Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

Tasios Melis

University of California - Berkeley

Thursday, 19 June 2014

Project ID # PD036

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

Overview

Timeline & Budget

- Project Start Date: 12/01/2004
- Project End Date: 01/31/2014
- No-cost extension: 09/30/2014
- Completion: 95%
- Total Funding Spent: $2,057 k
- Total Project Value: $2,175 k
- Cost Share Percentage: 20% of the total

Barriers

- Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier AN).

Consultations/ Beneficiaries

- The microalgal and cyanobacterial biotechnology field.

Relevance

The TLA concept

(TLA = Truncated Light-harvesting Antenna):

Minimize the light-harvesting antenna size of the photosystems to prevent over-absorption of sunlight and the ensuing wasteful dissipation of excess absorbed energy.

Relevance

Improves the sunlight-utilization efficiency and productivity of photosynthesis in microalgal and cyanobacterial cultures.

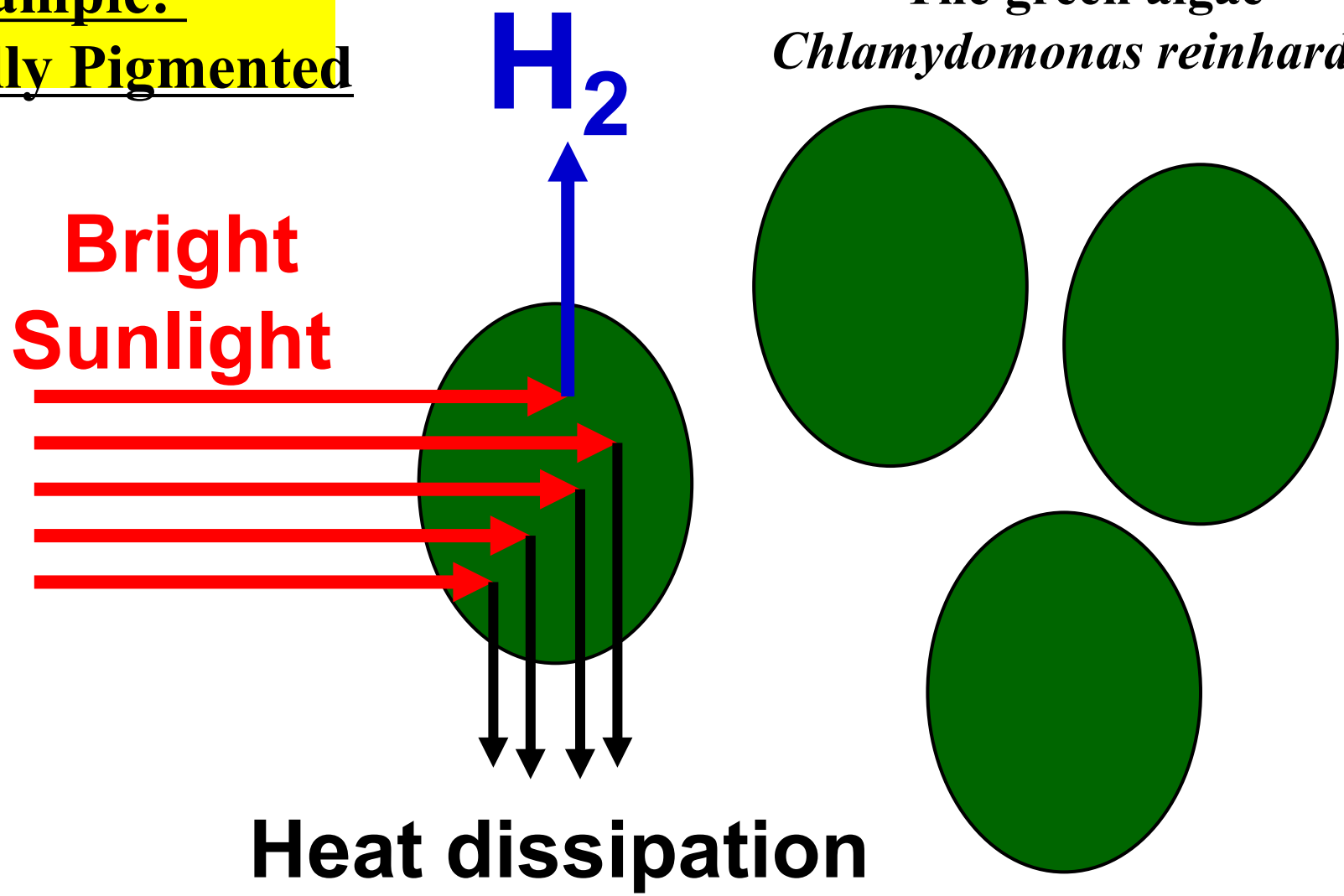
Specific Objectives in this Review Cycle

