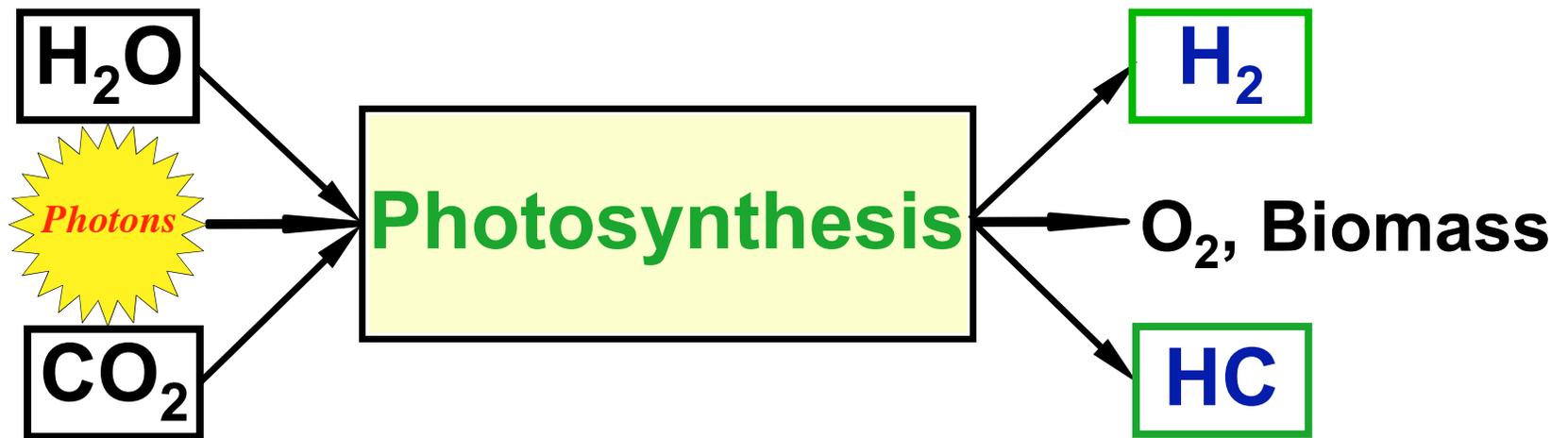


Photosynthesis for Hydrogen and Fuels Production

**Tasios Melis,
UC Berkeley
24-Jan-2011**

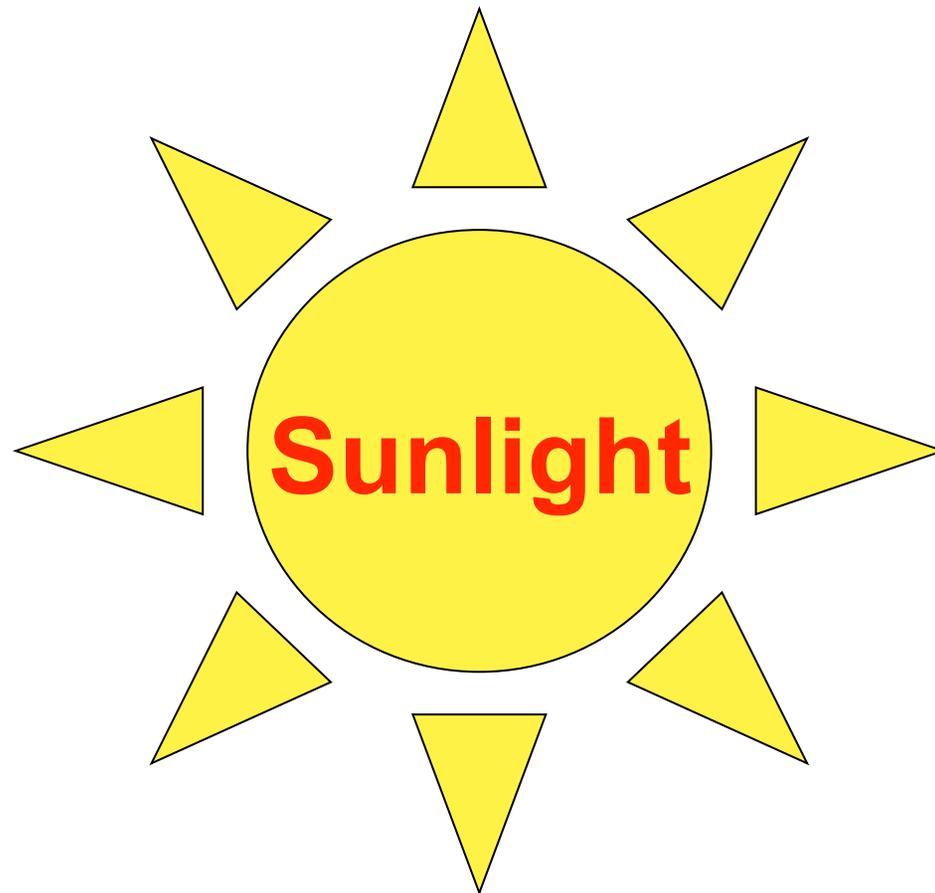


Feedstock and products



Process offers a renewable fuels supply
and mitigation of climate change.





Average US Solar insolation = 5 kWh m⁻² d⁻¹
CA household electricity consumption = 15 kWh d⁻¹



The relevant literature

Plant, Cell and Environment (2006) **29**, 315–330

doi: 10.1111/j.1365-3040.2005.01493.x

Can improvement in photosynthesis increase crop yields?

STEPHEN P. LONG¹, XIN-GUANG ZHU¹, SHAWNA L. NAIDU¹ & DONALD R. ORT²

¹*Departments of Crop Science and Plant Biology, University of Illinois, Urbana, IL 61801, USA, and* ²*Photosynthesis Research Unit, USDA-ARS, Urbana, IL 61801, USA*

“Six potential routes of increasing epsilon(c) by improving photosynthetic efficiency were explored, ranging from altered canopy architecture to improved regeneration of the acceptor molecule for CO₂. Collectively, these changes could improve epsilon(c) and, therefore, Y-p by c. 50%.”

**Gains upon improving the carbon reactions
of photosynthesis: up to 50%**



The relevant literature

Plant Science 177 (2009) 272–280



Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci



Review

Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency

Anastasios Melis *

Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA

Gains upon improving sunlight conversion efficiency: up to 300%





DOE Hydrogen Program

Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

**R&D project funded by the DOE-EERE
Hydrogen Program**

This presentation does not contain any proprietary, confidential, or otherwise restricted information



