

Cover

Federal Agency and Organization Element to Which Report is Submitted:

Department of Energy, Basic Energy Sciences

Federal Grant or Other Identifying Number Assigned by Agency:

DE-SC0005354

Project Title:

Photosystem II Water Oxidation: Mechanism, Efficiency and Flux in Diverse Oxygenic Phototrophs

PD/PI Name:

- G. Charles Dismukes, Principal Investigator
- Gennady M Ananyev, Co-Principal Investigator

Recipient Organization:

Rutgers University New Brunswick

Project/Grant Period:

09/01/2016 - 08/31/2017

Reporting Period:

09/01/2016 - 08/31/2017

Submitting Official (if other than PD\PI):

- G. Charles Dismukes
- Principal Investigator
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- 848-445-1489

Submission Date:

01/09/2018

Signature of Submitting Official (signature shall be submitted in accordance with agency specific instructions)

G. Charles Dismukes

Final Report

Accomplishments

* What are the major goals of the project?

We planned four aims: 1) extend the VZAD model to allow analysis of PSII chlorophyll fluorescence emission as modulated by interaction with the WOC; 2) compare the solar energy conversion efficiencies of PSII-WOCs from intact cells, isolated thylakoid membranes and PSII core complexes and crystals from cyanobacterium *Thermosynechococcus elongatus* (collaboration with Lawrence Berkeley National Laboratory); 3) determine whether PSII can store light energy by pumping protons across the thylakoid membrane (PSII-cyclic electron flow) and how it is regulated within the green alga *Chlorella ohadii* (collaboration with the Hebrew University of Jerusalem); and 4) genetically replace the native PSII-D1 protein subunit from a higher plant with two cyanobacterial D1 isoforms to test whether their functional advantages in growth and photoprotection can be transferred (collaboration with Rutgers University).

* What was accomplished under these goals (you must provide information for at least one of the 4 categories below)?

Major Activities: (omit)

Specific Objectives:

Aim 1: The VZAD model was partially extended but cannot yet obtain accurate S-state populations due to difficulty in establishing a universal baseline across all species. An improved VZAD software model has been made available on the PI's Web site and work continues on this objective. We additionally continued developing a fluorescence method for measurement of WOC cycle optimal transition times.

Aim 2: A manuscript is in preparation addressing this subject, in collaboration with a separate group after the original collaborator failed to supply viable samples.

Aim 3: A paper was published in *BBA Bioenergetics* addressing this point; research continues into the precise mechanism of energy transduction in PSII-CEF.

Aim 4: A manuscript is in preparation; the original goal was met.

In addition, we produced a paper in *Photosynthetica* detailing a method for selective removal of bicarbonate at multiple active sites around PSII and the roles of each site in the overall operation of the enzyme complex.

Significant Results:

Key outcomes or Other achievements:

Published manuscripts:

- Photosystem II-cyclic electron flow powers exceptional photoprotection and record growth in the microalga *Chlorella ohadii*. Ananyev, G., C. Gates, A. Kaplan, and G.C. Dismukes, *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 2017. 1858(11): p.873-883. Synopsis: We demonstrate the fastest growth rates observed in any phototroph, attributed to balancing of linear and cyclic PSII electron flow. We also provide a clearer picture of the mechanism of PSII-cyclic electron flow and its direct role in photoprotection.
- The Multiplicity of Roles for Dissolved Inorganic Carbon in Photosystem II Operation in the Hypercarbonate-requiring Cyanobacterium *Arthrospira maxima*. Ananyev, G., C. Gates, and G.C. Dismukes, *Photosynthetica* (Govindjee special edition), 2017. In press. Synopsis: Multiple active sites of bicarbonate functionality in PSII are definitively established. A new technique for selectively depleting bicarbonate from two classes of binding sites was demonstrated.

Submitted manuscripts under review:

- A non-invasive fluorescence method for investigating S state transition times. Gates, C., G. Ananyev, and G.C. Dismukes. In review at *Nature Methods*.

Manuscripts in preparation:

- The role of chloride in Photosystem II: *in vivo* bromide substitution reveals functionality in proton removal from the WOC active site. Gates, C., J. Williams, G. Ananyev, and G.C. Dismukes. Synopsis: We show that the function of chloride in WOC operation is to assist in removing protons and prevent damage to PSII stemming from proton accumulation at high light.
- Engineering Photosystem II Subunit D1 in *Nicotiana tabacum* to increase photosynthesis at extreme light intensities. Zhang, Y., G. Ananyev, P. Maliga, and G.C. Dismukes. Synopsis: We demonstrate that modification of the D1 subunit of PSII in a higher plant (tobacco) can be used to mimic cyanobacterial phenotypes and tune response to varying light intensities.

*** What opportunities for training and professional development has the project provided?**

- A new collaboration with Prof. Petra Fromme at Arizona State University allowed increased exposure in our lab to PSII microcrystals.
- A PhD student gave a presentation at the Eastern Regional Photosynthesis conference.

*** How have the results been disseminated to communities of interest?**

3 manuscripts published or submitted. Several local presentations at Rutgers University by affiliated students. One presentation at the Eastern Regional Photosynthesis Conference. Direct communication with interested researchers in the field following the aforementioned conference.

