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Title of research project:

Robust Microalgal Production Strains for High Yield Growth on Fossil Fuel Gas: Toward Cost Effective Biofuels and CO_2 Mitigation.

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Abstract

Our research focus in this project has been on the development of transgenic algal strains that can grow efficiently under mass cultivation conditions, while producing higher yields of oils (lipids) or biomass. *Nannochloropsis oceanica* CCMP1779 (*N. oceanica*), a highly oleaginous heterokont, was chosen for genetic manipulation by both random and targeted mutagenesis approaches.

The importance of photosynthetically assimilated carbon intermediates on the downstream steps of fatty acid biosynthesis and accumulation of triacylglycerols (TAG) was investigated by genetically targeting two key enzymes at branch-points between metabolic pathways of *N. oceanica*: citrate synthase (CIS) in the TCA cycle as a drain on accumulation of acetyl-co-A and malonyl-coA (-co-enzyme A), precursors to fatty acid biosynthesis, and cytosolic glycerol 3-phosphate dehydrogenase (G3PDH), the rate-limiting enzyme in gluconeogenesis, for diverting glyceride metabolites into triacylglycerols (TAG). Four robust transgenic strains were created as tests: A strain to inhibit protein synthesis via downregulating the predicted CIS-encoding endogenous gene using RNA interference (RNAi) technology; a strain to improve TAG assembly via heterologous expression of a yeast gene coding cytosolic G3PDH (EC 1.1.1.8), and the double mutant. All three strategies proved to be effective for enhancing neutral lipid production, increasing TAG accumulation by 2- to 3-fold. However, both the photosynthetic carbon fixation rate and biomass yield were slightly sacrificed in all mutants. The likely metabolic control points were identified by a comprehensive analysis of photosynthetic flux from water to CO_2, photosynthate partitioning into carbohydrates, proteins and lipids, and transcriptional analysis. Increasing light energy conversion into NADPH and ATP and improving their utilization for carbon fixation are identified as the critical needs for further improving both oil and biomass yields by metabolic engineering.

Supplementing algal cultures with CO_2 from flue gas has been investigated for increasing algal biomass productivity. Therefore, it is necessary to identify robust algal strains that can tolerate the resulting acidic medium caused by the larger amount of dissolved inorganic carbon (DIC), while ensuring efficient carbon utilization. In the present study, the strategy of insertional mutagenesis was employed to create a random mutant library in *N. oceanica* consisting of 1200 strains, followed by high throughput screening for desired phenotypes, specifically, robust growth at acidic pHs (5.5 and 6.4). Two strains (LpH23 and B1), that outcompeted WT in the screening process, were further evaluated for their biomass yields and photosynthetic traits using a number of biophysical approaches. Both LpH23 and B1 show advantages in photoautotrophic growth and intrinsic photosynthetic efficiency in terms of water oxidation and CO_2 fixation rates in acidified media using air bubbling. However, only B1 outperformed WT when supplied with 15% CO_2 bubbling in a 100 L laboratory raceway open pond reactor. While the random mutation in each strain leads to an enhanced tolerance to low pH, this only benefits the growth of LpH23 under low CO_2/DIC availability, while B1 additionally suppresses the toxicity of excess CO_2. Going forward, genetic characterization will be employed to pinpoint the mutation site in each strain to rationalize the observed phenotypes. Neither strain exhibits improved growth relative to WT under ambient CO_2/DIC in marine media.

A third genetic strategy was examined to improve photosynthetic biomass yield using a higher plant model organism (tobacco) based on knowledge of the cyanobacterial reaction center design

principle. By introducing single point mutations into tobacco *psbA* gene (coding for the reaction center D1 subunit of Photosystem II) to mimic the cyanobacterial high-light and low-light D1 isoforms, the tobacco mutants exhibit the same biophysical traits as the prokaryotic PSIIs. Incorporating the cyanobacterial low light mutation into the tobacco D1 protein improved the catalytic efficiency of linear electron flux from water oxidation to plastoquinone reduction, increased the rate of CO_2 fixation, and resulted in significant biomass gain at continuous low light intensity (+16%). Relative to the control strain, the tobacco mutant expressing the engineered high-light isoform exhibits more PSII charge separation but also more charge recombination (less linear electron flux), a marginally higher tolerance to photoinhibition and no significant change in biomass yield under the tested light conditions. The selection marker gene interferes with growth and must be removed for reliable evaluation of the biophysical and physiological (growth) performance of transgenic plants.