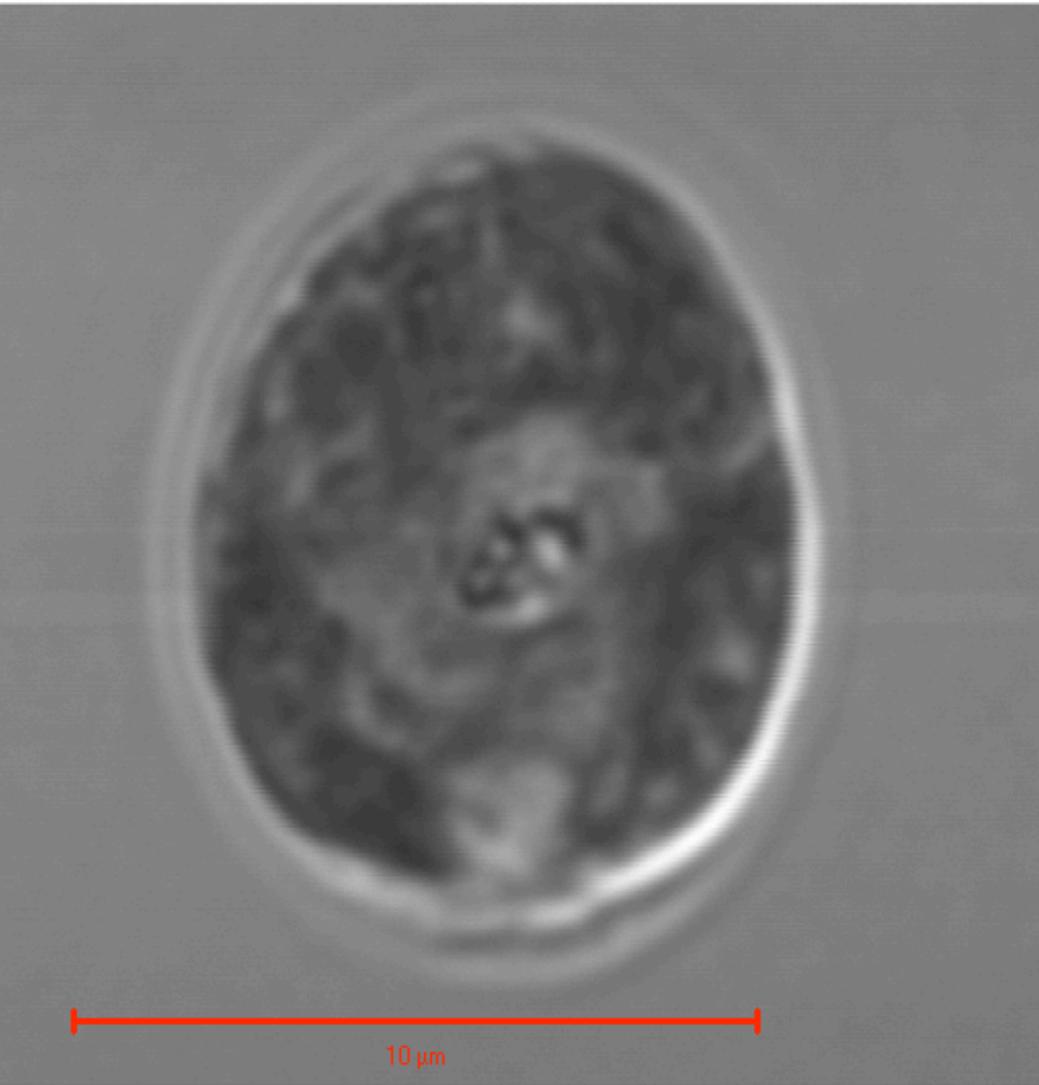
View of a microalga cross-section

The unicellular green alga
Chlamydomonas reinhardtii

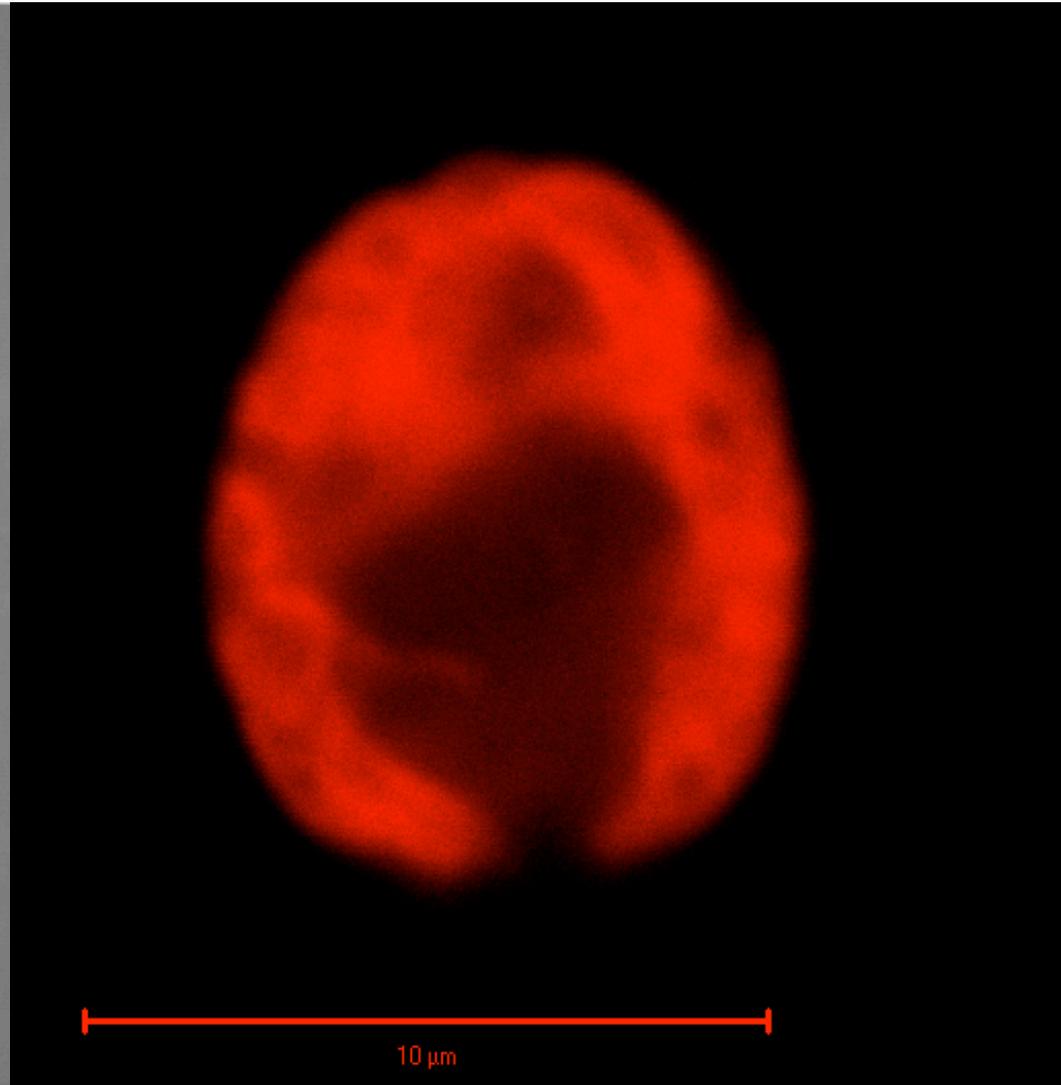


Self-repairing and
replicating microstructure

The green microalga *Chlamydomonas reinhardtii*

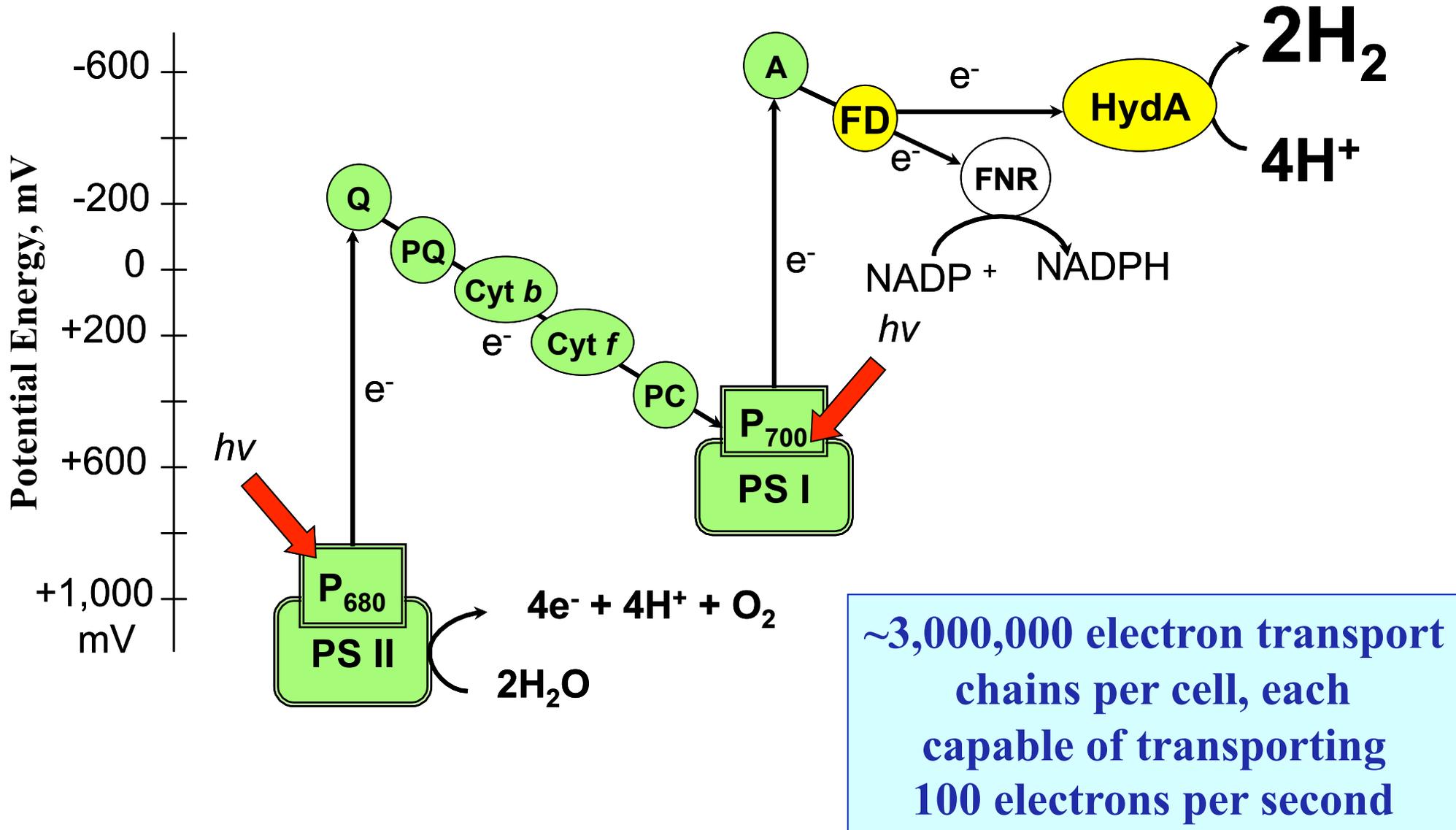


Differential Interference Contrast

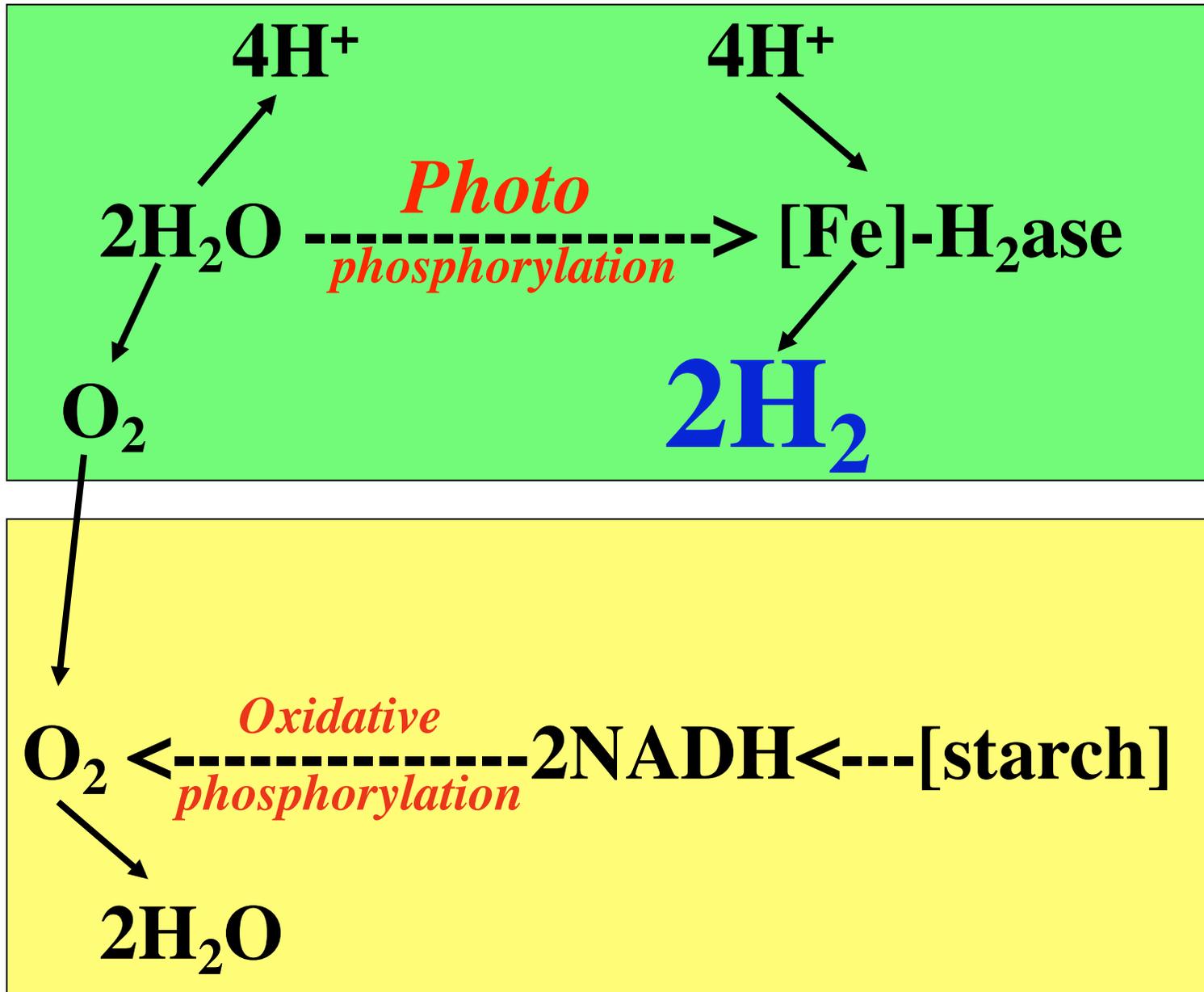


Chlorophyll Fluorescence

