jiang, Isha Kalra, Xin Wang, Melissa Cano, PinChing Maness, Jianping Yu, Wei Xiong (2020) A generalized computational framework to streamline thermodynamics and kinetics analysis of metabolic pathways. Metabolic Engineering. DOI: 10.1016/j.ymben.2019.08.006

5. Bo Wang, Tao Dong, Aldon Myrlie, Liping Gu, Huilan Zhu, Wei Xiong, PinChing Maness, Ruanbao Zhou, Jianping Yu (2019) Photosynthetic Production of Nitrogen-Rich Compound Guanidine. Green Chemistry. 21, 2928 – 2937. DOI: 10.1039/c9gc01003c.

6. Damien Douchi, Feiyan Liang, Melissa Cano, Wei Xiong, Bo Wang, Pin-Ching Maness, Peter Lindblad, Jianping Yu (2019) Membrane-Inlet Mass Spectrometry enables a quantitative understanding of inorganic carbon uptake flux and carbon concentrating mechanisms in metabolically engineered cyanobacteria. Frontiers in Microbiology. DOI: 10.3389/fmicb.2019.01356.

7. Jianping Yu, Bo Wang. Guanidine degradation enzyme and methods of use. (2020) US Provisional Patent application. No. 63/126,828.

8. Invited seminar speaker: Las Alamos National Laboratory. October 6th, 2020. Title: Overcoming genetic instability in cyanobacterial ethylene production.

9. Invited seminar speaker: University of Missouri Kansas City. March 6th, 2020. Title: Cyanobacteria photosynthesis and biotechnology.

# Other strategies to enhance algal productivity: (1) Increasing $CO_2$ uptake via overexpression of FbaA

- We developed a MIMS method to quantify $CO_2$ uptake and fixation rates simultaneously in real time
- FbaA (a key enzyme in CBB cycle) over-expression enhanced both rates, indicating a regulatory link between CBB flux and carbon concentrating mechanism

Douchi et al (2019) Membrane-Inlet Mass Spectrometry enables a quantitative understanding of inorganic carbon uptake flux and carbon concentrating mechanisms in metabolically engineered cyanobacteria. Frontiers in Microbiology. DOI: 10.3389/fmicb.2019.01356.

# (2) Increasing $CO_2$ fixation and ethylene production by overexpression of PEP Carboxylase

- Overexpression of an alternative carbon fixation enzyme PEPC complemented $CO_2$ fixation by Rubisco
- Increased $CO_2$ fixation led to 3X increase in ethylene productivity in engineered cyanobacteria

Durall et al (2020) Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the cyanobacterium Synechocystis PCC 6803. Biotechnology for Biofuels 13:16. DOI: 10.1186/s13068-020-1653-y

# (3) Enhancing ethylene production with guanidine degradation

- A decades-long puzzle in cyanobacterial ethylene production is the cause of genomic instability in Synechococcus 7942.
- We found that a co-product guanidine accumulates in ethylene-producing cyanobacteria.
- We identified a new class of enzyme from Synechocystis 6803 that degrades guanidine (manuscript under review).
- Heterologous expression of this enzyme enabled genomic stability and higher productivity in ethylene-producing cyanobacteria.


- Wang et al (2019) Photosynthetic Production of Nitrogen-Rich Compound Guanidine. Green Chemistry. 21, 2928 – 2937. DOI: 10.1039/c9gc01003c.
- Guanidine degradation enzyme and methods of use. (2020) US Provisional Patent application. No. 63/126,828.