In the course of the foregoing studies, we developed two general methods that can serve researchers investigating CO_2/DIC in photosynthetic organisms. We published a new method for removing DIC from intact cells based on a (bi)carbonate chelator and magnesium ion for (bi)carbonate ion-pairing. We identified the relative affinities of three sites: the water-oxidizing complex (WOC), non-heme iron/Q_A^- and solvent-accessible arginines throughout PSII. Full reversibility was achieved and (bi)carbonate uptake was shown to require light.

A non-invasive method is being developed to measure the capacity and kinetics of CO2 fixation in aquatic and terrestrial oxygenic phototrophs using chlorophyll fluorescence emission yield. The variable portion of fluorescence yield (Fv from P ↔ P*) produced in competition with primary charge separation (P ↔ P* → $P^+Q_A^-$) in Photosystem (PS) II is used to measure the transit times for electrons/protons produced by water oxidation to fill the major intermediate pools of carriers (PQ, Fd^+, $NADP^+$). The resulting bottlenecks cause Fv to decrease and subsequently recover as filled pools are successive emptied (reoxidized, ultimately reaching the terminal reaction of CO2 fixation at RuBisCO. The method measures the transit time needed to photogenerate ATP and NADPH from water oxidation (the light reactions) and their consumption by the subsequent dark reaction of CO_2 fixation. Application of the method to several oxygenic phototrophs is illustrated and reveals large phenotypic differences in the light and dark reaction kinetics across the photosynthetic tree of life.

Introduction

The purpose of this research project was to investigate genetic approaches to improve the low efficiency of solar to biomass conversion of photosynthetic organisms and to alter the composition of products. Three genetic approaches were investigated using both random and specific targeting strategies, as described in this report.

Background

In this project, incremental improvement in photosynthetic growth rate and yield was achieved under various environmental stress conditions, or biomolecule composition could be shifted to

more desired products. However, no improvement in biomass yield under non-stressed optimal environmental conditions was achieved. The general conclusion reached is that it is very difficult to improve solar conversion efficiency of natural photosynthesis in any of the three branches of oxygenic photosynthesis.

The fundamental limitation of energy conversion and storage using conventional (natural) cellular organelles, chemiosmotic gradients for ionic energy storage and redox energy differences to create unidirectional chemical reactions are constrained both by the laws of thermodynamics and by the incommensurate time scales of the photonic steps vs enzymatic steps in photosynthesis. These rules limit the range of possible solutions using conventional cell biology.

The technical literature over the previous four years since submission of this proposal has extensively reported on the progress and limitations to commercialization of algal biomass and biofuels. A survey of this literature is beyond the scope of this report. The general consensus is that there remain multiple unsolved challenges to environmental sustainability and economic feasibility of biofuels and feedstock chemicals from biomass. These reports align with the conclusions of this project.

Results

Publications:

The multiplicity of roles for (bi)carbonate in photosystem II operation in the hypercarbonate-requiring cyanobacterium *Arthrospira maxima*. *Photosynthetica* 56, 217-228 (2018). Gennady Ananyev, Colin Gates, G. Charles Dismukes, https://doi.org/10.1007/s11099-018-0781-0