Photosynthetic water oxidation and H₂-production



Hypoxic photosynthesis: O_2 is consumed by mitochondria





H₂ bubbles



Microalgal H₂-production facility in the laboratory



UCB-Melis



vent



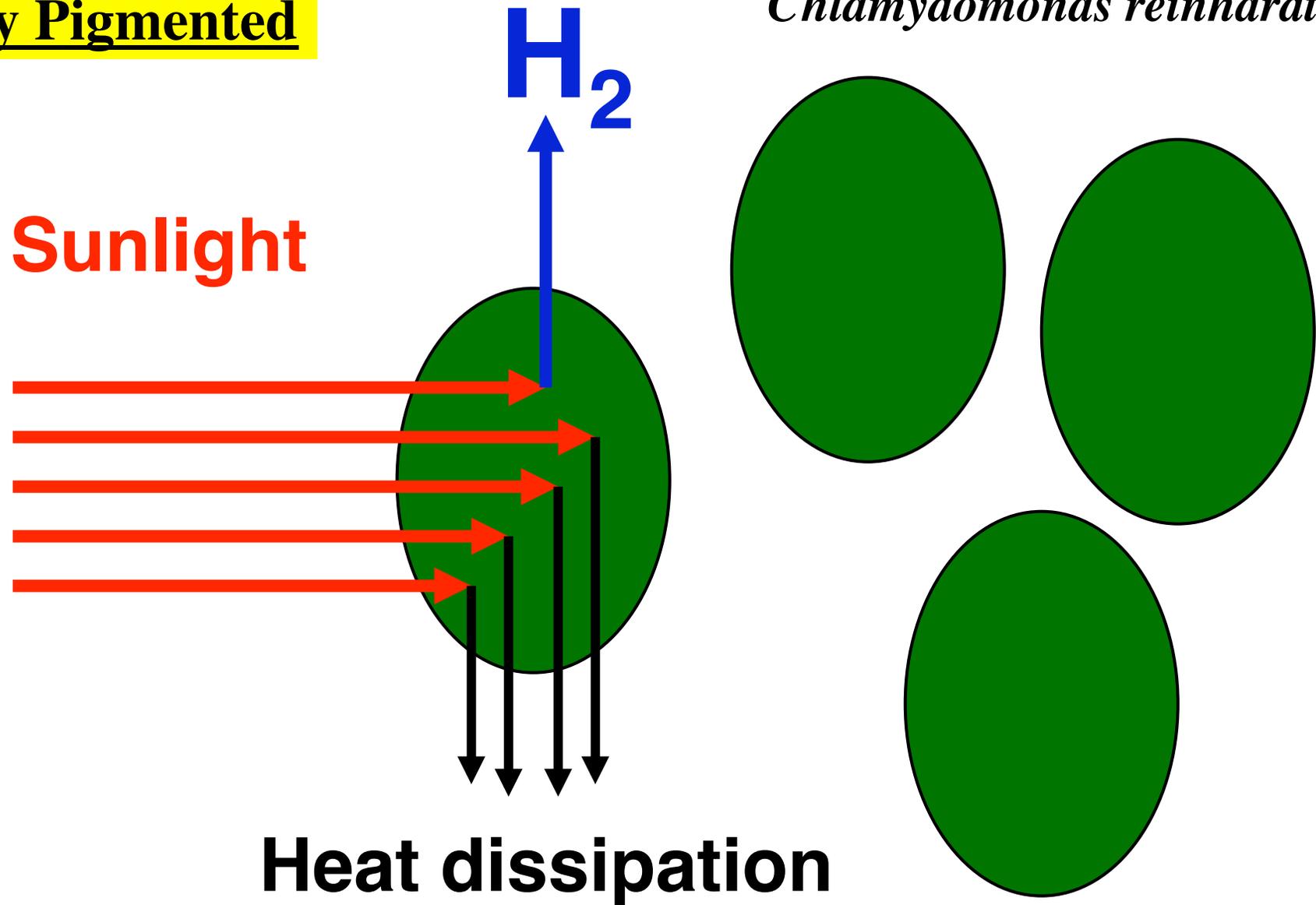
**Hydrogen production
in a backyard**

Chlamydomonas reinhardtii mass culture



Example: Cells
Fully Pigmented

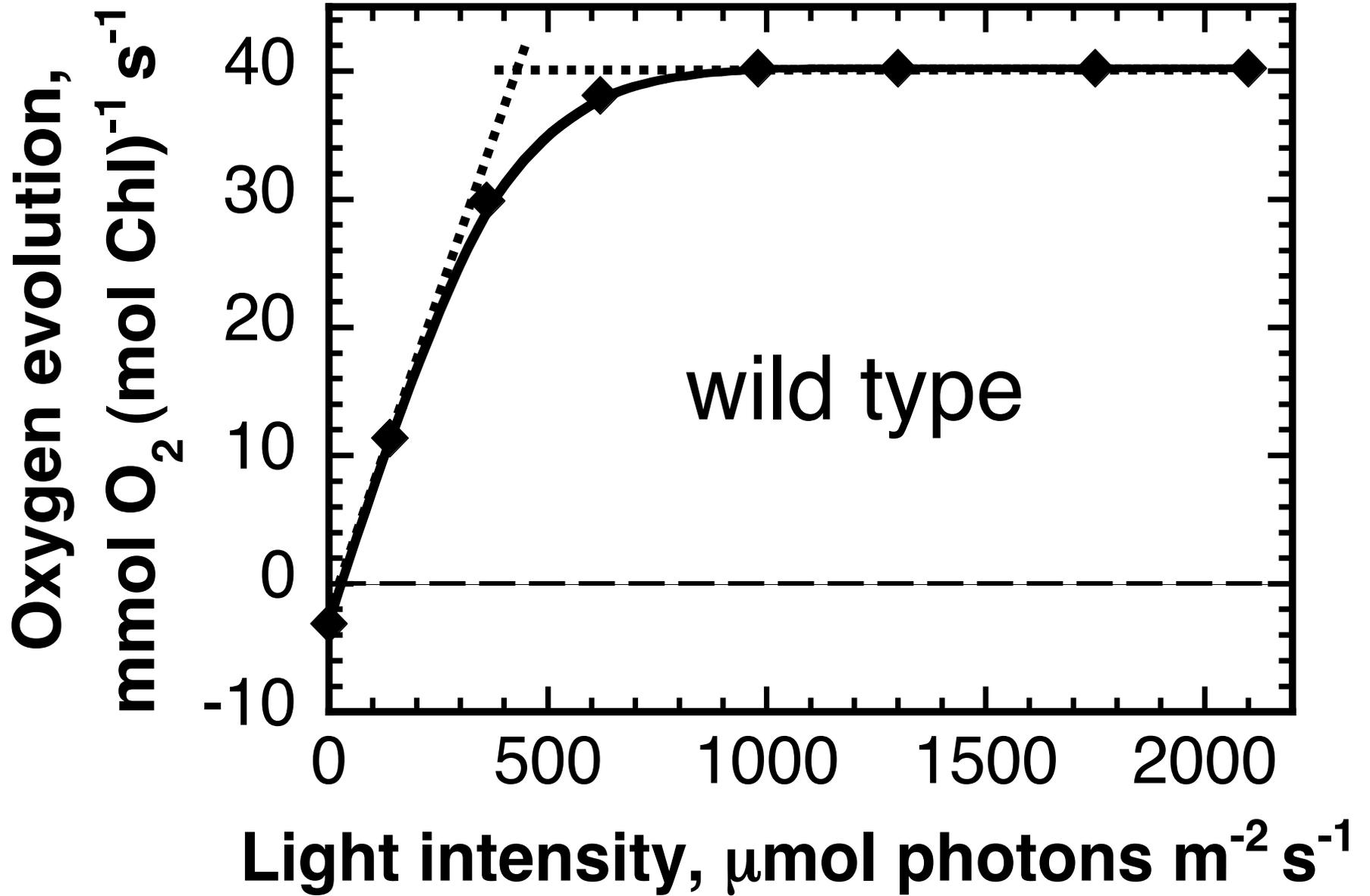
The green algae
Chlamydomonas reinhardtii