Assess the applicability of the TLA concept in cyanobacteria.

Relevance

Example:
Fully Pigmented

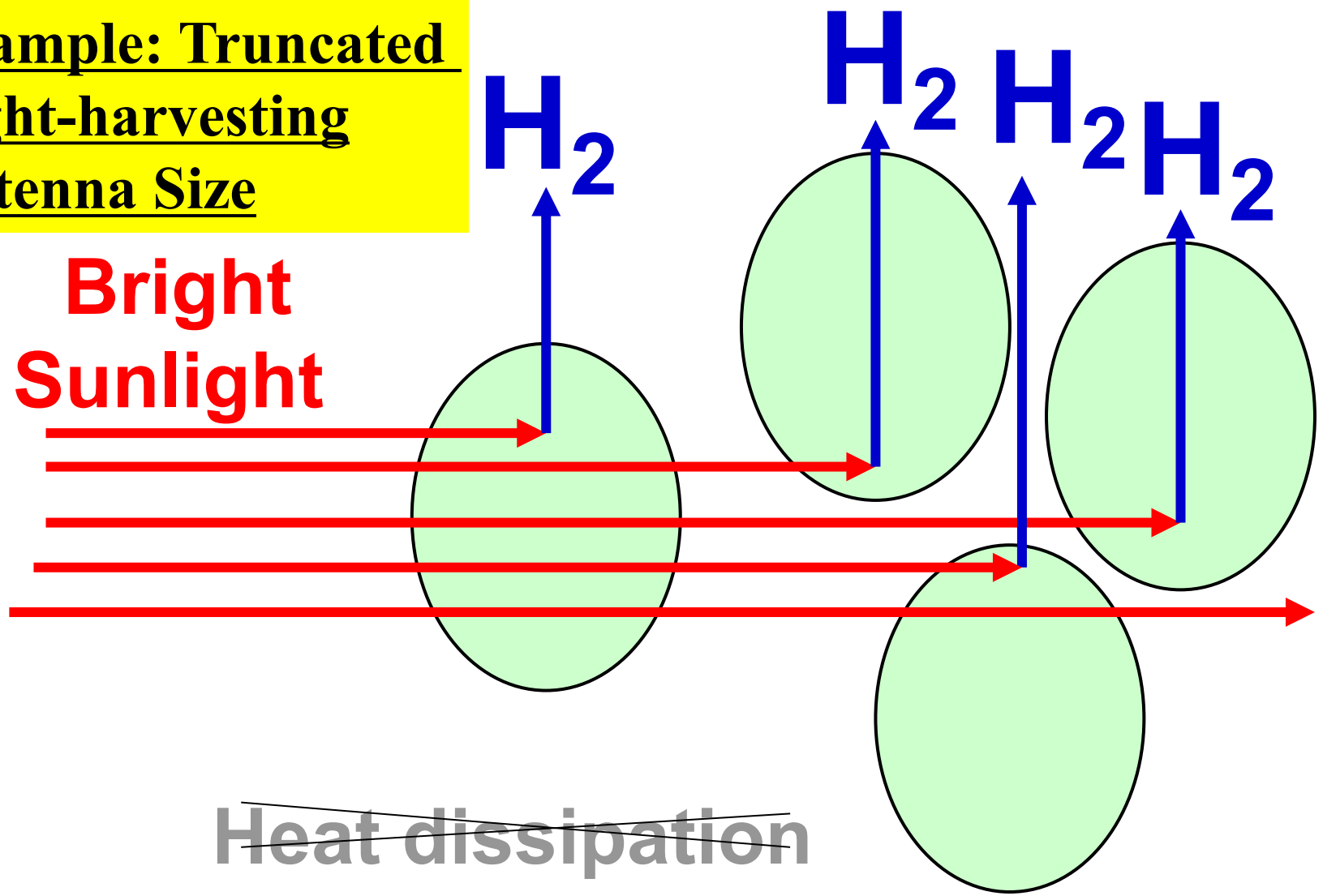
The green algae
Chlamydomonas reinhardtii



Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.

Relevance

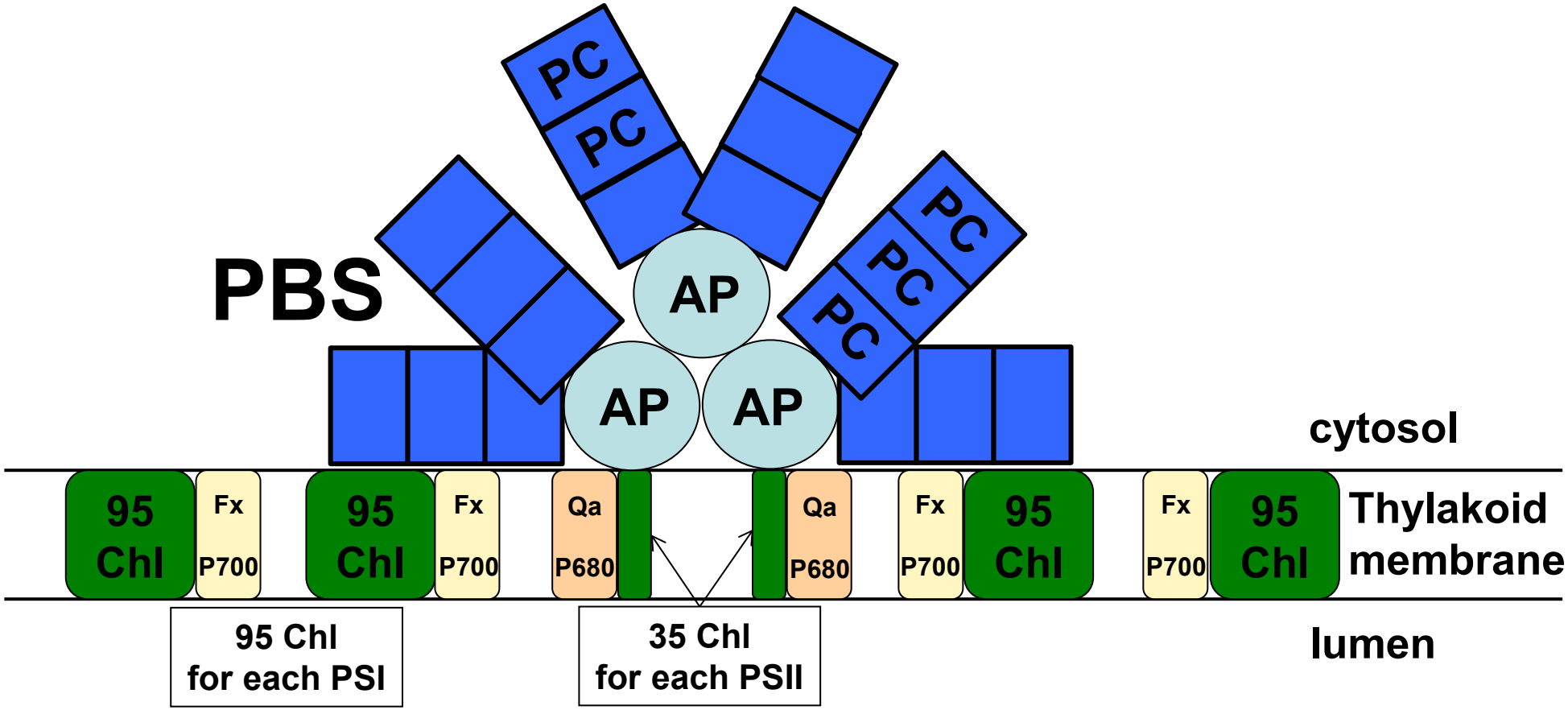
Example: Truncated Light-harvesting Antenna Size