*** What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to report- this is a terminal award.

Conference Papers and Presentations

- Photosystem II-cyclic electron flow powers exceptional photoprotection and record growth in the microalga *Chlorella ohadii*. Ananyev, G., C. Gates, A. Kaplan, and G.C. Dismukes, *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 2017. 1858(11): p.873-883. Synopsis: We demonstrate the fastest growth rates observed in any phototroph, attributed to balancing of linear and cyclic PSII electron flow. We also provide a clearer picture of the mechanism of PSII-cyclic electron flow and its direct role in photoprotection. Federal support acknowledged.
- The Multiplicity of Roles for Dissolved Inorganic Carbon in Photosystem II Operation in the Hypercarbonate-requiring Cyanobacterium *Arthrospira maxima*. Ananyev, G., C. Gates, and G.C. Dismukes, *Photosynthetica* (Govindjee special edition), 2017. In press. Synopsis: Multiple active sites of bicarbonate functionality in PSII are definitively established. A new technique for selectively depleting bicarbonate from two classes of binding sites was demonstrated. Federal support acknowledged.
- (Presentation) A non-invasive fluorescence method for investigating S state transition times. By Colin Gates; presented at the 2017 Eastern Regional Photosynthesis Conference, Woods Hole, MA. Awarded; Federal support acknowledged.

Websites

- Hardware: <https://www.waksman.rutgers.edu/dismukes/pages/instrument>
- Software: <http://chem.rutgers.edu/dismukes-developed-software-available-research>

Participants/Organizations

What individuals have worked on the project?

Name	Most Senior Project Role	Nearest Person Month Worked
Dismukes, G. Charles	PD/PI	1
Ananyev, Gennady	Co PD/PI	3
Gates, Colin	Graduate Student (research assistant)	6
Khue Tu Ho-Nguyen	Graduate Student (research assistant)	1
Jonah Williams	Undergraduate Student	1
Brendan Cullinane	Undergraduate Student	8
Areeb Zaidi	Undergraduate Student	1

Full details of individuals who have worked on the project:

G. Charles Dismukes

Email: dismukes@chem.rutgers.edu

Most Senior Project Role: PD/PI

Nearest Person Month Worked: 1

Contribution to the Project: Principal investigator

Funding Support: Rutgers

International Collaboration: No

International Travel: No

Gennady M Ananyev

Email: ananyev@waksman.rutgers.edu

Most Senior Project Role: Co PD/PI

Nearest Person Month Worked: 3

Contribution to the Project: Experimentation, training, data processing, writing

Funding Support: this grant

International Collaboration: No

International Travel: No

Colin Gates

Email: cmgates@rutgers.edu

Most Senior Project Role: Graduate Student (research assistant)

Nearest Person Month Worked: 6

Contribution to the Project: Experimentation, training, data processing, writing

Funding Support: Rutgers teaching assistantship

International Collaboration: No

International Travel: No

Khue Tu Ho-Nguyen

Email: kt.hn@rutgers.edu

Most Senior Project Role: Graduate Student (research assistant)

Nearest Person Month Worked: 1

Contribution to the Project: Experimentation

Funding Support: Rutgers first-year fellowship

International Collaboration: No

International Travel: No

Jonah Williams

Email: jonah.williams96@gmail.com

Most Senior Project Role: Undergraduate student

Nearest Person Month Worked: 1

Contribution to the Project: Data processing

Funding Support: n/a

International Collaboration: No

International Travel: No

Brendan Cullinane

Email: btc46@chem.rutgers.edu

Most Senior Project Role: Undergraduate student

Nearest Person Month Worked: 8

Contribution to the Project: Experimentation, data processing

Funding Support: This grant

International Collaboration: No

International Travel: No

Areeb Zaidi

Email: arz31@scarletmail.rutgers.edu

Most Senior Project Role: Undergraduate student

Nearest Person Month Worked: 1

Contribution to the Project: Data processing

Funding Support: n/a

International Collaboration: No

International Travel: No

What other organizations have been involved as partners?

Name	Type of Partner Organization	Location
Arizona State University	Academic Institution	Tempe, AZ, USA

Full details of organizations that have been involved as partners:

Arizona State University

Organization Type: Academic Institution

Organization Location: Tempe, AZ, USA

Partner's Contribution to the Project:

Collaborative Research

More Detail on Partner and Contribution: Prof. Petra Fromme at ASU supplies us with PSII microcrystals; we characterize them biophysically for improved understanding of crystal function, especially under crystallographic conditions.

What other collaborators or contacts have been involved?

Aaron Kaplan – Hebrew University of Jerusalem, Israel. Solely involved in writing a paper during this time.

Derrick Kolling – Marshall University, WV, USA. We allowed him to use our equipment for his own projects.

Impacts

What is the impact on the development of the principal discipline(s) of the project?

- Improved understanding of the role of bicarbonate in PSII – a clearer answer to a 60-year-old question.
- Improved ability to examine PSII activity fluorometrically.
- Conclusively demonstrated the existence and relevance of PSII-cyclic electron flow.
- Characterized *Chlorella ohadii*, the world's fastest-growing phototroph.
- Inserted cyanobacterial-type PSII into a higher plant and characterized functionality and regulation.

What is the impact on other disciplines?

Broad translational impact on agriculture/biomass, bioproduction, bioenergy, and catalyst development.

What is the impact on the development of human resources?

Two graduate students and three undergraduate student trainees.

What is the impact on physical resources that form infrastructure?

Nothing to report.

What is the impact on institutional resources that form infrastructure?

Nothing to report.

What is the impact on information resources that form infrastructure?

Nothing to report.

What is the impact on technology transfer?

Nothing to report.

What is the impact on society beyond science and technology?

Nothing to report.

Changes/Problems

Changes in approach and reason for change

Switched collaborators for comparison of PSII-containing biological samples due to original collaborator's lack of enthusiasm.

Actual or Anticipated problems or delays and actions or plans to resolve them

Discovered a high degree of entanglement between PSII operation on donor and acceptor sides which prevented complete construction of a VZAD model for fluorescence. Resolution may require chemical supplementation of samples to be examined.

Changes that have a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use or care of biohazards

Nothing to report.

SPECIAL REPORTING REQUIREMENTS:

None.

BUDGETARY INFORMATION:

To be completed by Contracting Officer.