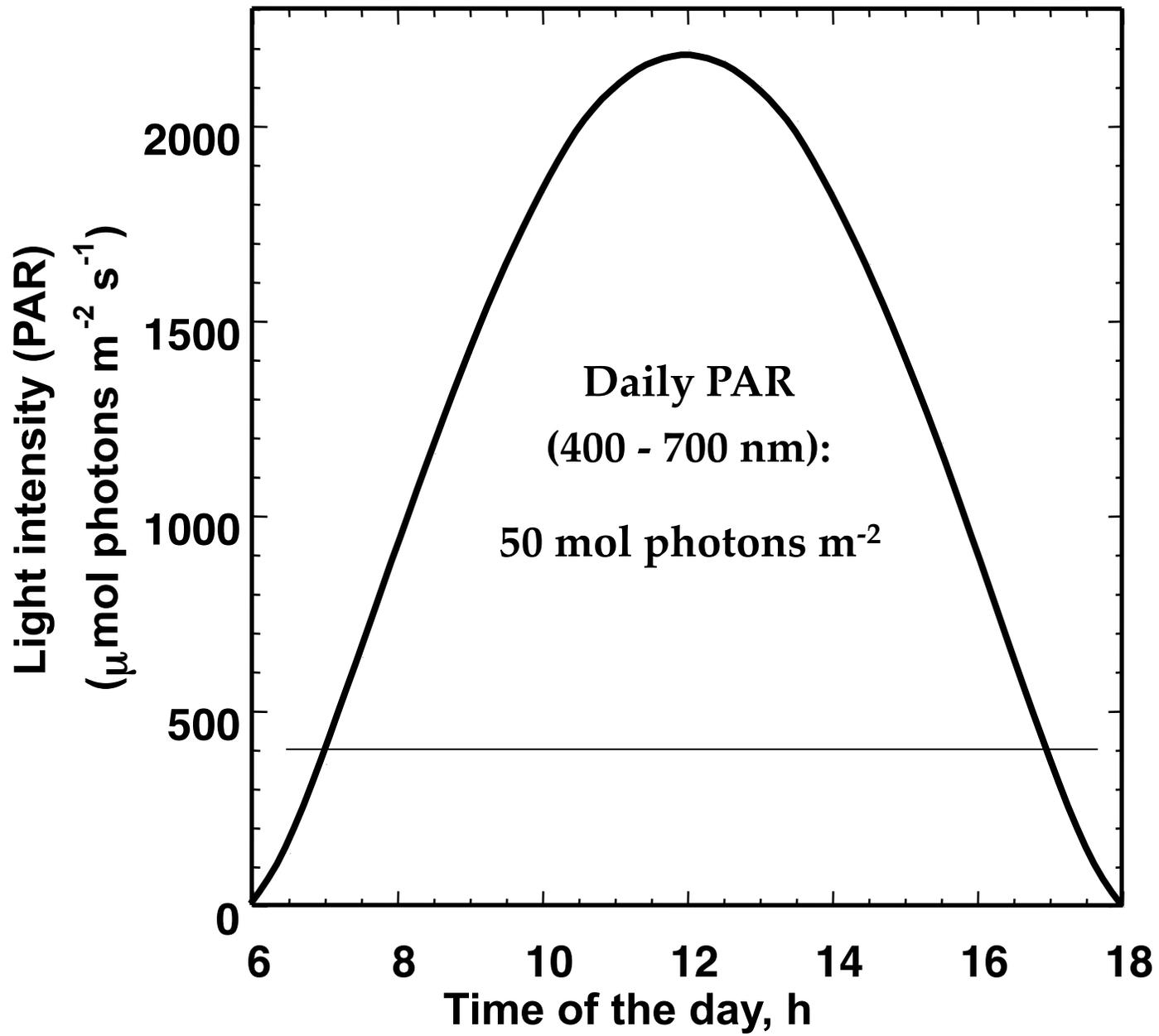


Fully pigmented cells over-absorb and wastefully dissipate bright sunlight



The light-saturation curve of photosynthesis





The problem of the early light-saturation of photosynthesis

Severe: *Green and purple bacteria*

Cyanobacteria & red algae

Green & brown algae

Less severe: *C3 plants & C4 plants*



Photosynthetic Productivities

Theoretical productivity:

~75 g dry weight m⁻² d⁻¹

(8-10% solar energy conversion efficiency)

(based on the average US solar insolation = 5 kWh m⁻² d⁻¹)



Photosynthetic Productivities

Theoretical productivity:

~75 g dry weight $\text{m}^{-2} \text{d}^{-1}$

(8-10% solar energy conversion E)

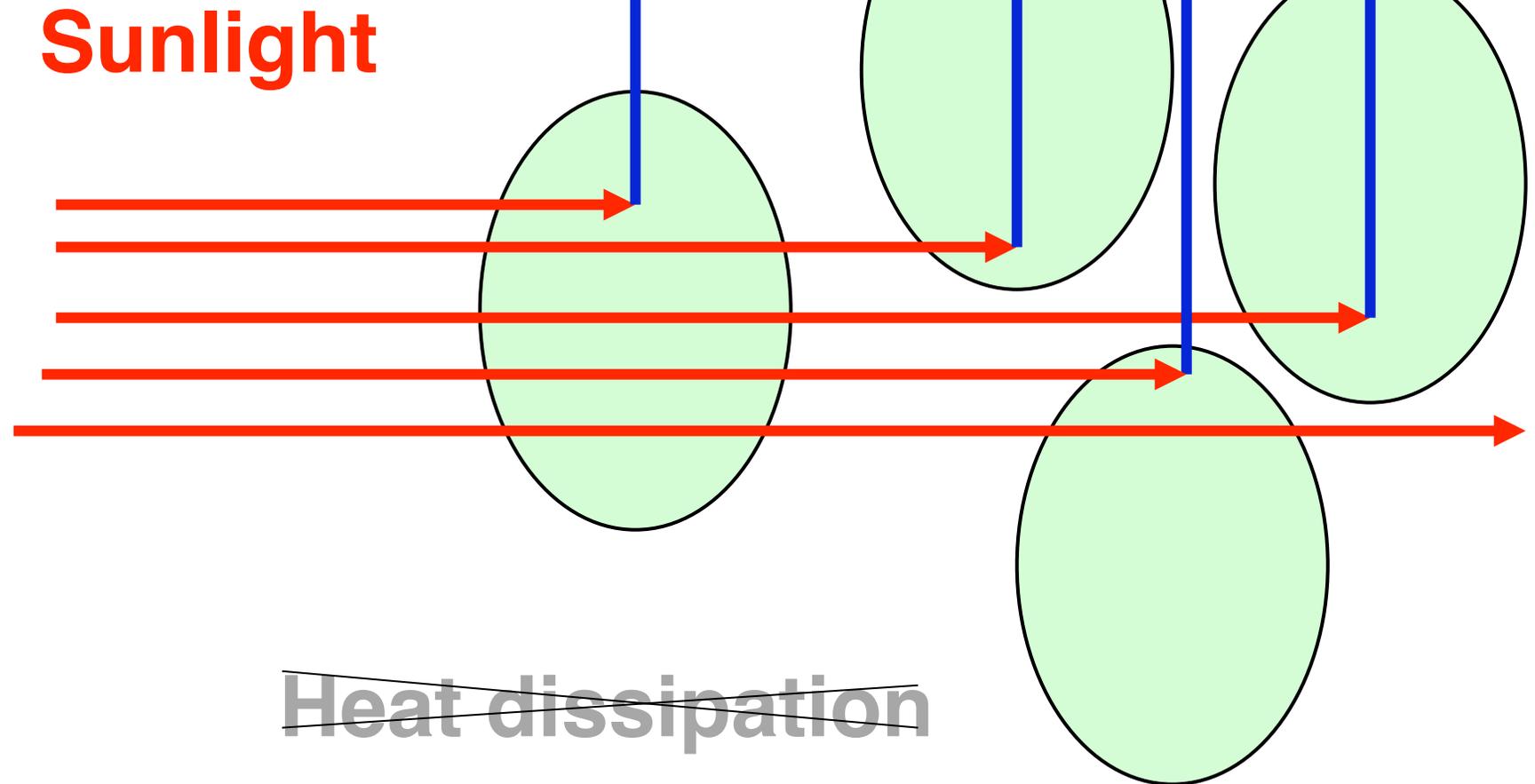
Measured productivities:

Less than 25 g dry weight $\text{m}^{-2} \text{d}^{-1}$

(3-4% solar energy conversion E)