Truncated antenna cells permit greater transmittance of sunlight and overall better solar utilization by the culture.

Cyanobacterial TLA Approach

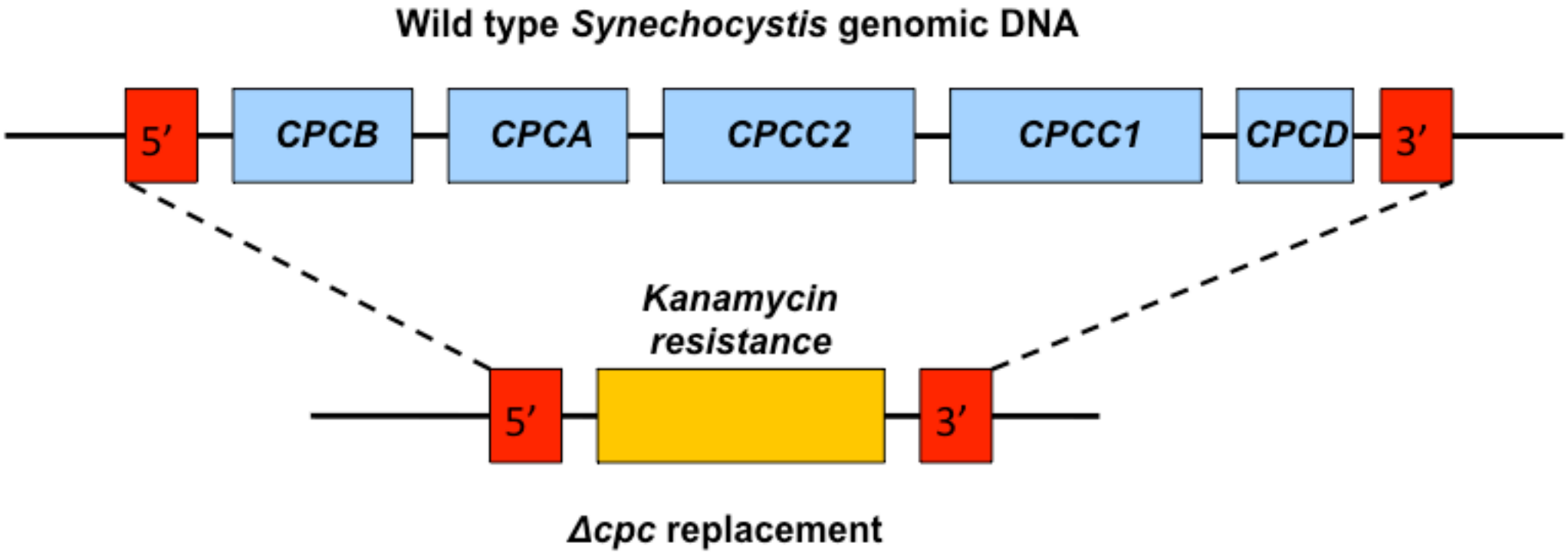
Unit photosynthetic apparatus and cyanobacterial antenna organization



Photosystem stoichiometry and phycobilisome-chlorophyll antenna organization in the thylakoid of cyanobacteria. Cyanobacteria may possess up to **850** phycocyanin (PC), allophycocyanin (AP), and chlorophyll (Chl) molecules per unit photosynthetic apparatus. *Phycobilisome (PBS) schematic adapted from Glazer and Melis 1987*.