Abstract: *Arthrospira maxima* is unique among cyanobacteria, growing at alkaline pH (<11) in concentrated (bi)carbonate (1.2M saturated) and lacking carbonic anhydrases. We investigate dissolved inorganic carbon (DIC) roles within photosystem II of *A. maxima* cells oximetrically and fluorometrically, monitoring the light reactions on the donor and acceptor sides of PSII. We develop new methods for removing DIC based on a (bi)carbonate chelator and magnesium for (bi)carbonate ion-pairing. We establish relative affinities of three sites: the water-oxidizing complex (WOC), non-heme iron/Q_A^- and solvent-accessible arginines throughout PSII. Full reversibility is achieved but (bi)carbonate uptake requires light. DIC depletion at the non-heme iron site and solvent-accessible arginines greatly reduces the yield of O_2 due to O_2 uptake, but accelerates the PSII-WOC cycle, specifically the S2→S3 and S3→S0 transitions. DIC removal from the WOC site abolishes water oxidation and appears to influence free energy stabilization of the WOC from a site between CP43-R357 and Ca^{2+}.

Mutation of Spirulina sp. by nuclear irradiation to improve growth rate under 15% carbon dioxide in flue gas. *Bioresour Technol*, **238**, 650-656 (2017). Jun Cheng, Hongxiang Lu, Xin He, Weijuan Yang, Junhu Zhou, Kefa Cen, DOI:10.1016/j.biortech.2017.04.107

Abstract: Spirulina sp. Was mutated by c-rays from 60Co nuclear irradiation to improve growth and CO2 fixation rate under 15 vol.% CO2 (in flue gas from a power plant). Mutants with enhanced growth phenotype were obtained, with the best strain exhibiting 310% increment in biomass yield on day 4. The mutant was then domesticated with elevated CO2 concentration, and the biomass yield increased by 500% after domestication under 15 vol.% CO2, with stable inheritance. Ultrastructure of Spirulina sp. shows that the fractal dimension of Spirulina cells decreased by 23% after mutation. Pore size in the cell wall of Spirulina mutant increased by 33% after 15 vol.% CO2 domestication. This characteristic facilitated the direct penetration of CO2 into cells, thus improving CO2 biofixation rate.

Manuscripts submitted for peer review:

PSII reaction center engineering inspired by cyanobacterial design enhances biomass production in higher plants. Yuan Zhang, Gennady Ananyev, and Pal Maliga and G. Charles Dismukes,
Submitted to Nature Plants, NPLANTS-18075058. https://mts-nplants.nature.com/cgi-bin/main.plex?el=A7Ce4CIp5A1KFc3J3A9ftdrYjiQ0uVpdjz2C8SOGe6dwZ
The significance of our findings may be summarized as follows:

- By introducing single point mutations into tobacco *psbA* gene (coding for the reaction center D1 subunit of Photosystem II) to mimic the cyanobacterial high-light and low-light D1 isoforms, the tobacco mutants exhibit the same biophysical traits as the prokaryotic PSIIs.
- Incorporating the cyanobacterial low light mutation into the tobacco D1 protein improved the catalytic efficiency of linear electron flux from water oxidation to plastoquinone reduction, increased the rate of CO_2 fixation, and resulted in significant biomass gain at continuous low light intensity (+16%).
- Relative to the control strain, the tobacco mutant expressing the engineered high-light isoform exhibits more PSII charge separation but with more charge recombination (less linear electron flux), a marginally higher tolerance to photoinhibition and no significant change in biomass yield under the tested light conditions.
- Excision of the antibiotic marker gene is necessary for reliable evaluation of the biophysical and physiological performance of transgenic plants.

Manuscripts in preparation:

Metabolic engineering of algal central carbon metabolism for substantial accumulation of storage lipids (TAGs)
Prospective Journal: Plant Biotechnology
Yuan Zhang[1], Yunbing Ma[1], Eric Poliner[2], Tomomi Takeuchi[2], Jonah Williams[1], Christoph Benning[2] and G. Charles Dismukes[1,3]
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Abstract: The importance of photosynthetically assimilated carbon intermediates on the downstream steps of fatty acid biosynthesis and accumulation of triacylglycerols (TAG) is