Example: Truncated Chl Antenna Size



Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture



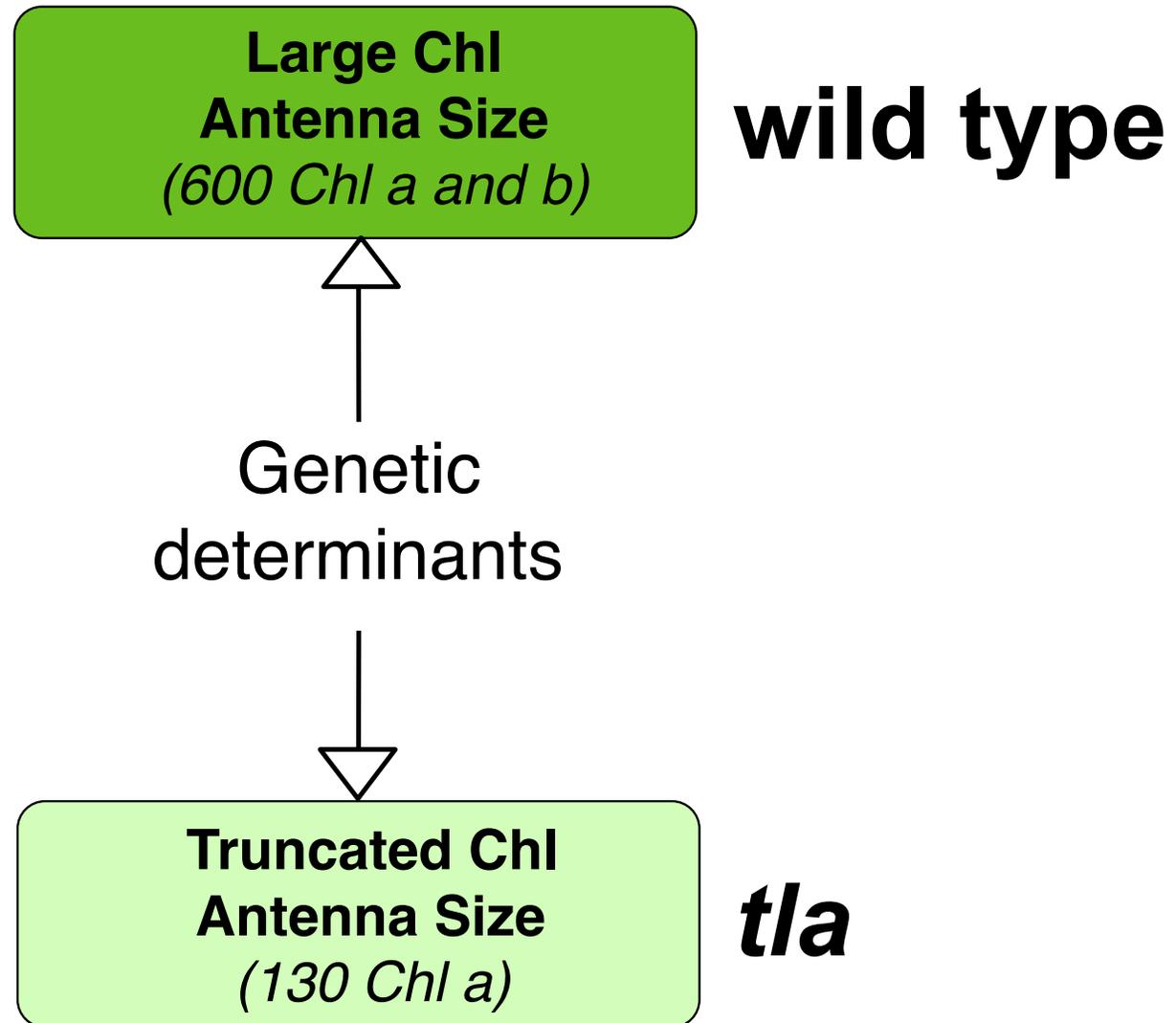
The *Tla* concept

(*Tla* = Truncated light-harvesting antenna)

Minimize the chlorophyll antenna size of photosynthesis to prevent the early light-saturation effect.



Regulation of the Chl antenna size



Interference with the genetic mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.



Approach

- **Identify genes that confer a truncated antenna in a model organism.**
- **Apply these genes to other organisms of interest.**
- **Improve photosynthesis, hydrogen, or fuels production by up to 300%.**



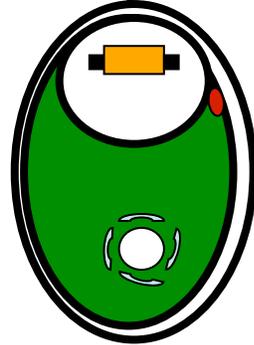
DNA insertional mutagenesis and screening

ARG7

Plasmid DNA

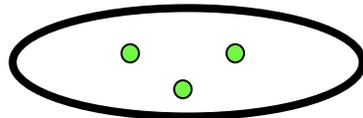
TRANSFORMATION

(Random insertion of plasmid DNA
in the nuclear genome)



Chlamydomonas reinhardtii

GROW TRANSFORMANTS

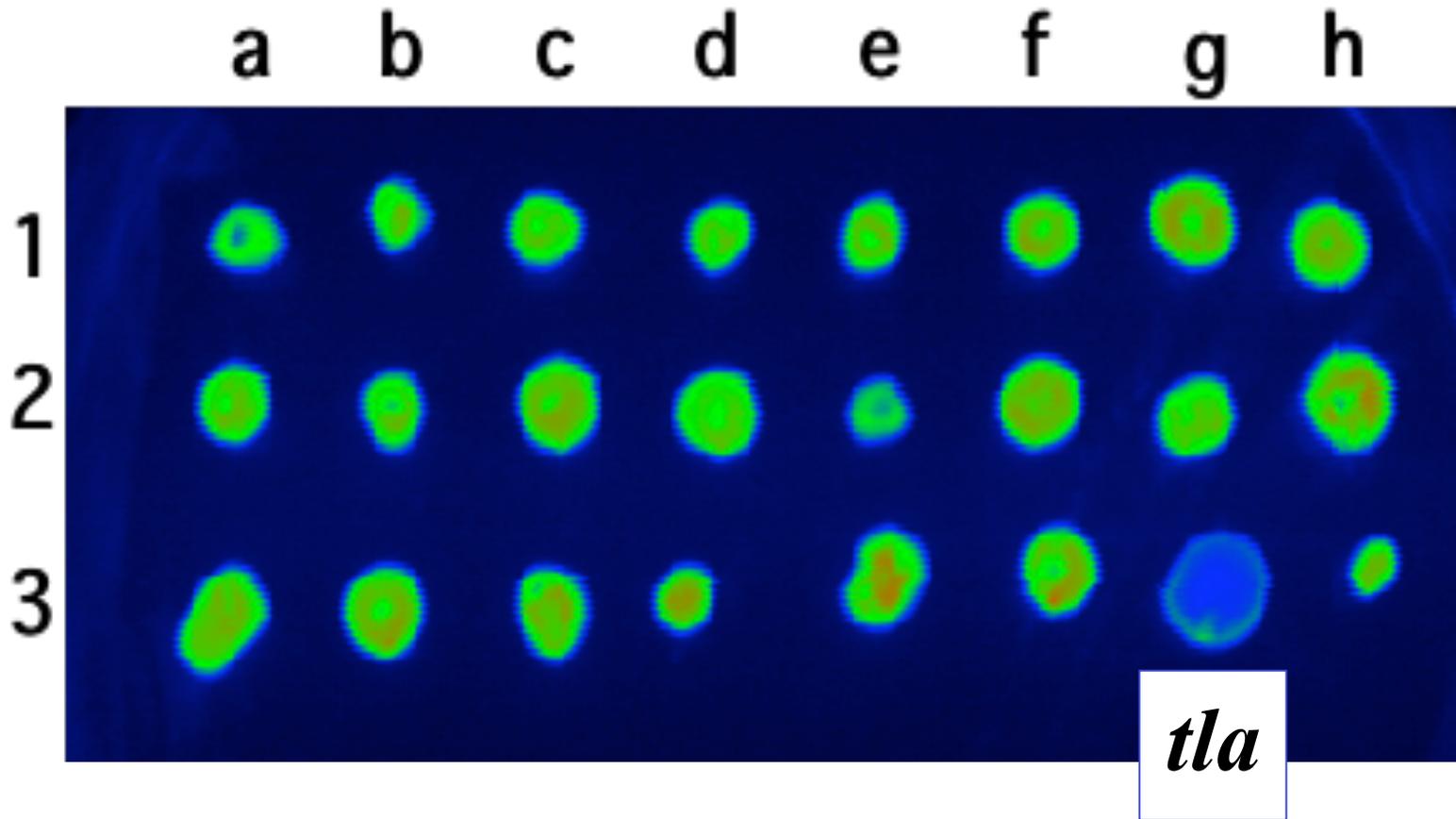


SCREEN TRANSFORMANTS

SELECT TRUNCATED ANTENNA STRAINS



DNA insertional mutagenesis and screening

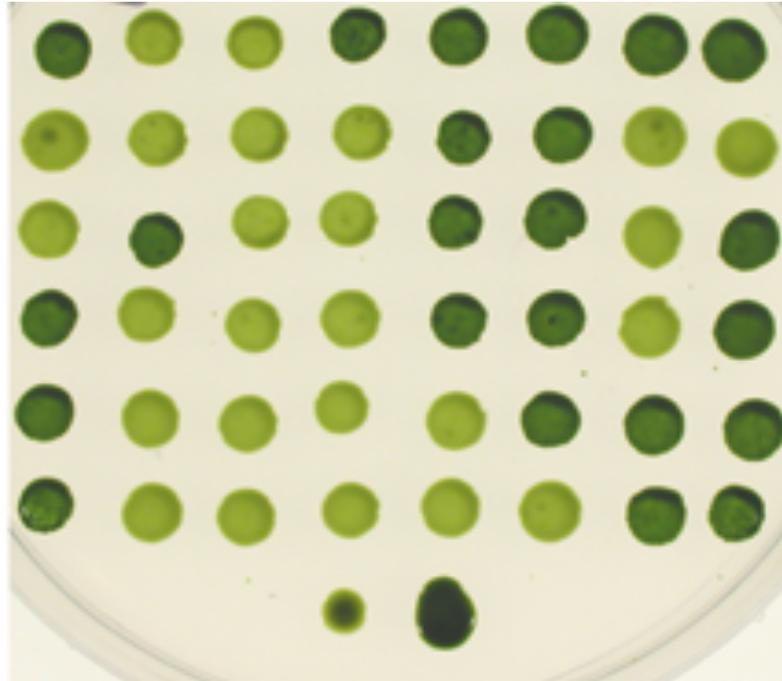


Chlorophyll *a* fluorescence imaging analysis

UCB-Melis



DNA insertional mutagenesis and screening for *truncated light-harvesting antenna (tla)* mutants



**Dot-Spot Colonies of
*Chlamydomonas reinhardtii***

Secondary screening for *tla* mutants

Criteria:

- Functional photosystem antenna size *smaller than wild type*
- Number of photosystems per chloroplast should be *the same or greater than wild type*
- High quantum yield of photosynthesis is maintained
- Photosynthesis & productivity per chlorophyll: *inversely proportional to antenna size*

Objective:

- Identify “true positive” *tla* mutants with **improved sunlight utilization efficiency**.