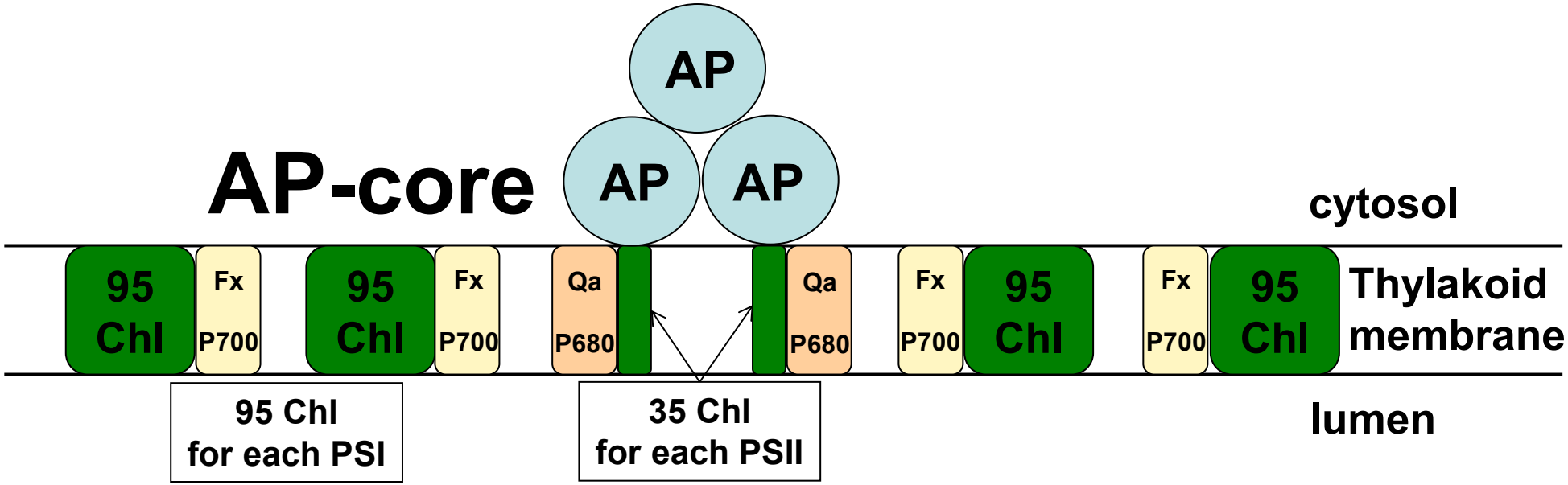
TLA Cyanobacterial Approach

Replacement of the phycocyanin-encoding *cpc* operon



Cyanobacterial TLA Approach

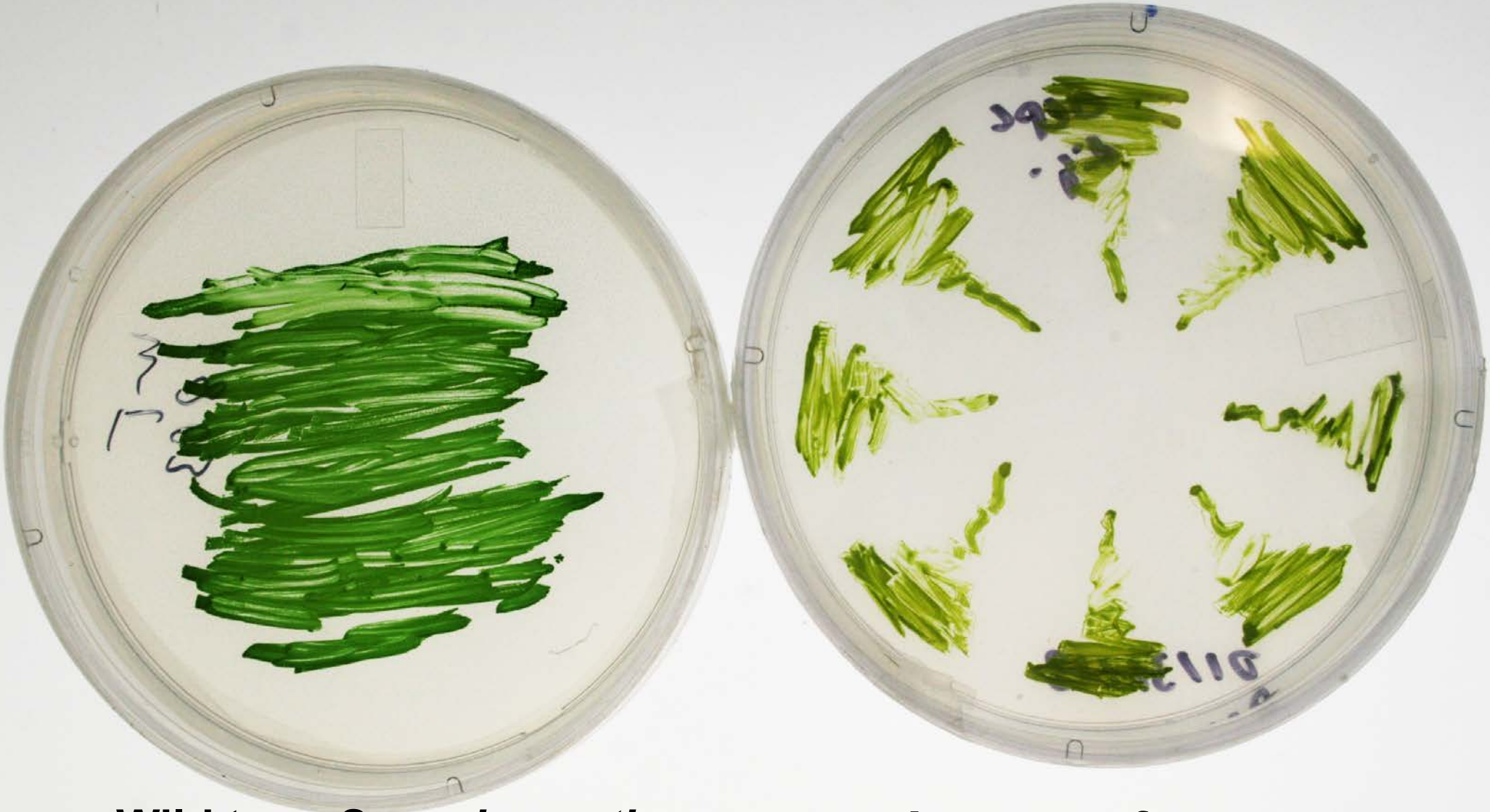
Unit photosynthetic apparatus and cyanobacterial TLA organization
The model shows the anticipated antenna size in *Δcpc* cyanobacteria



Go/No-Go Decision: Present evidence at the **molecular** and **biochemical** levels to demonstrate proof-of concept and capability of gene replacement in cyanobacteria, as the method of choice for the generation of TLA mutants in these photosynthetic microorganisms.