investigated by genetically targeting two key enzymes at branch-points between metabolic pathways: citrate synthase (CIS) in the TCA cycle as a drain on accumulation of acetyl-co-A and malonyl-coA (-co-enzyme A), precursors to fatty acid biosynthesis, and cytosolic glycerol 3-phosphate dehydrogenase (G3PDH), the rate-limiting enzyme in gluconeogenesis, for diverting glyceride metabolites into triacylglycerols (TAG). Four robust transgenic strains were created in *Nannochloropsis oceanica* CCMP1779 (*N. oceanica*) as tests: A strain to inhibit protein synthesis via downregulating the predicted CIS-encoding endogenous gene using RNA interference (RNAi) technology; a strain to improve TAG assembly via heterologous expression of a yeast gene coding cytosolic G3PDH (EC 1.1.1.8), and the double mutant. All three strategies proved to be effective for enhancing neutral lipid production, increasing TAG accumulation by 2- to 3-fold. However, both the photosynthetic carbon fixation rate and biomass yield were slightly sacrificed in all mutants. The likely metabolic control points were identified by a comprehensive analysis of photosynthetic flux from water to CO_2, photosynthate partitioning into carbohydrates, proteins and lipids, and transcriptional analysis. Increasing light energy conversion into NADPH and ATP and improving their utilization for carbon fixation are identified as the critical needs for further improving both oil and biomass yields by metabolic engineering.

Random Mutagenesis of the Heterokont *Nannochloropsis oceanica* CCMP1779 for Screening of Acidophilic Microalgae

Prospective Journal: Bioresource Technology

Yuan Zhang[1], Jonah M. Williams[1,2], Yunbing Ma[1], Hoa Vu[1], Gennady Ananyev[1], Christoph Benning[4], G. Charles Dismukes[1,3 ; 1]Waksman Institute of Microbiology, [2]Department of Chemical and Biochemical Engineering, [3]Department of Chemistry and Chemical Biology, Rutgers, the State University of New Jersey, NJ 08854, USA, [4]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824.

Abstract: Supplementing algal cultures with CO_2 from flue gas produced by fossil power plants has been adopted for increasing algal biomass productivity. Therefore, it is necessary to identify robust algal strain that can tolerate the resulting acidic medium caused by the large amount of dissolved inorganic carbon (DIC), while ensuring efficient carbon utilization. The marine heterokont *Nannochloropsis oceanica* CCMP1779 is drawing considerable interest as resource for biodiesel production due to its robust growth in open cultures and naturally high lipid content. In the present study, the strategy of insertional mutagenesis was employed to create a *N.o1779* random mutant library consisting of 12,00 strains, followed by high throughput screening for desired phenotypes, specifically, robust growth at acidic pHs (5.5 and 6.4). Two strains (LpH23 and B1), that outcompeted WT in the screening process, were further evaluated for their biomass yields and photosynthetic traits using a number of biophysical approaches. Both LpH23 and B1 show advantages in photoautotrophic growth and intrinsic photosynthetic efficiency in terms of water oxidation and CO_2 fixation rates in acidified media using air bubbling. However, only B1 outperformed WT when supplied with 15% CO_2 bubbling in a 100 L laboratory raceway open pond reactor. We speculate that while the random mutation in the respective strains lead to an enhanced tolerance to low pH, this only benefits the growth of LpH23 under low CO_2/DIC availability, while B1 additionally suppresses the toxicity of excess CO_2. Going forward, genetic characterization will be employed to pinpoint the mutation site in each strain to rationalize the observed phenotypes.

Rapid non-invasive fluorescence induction technique for determining CO2-fixation rates and light driven steps from water to $NADP^+$ in photosynthetic organisms.

Prospective Journal: BBA-Bioenergetics

Gennady Ananyev[1,2] Apostolos Zournas, Colin Gates[1,2], , and G. Charles Dismukes[1,2]* [1]Dept of Chemistry & Chemical Biology, and [2] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ.