Progress achieved vs targets set

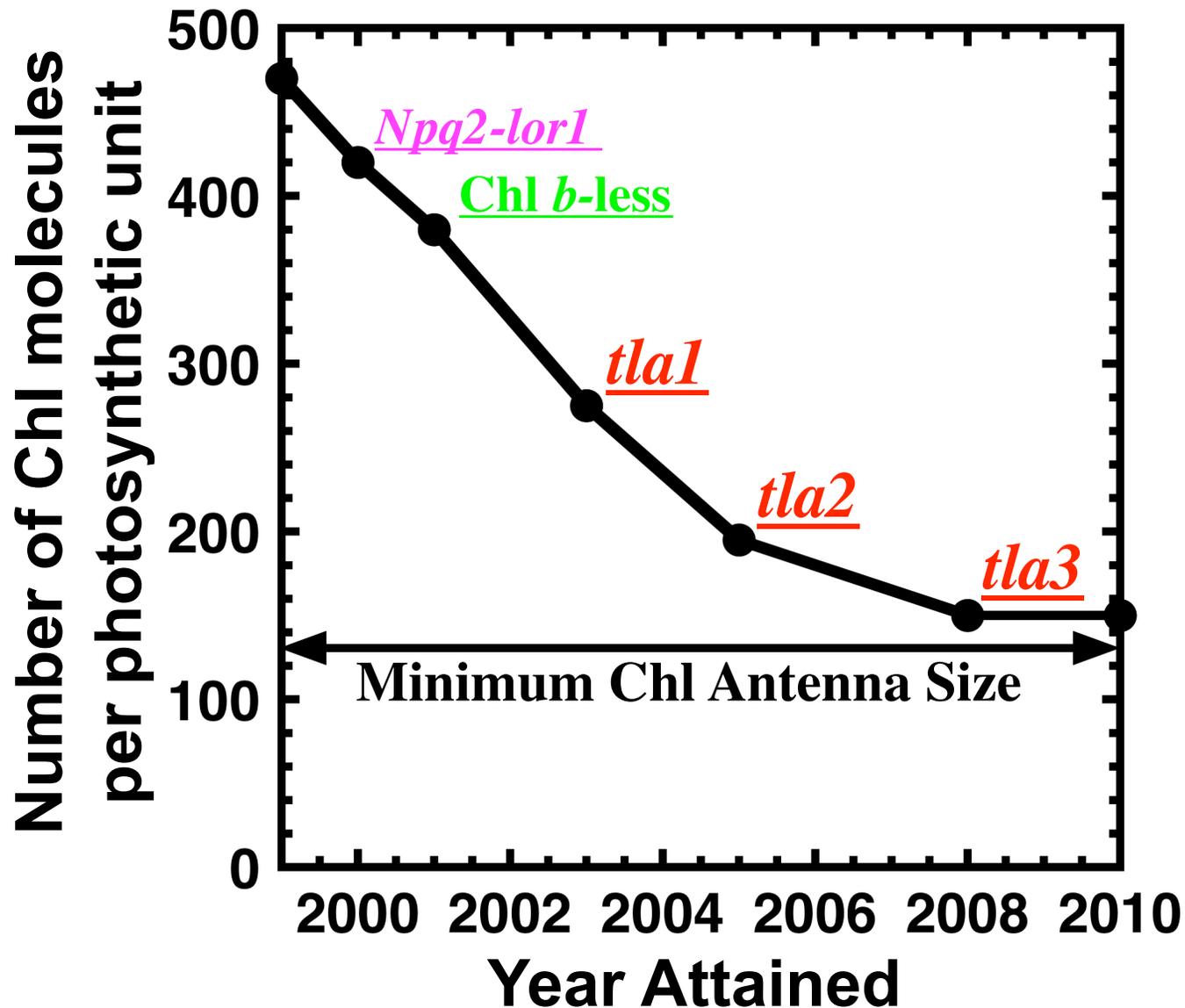
Chlorophyll antenna size in wild type and mutants

	2000	2003	2005	2008	2010	2015
Targets <i>(Chl Antenna size)</i>	600 (WT)		300		200	150
Progress Achieved	600 (WT)	275 <i>tla1</i>	195 <i>tla2</i>	150 <i>tla3</i>		

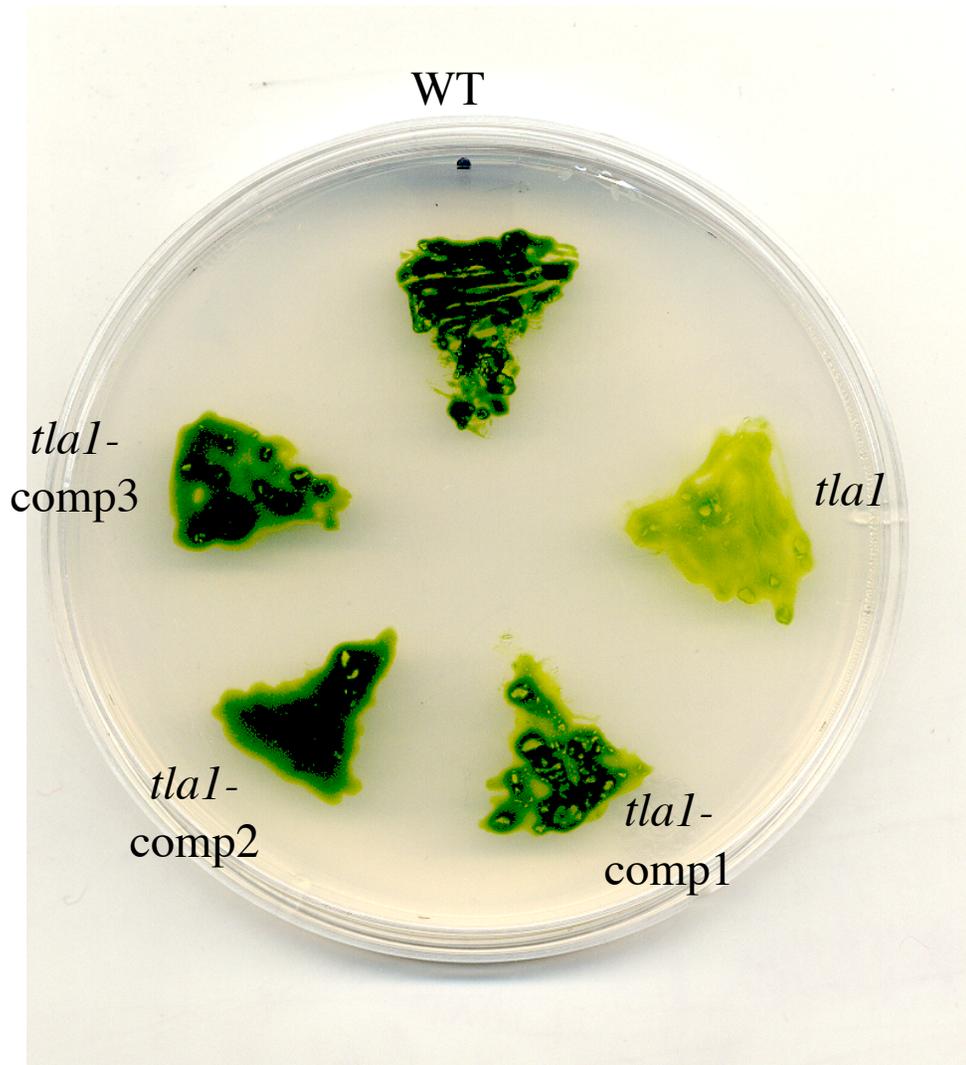


Project Timeline

Chlorophyll Antenna Size in *Chlamydomonas*



Chlamydomonas tla1 mutant phenotype and its complementation



Complemented *tla1* strains with the wild type *Tla1* gene (*tla1*-comp1, *tla1*-comp2, and *tla1*-comp3) recovered the green pigmentation phenotype.