TLA Cyanobacterial Analysis (2013 AMR)

Molecular and Physiological Evidence of Transformation

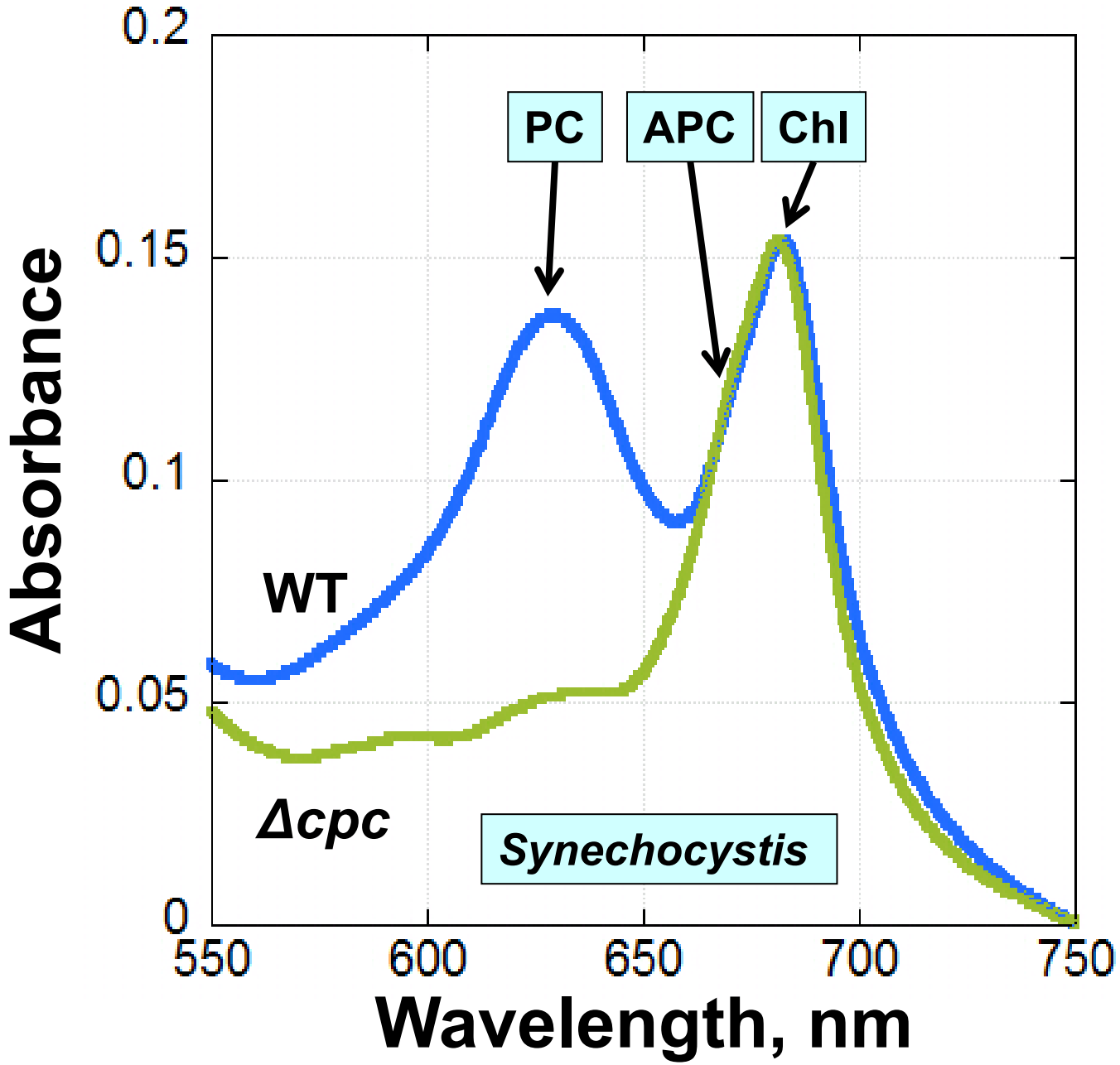


Wild type *Synechocystis*
Blue-green phenotype
due to PC and Chl pigments