Abstract: We describe a rapid, non-invasive method to measure the capacity and kinetics of CO2 fixation in aquatic and terrestrial oxygenic phototrophs using chlorophyll fluorescence emission yield. The variable portion of fluorescence yield (Fv from $P \leftrightarrow P^*$) produced in competition with primary charge separation ($P \leftrightarrow P^* \rightarrow P^+Q_A^-$) in Photosystem (PS) II is used to measure the transit times for electrons/protons produced by water oxidation to fill the major intermediate pools of carriers (PQ, Fd^+, $NADP^+$). The resulting bottlenecks cause Fv to decrease and subsequently recover as filled pools are successive emptied (reoxidized, ultimately reaching the terminal reaction of CO2 fixation at RuBisCO. The method measures the transit time needed to generate ATP and NADPH from water oxidation (the light reactions) and their consumption by the subsequent dark reaction of CO_2 fixation. Application of the method to several oxygenic phototrophs is illustrated and reveals large phenotypic differences in the light and dark reaction kinetics across the photosynthetic tree of life.

Educational Products:

Postdoctoral researcher: Dr. Yunbing Ma

PhD thesis: Yuan Zhang
From Light To Life: The Energy Conversion And Storage In Plant And Algae

Abstract: Photosynthesis, the physico-chemical process converting sunlight into chemical energy, is the basis to feed the world and fuel the planet. To satisfy the growing demand for food and fuel, the efficiency of the natural photosynthesis needs to be optimized for maximum crop yield, while the photosynthetically assimilated carbon needs to be more sophisticatedly recruited for generating energy-dense renewable products. There are two objectives of this dissertation, the first is to explore the feasibility to boost the biomass yield of the crop plant by genetically engineer their photosystem II (PSII), and the second is to create robust microalgal transgenic strains with enhanced lipid content and CO_2 utilization efficiency for biofuel production as well as CO_2 mitigation.

In Chapter 2, we explore whether the prokaryotic design principal of PSII D1 subunit is applicable in a higher plant model *Nicotiana tabacum*. By introducing the single point mutation into tobacco *psbA* gene at A152S mimicking the cyanobacterial HL D1:2 strain, we created a high light mutant exhibiting higher photosynthetic efficiency, higher tolerance to photoinhibition and modestly increased biomass potential under high light conditions. By contrast, the only benefit of incorporating the E130Q point mutation mimicking the LL isoform is restricted to improving WOC cycling efficiency at low light intensity, while the biomass yield was impaired at low light intensity. Our findings indicate that at all light intensities increasing charge separation yield and

photochemical conversion produces more biomass in a higher plant, and that photosynthetic designs from prokaryotic phototrophs can be employed to improve the productivity of crop plants.

In Chapter 3, *Nannochloropsis oceanica* CCMP1779 (*N.o1779*), the emerging oleaginous model algae, is chosen for application of the "push and pull" strategy to enhance the lipid production by metabolic engineering. The regulatory importance of citrate synthase (CIS) in partitioning the carbon flux towards the pathways of protein and FA biosynthesis, and the functional role of glycerol 3-phosphate dehydrogenase (G3PDH) in diverting carbon precursors from glycolysis to TAG assembly are fully examined in the *N.o1779* transgenic strains. Downregulation of a putative endogenous gene encoding citrate synthase (*N.oCIS*) via RNA interference technology and expression of a yeast gene encoding the cytosolic *G3PDH* (*S.cG3PDH*) lead to higher accumulation of TAG and increased abundance of free FA, advancing our understanding on the genetic and molecular basis of algal TAG metabolism.

In Chapter 4, the goal was to create a robust industrial strain that be cultivated in the open culture using flue gas as the carbon source. Applying the insertional random mutagenesis strategy combined with the high throughput screening approach to the same *N.o1779* oleaginous microalgae, a winning mutant was successfully isolated for its great advantages in photoautotrophic growth and intrinsic photosynthetic efficiency not only under the normal growth conditions but also in the acidic environment. The genome sequencing project currently in progress will potentially unlock the regulatory mechanism responsible for this beneficial phenotype.