Photosynthetic unit chlorophyll antenna size of wild type and *tla1* mutant

Wild type Chl Antenna Size

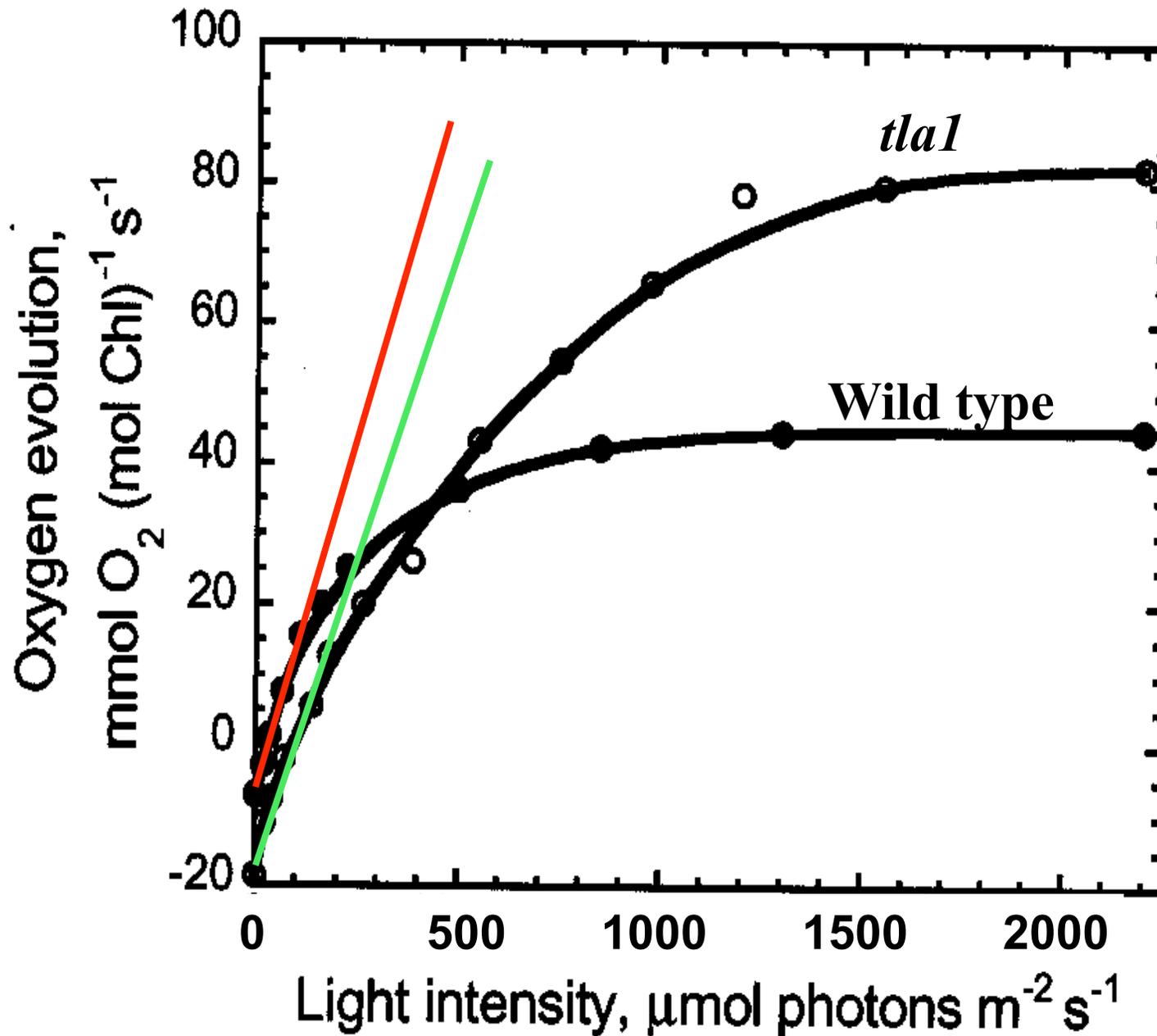
- ~ 600 Chl *a* + *b* molecules

tla1 mutant Chl antenna size

- 300 Chl *a* + *b* molecules



The light-saturation curve of photosynthesis

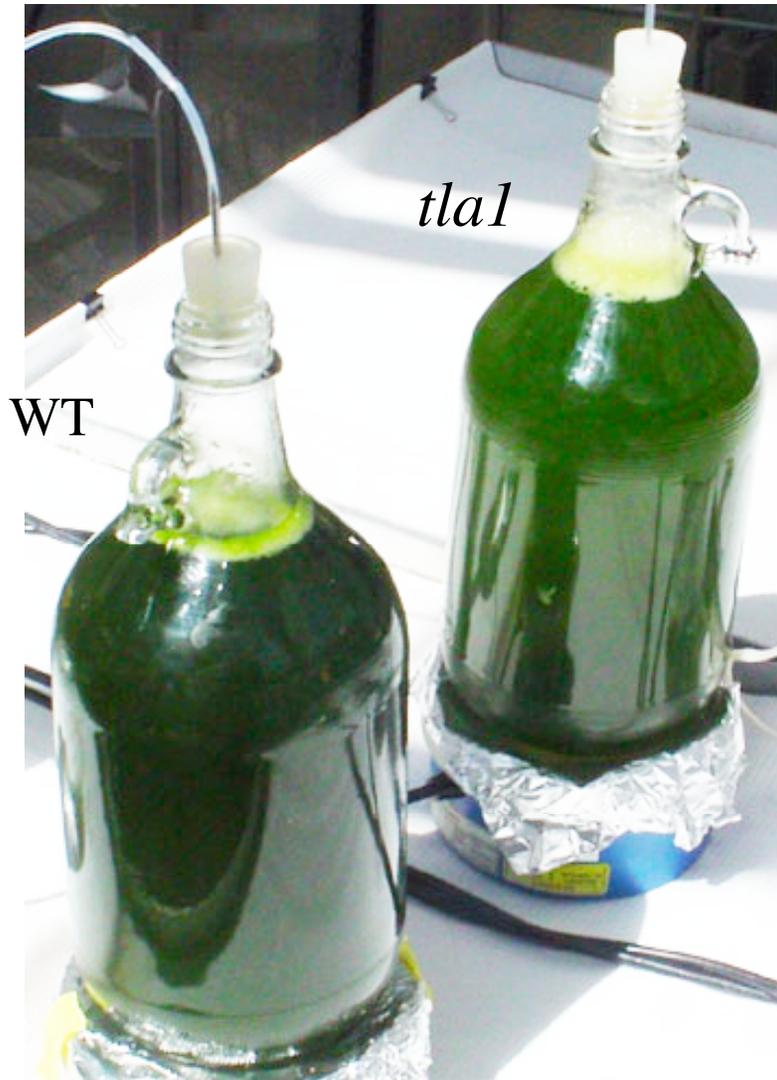


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Productivity in Mass Culture