In summary, my dissertation advances the understanding of the PSII design principal and the metabolic network of photosynthetic carbon distribution. New genetic engineering strategies have been developed throughout this dissertation to improve biomass productivity in higher plant and enhance lipid productivity and carbon utilization in eukaryotic microalgae.

PhD Thesis: Colin Gates
Title: Kinetic and Energetic Constraints on Electron Transfer in Photosystem II
Ch X. The multiplicity of roles for (bi)carbonate in photosystem II operation in the hypercarbonate-requiring cyanobacterium *Arthrospira maxima*. Published manuscript summarized herein.

Master Thesis: Hoa Vu
Screening and Characterization of a Mutant Library of the Microalga *Nannochloropsis oceanica* for Growth and Lipid Production at High CO_2 Conditions.

Abstract: *Nannochloropsis* is a genus of microalgae that produces substantial amounts of storage lipids referred to as triacylglycerides that are derived from fatty acids. These products are precursors to important dietary lipids for use in oils and as replacements for hydrocarbon fuels. To enhance lipid production in *Nannochloropsis oceanica* CCMP 1779 we applied an insertional mutagenesis approach to produce a library of 1200 strains containing single insertions located randomly in the genome. The collection was screened for growth at high CO_2 (≤10%) and low pH, and for high lipid content. Seven mutants were selected for quantitative assays and characterization of photosynthetic efficiency. All 7 strains and the wild type (WT) grow fastest as measured by growth rate and final dry biomass at 2% CO_2. The WT had a higher growth rate and biomass

production compared with the mutant strains at 2% and 10% CO_2. There was pH and CO_2-dependence of the growth rate of mutants and the WT. A level of 10% CO_2 was a stress condition for *Nannocholoropsis oceanica* CCMP 1779. One strain (G2) grown at 2% and 10% CO_2 had a higher lipid content by Nile Red fluorescence than the WT. When quantified by lipid extraction, this strain did not show a higher lipid content than the WT by day 15 of growth.

Undergraduate honor thesis:

Jonah Williams

Random Mutagenesis of Nannochloropsis oceanica CCMP1779 to Provide More Robust Algal Bioenergy Crops

Abstract: Aquatic microbial oxygenic photoautotrophs (AMOPs) are the most productive photosynthetic organisms at solar energy conversion, by far. The marine heterokont *Nannochloropsis oceanica* CCMP 1779 is drawing considerable interest as resource for biodiesel production due to its robust growth in open cultures and naturally high lipid content. Currently, the only practical means for delivering CO_2 at large scale is to site algal production facilities near fossil power plants and utilize flue gas for algal cultivation, which requires robust algae with substantial tolerance to the acidic environment. In this study, the strategy of insertional mutagenesis was employed to create a random mutant library of 1200 strains, followed by high throughput screening for desired phenotypes, namely robust growth and enhanced tolerance to low pH. Two winning strains were further evaluated for their growth potential and photosynthetic capacities using a number of biophysical measurements, showing intrinsic photosynthetic advantage in both water oxidation and CO_2 fixation rates over the WT strain at both the optimal growth conditions and under stress at low pH. Whole genome sequencing to pinpoint the mutation site in the two winning strains is in progress and will allow a deeper understanding of the observed phenotypic responses. This work will provide useful insights into the metabolic engineering of eukaryotic algae and the kinetic chokepoints within the photosynthetic machinery.

Conference Presentations:

Metabolic engineering of *Nannochloropsis Oceanica CCMP1779* for biofuel production, Yuan Zhang, Yunbing Ma, Eric Poliner, Tomomi Takeuchi, Jonah Williams, Gennady Annayev, Christoph Benning and G. Charles Dismukes, September 2018, Waksman Retreat, Rutgers University, NJ.

Robust Microalgal Production Strains for High Yield Growth on Fossil Flue Gas, G. Charles Dismukes, GCEP Annual Meeting, Stanford University, Palo Alto, CA, November 2016.

Conference Posters:

Random Mutagenesis of the Heterokont *Nannochloropsis oceanica* CCMP1779 for Bioenergy Applications, Yuan Zhang, Jonah Williams, Yunbing Ma, Hoa Vu, Gennady Annayev, Christoph Benning and G. Charles Dismukes, May 2018, Eastern Regional Photosynthesis Conference, Woods Hole, MA

Applied Random Mutagenesis of the Heterokont *Nannochloropsis Oceanica* for Bioenergy Applications, Jonah Williams, Yuan Zhang, Yunbing Ma, Hoa Vu, Gennady Annayev, Christoph Benning and G. Charles Dismukes, March 2018, 2018 Microbiology at Rutgers Symposium, Rutgers University, NJ

Yuan Zhang, Yunbing Ma, Eric Poliner, Jonah Williams, Gennady Ananyev, Hoa Vu, Christoph Benning, G. Charles Dismukes, Metabolic engineering of *Nannochloropsis Oceanica* CCMP1779 for high lipid production, October 2017, GCEP symposium, Stanford University, CA

Hoa Vu, Yunbing Ma, Yuan Zhang, Khue Tu Ho-Nguyen, Gennady Ananyev, Zhi-Yan Du, Jun Cheng, Christoph Benning, Charles Dismukes, High Throughput Screening of a Mutant Library of the microalga *Nannochloropsis oceanica* for Growth and Lipid Production at High CO_2 Conditions, October 2017, GCEP symposium, Stanford University, CA

Yuan Zhang, Gennady Ananyev, Yunbing Ma, Hoa Vu, Jun Cheng, G. Charles Dismukes, Robust Microalgal Production Strains for High Yield Growth on Fossil Flue Gas: Toward Cost Effective Biofuels and CO_2 Mitigation, September 2017, Waksman Retreat, Rutgers University, NJ

Yuan Zhang, Gennady Ananyev, Yunbing Ma, Hoa Vu, Jun Cheng, G. Charles Dismukes, 2017, Forward and reverse genetic engineering in *Nannochloropsis oceanica* for higher lipid production, September 2017, Waksman Retreat, Rutgers University, NJ

Gennady Ananyev, Colin Gates, Haim Treves, and G. Charles Dismukes, Record Growth Rate and Exceptional Photoprotection Powered by an Adaptable Light Conversion Mechanism in the Microalga *Chlorella ohadii*, September 2017, Molecular Biosciences Symposium, Rutgers University, NJ

Gennady Ananyev, Colin Gates, Haim Treves, and G. Charles Dismukes, Record Growth Rate and Exceptional Photoprotection Powered by an Adaptable Light Conversion Mechanism in the Microalga *Chlorella ohadii*, September 2017, Microbiology Symposium, SEBS, Rutgers University, NJ

Gennady Ananyev, Colin Gates, Haim Treves, and G. Charles Dismukes, Record Growth Rate and Exceptional Photoprotection Powered by an Adaptable Light Conversion Mechanism in the Microalga *Chlorella ohadii*, July 2017, the Gordon Research Conference in Photosynthesis, Newry, ME

Gennady Ananyev, Colin Gates, Aaron Kaplan, and G. Charles Dismukes, Photosystem II-Cyclic Electron Flow Powers Exceptional Photoprotection and Record Growth in the Microalga *Chlorella ohadii*, April 2017, Eastern Regional Photosynthesis Conference, Woods Hole, MA

Gennady Ananyev, Colin Gates, Haim Treves, and G. Charles Dismukes, Record Growth Rate and Exceptional Photoprotection Powered by an Adaptable Light Conversion Mechanism in the Microalga *Chlorella ohadii*, April 2017, Eastern Regional Photosynthesis Conference, Woods Hole, MA

Yuan Zhang, Gennady Ananyev, Yunbing Ma, Hoa Vu, Jun Cheng, G. Charles Dismukes, Robust Microalgal Production Strains for High Yield Growth on Fossil Flue Gas: Toward Cost Effective Biofuels and CO_2 Mitigation, Sept 2016, Waksman Retreat, Rutgers University, NJ