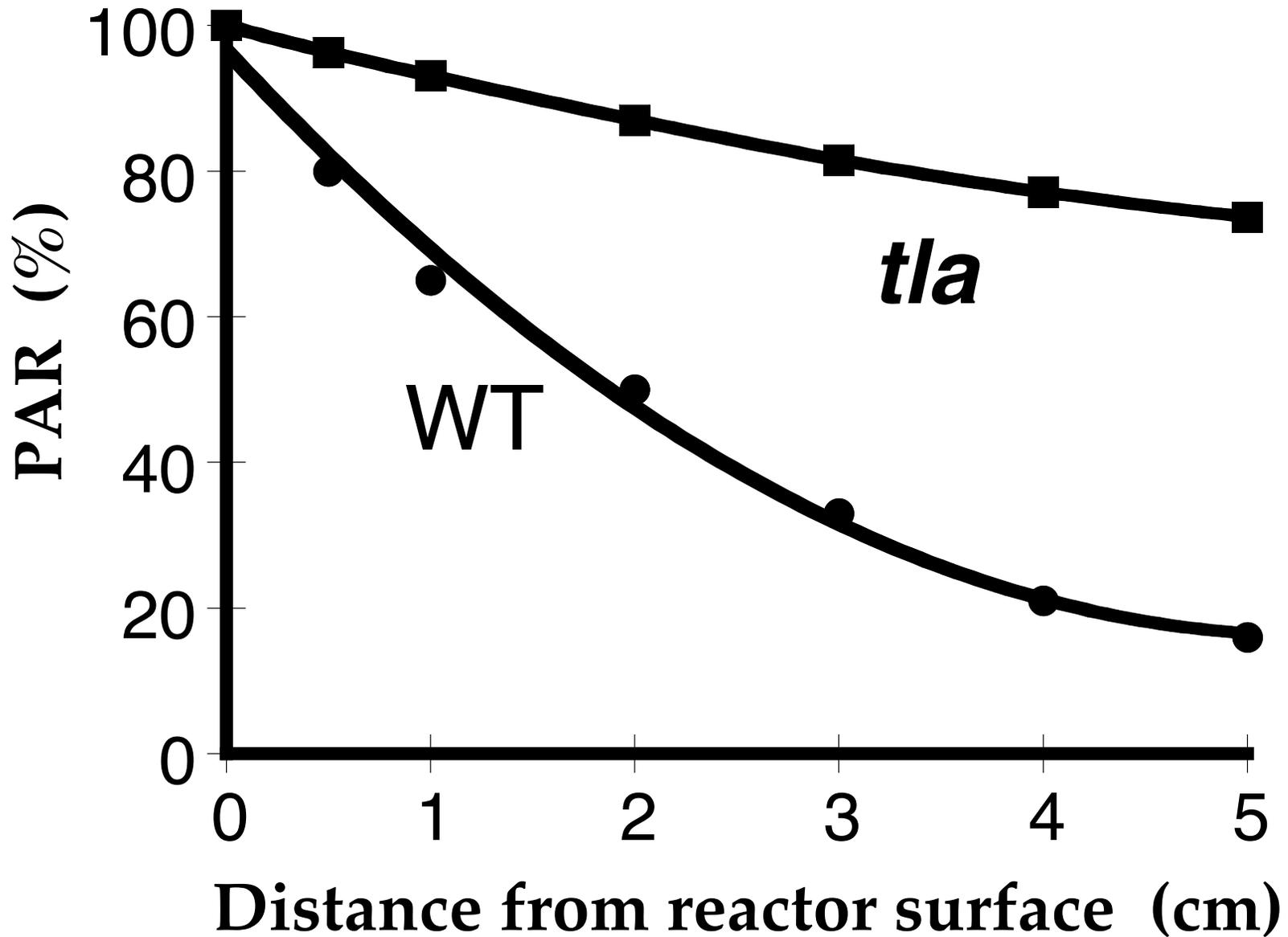
Cultures in the Greenhouse



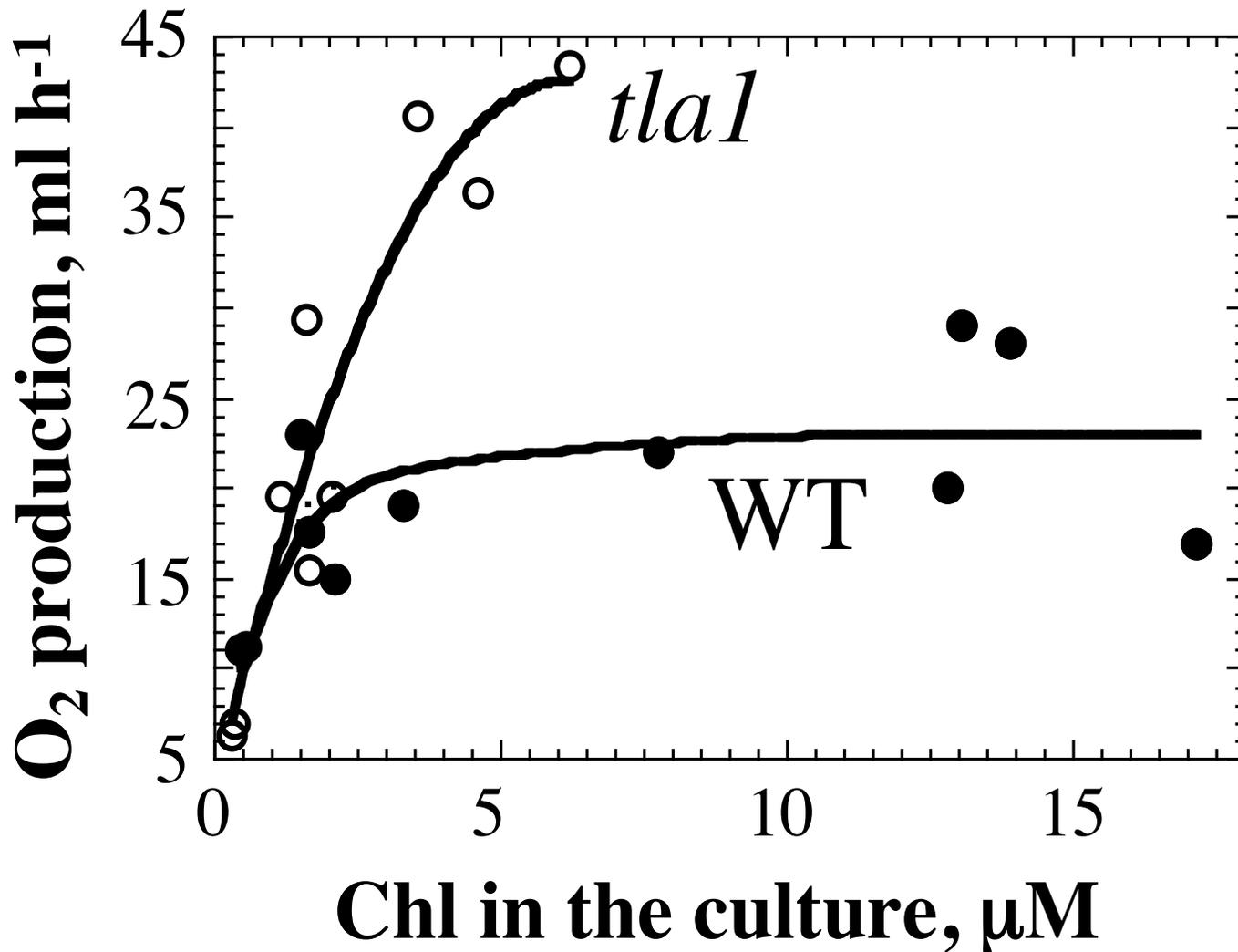
<u>Parameter</u>	<u>WT</u>	<u>tla1</u>
Cell/mL (x10 ⁶)	6.36	10.0
[Chl] (uM)	25.6	15.4

The *tla1* strain shows greater productivity than the wild type cells under bright sunlight conditions.
(Note relative amounts of gas bubbles produced by the two samples.)

Gradient of sunlight penetration through a high density wild type (WT) and *tla* culture



Photosynthetic productivity of wild type (WT) and *tla1* mutant

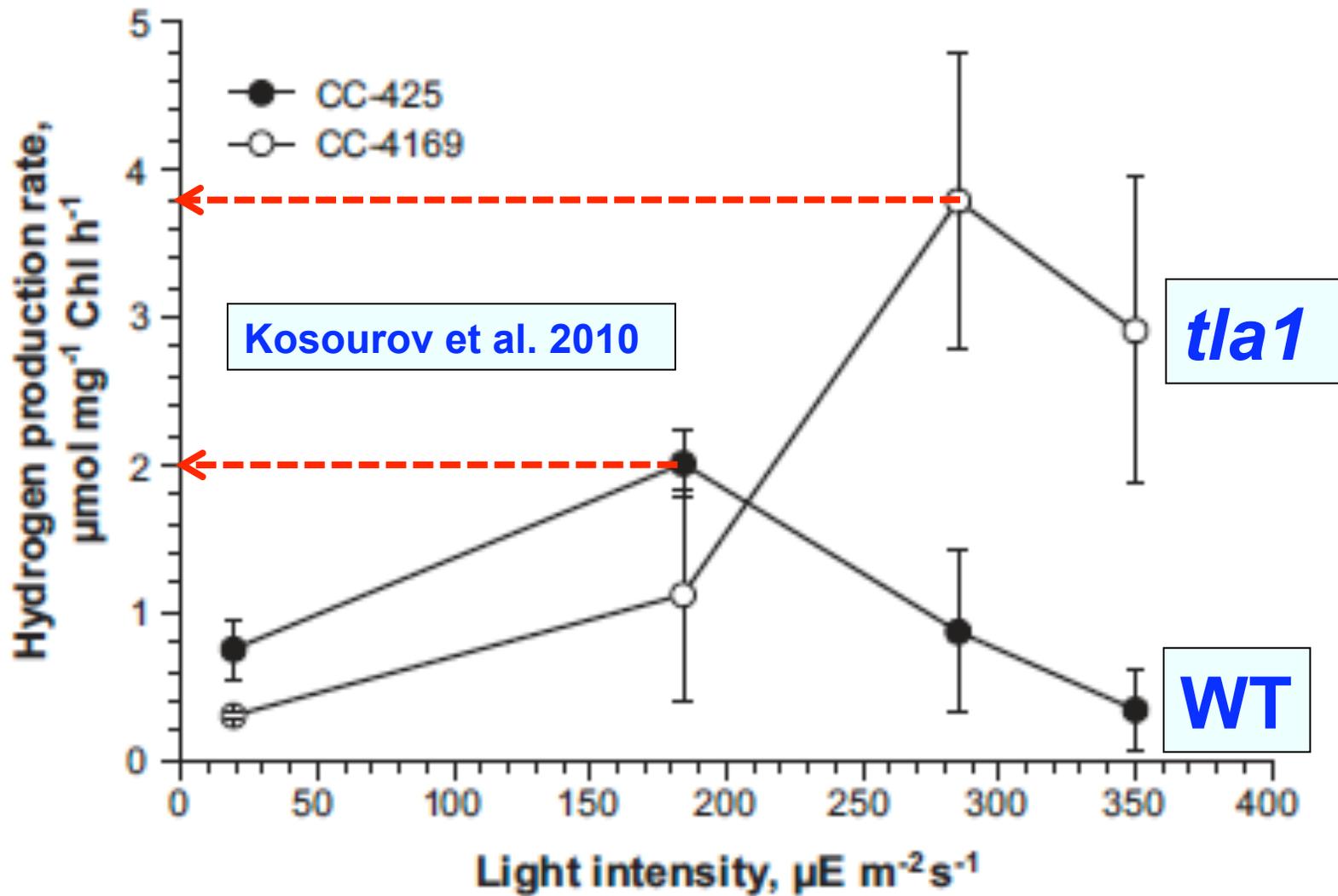


From Concept to Application

- The *Tla* concept is commercially applied in green microalgae:
 - *Chlamydomonas* for biomass production; and
 - *Nannochloropsis* for commercial production of polyunsaturated fatty acids (PUFAs).
- The *tla1 mutant* strain was requested and acquired by universities (x5), industry (x5), and government labs (x4).
- Successful application of the *Tla1* gene at NREL for enhanced H₂-production.



Light intensity-dependent rate of H₂-production by immobilized wild type (CC-425) and *tla1* antenna mutant (CC-4169)



Increased *Tla* awareness in the field

Many labs in several countries are now engaged in *tla* research.

(green microalgae, cyanobacteria, photosynthetic bacteria)



Related DOE workshops

- 1) Office of Basic Energy Sciences workshop on
“What is the Efficiency of Photosynthesis?”
May 23-24, 2009
- 2) ARPA-E workshop, "Applied Biotechnology
for Transportation Fuels: Meeting Today's
Energy Needs by Maximizing Photon Capture".
December 2-3, 2010



Summary and Conclusions

A *Tla* property in mass culture:

- ***Prevents the early light-saturation of photosynthesis.***
- ***Facilitates better sunlight penetration.***
- ***Enhances solar energy conversion efficiency and productivity (up to 300% in green microalgae).***



Future Tla extensions

- ***Development of non-genetic approaches to generating Tla strains.***
- ***Application of Tla technologies for biomass, H₂ and fuels production in cyanobacteria and photosynthetic bacteria.***
- ***Extension of Tla R&D to include generation of cell-fuel* molecules for application in fuel-cells.***

*** cell-fuels = small-size high-energy bio-products**



Thank You for Listening!