***Δcpc* transformants**
Green phenotype
indicating loss of PC

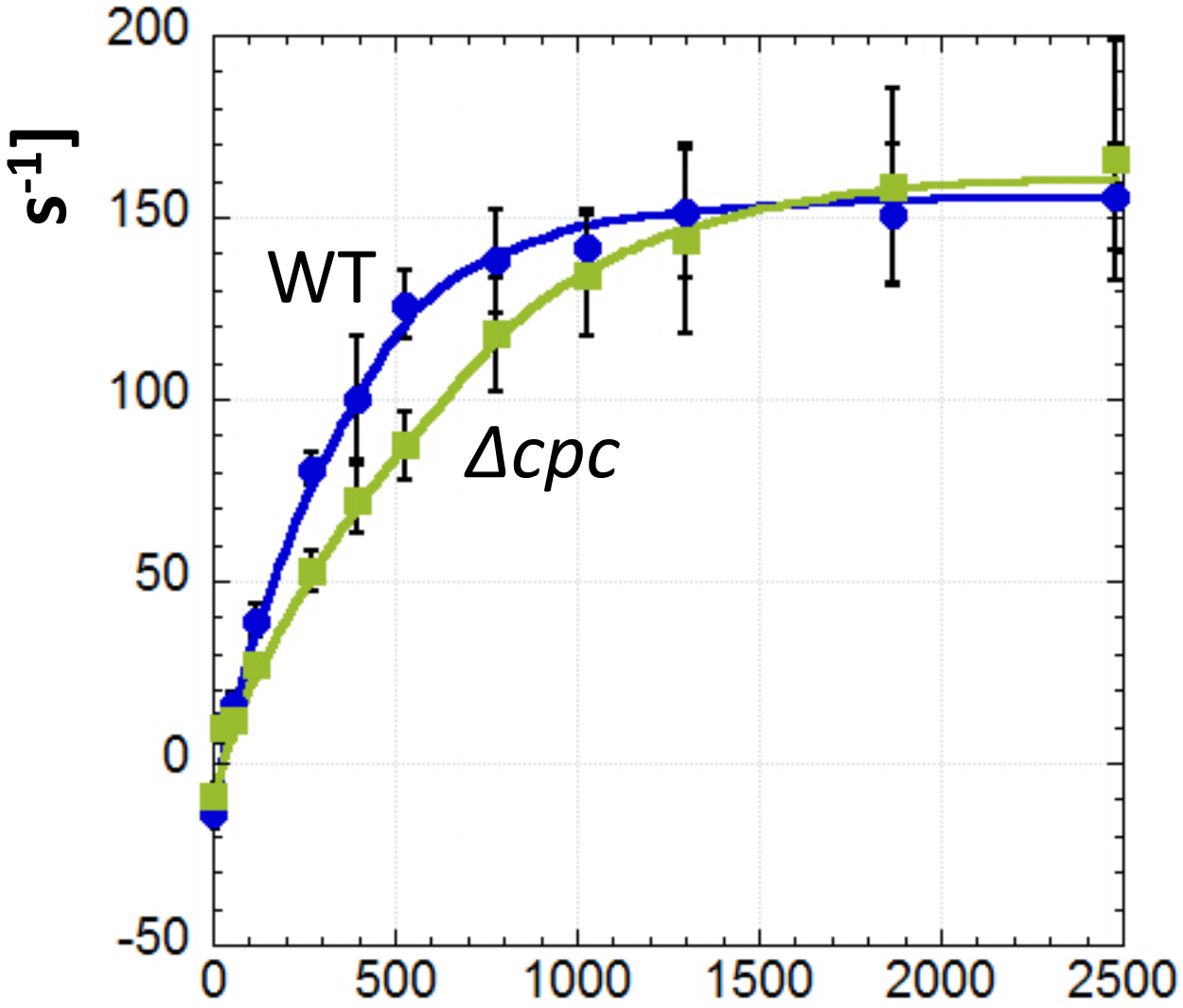
TLA Cyanobacterial Analysis (2013 AMR)

Biochemical Evidence of Transformation



TLA Cyanobacterial Analysis

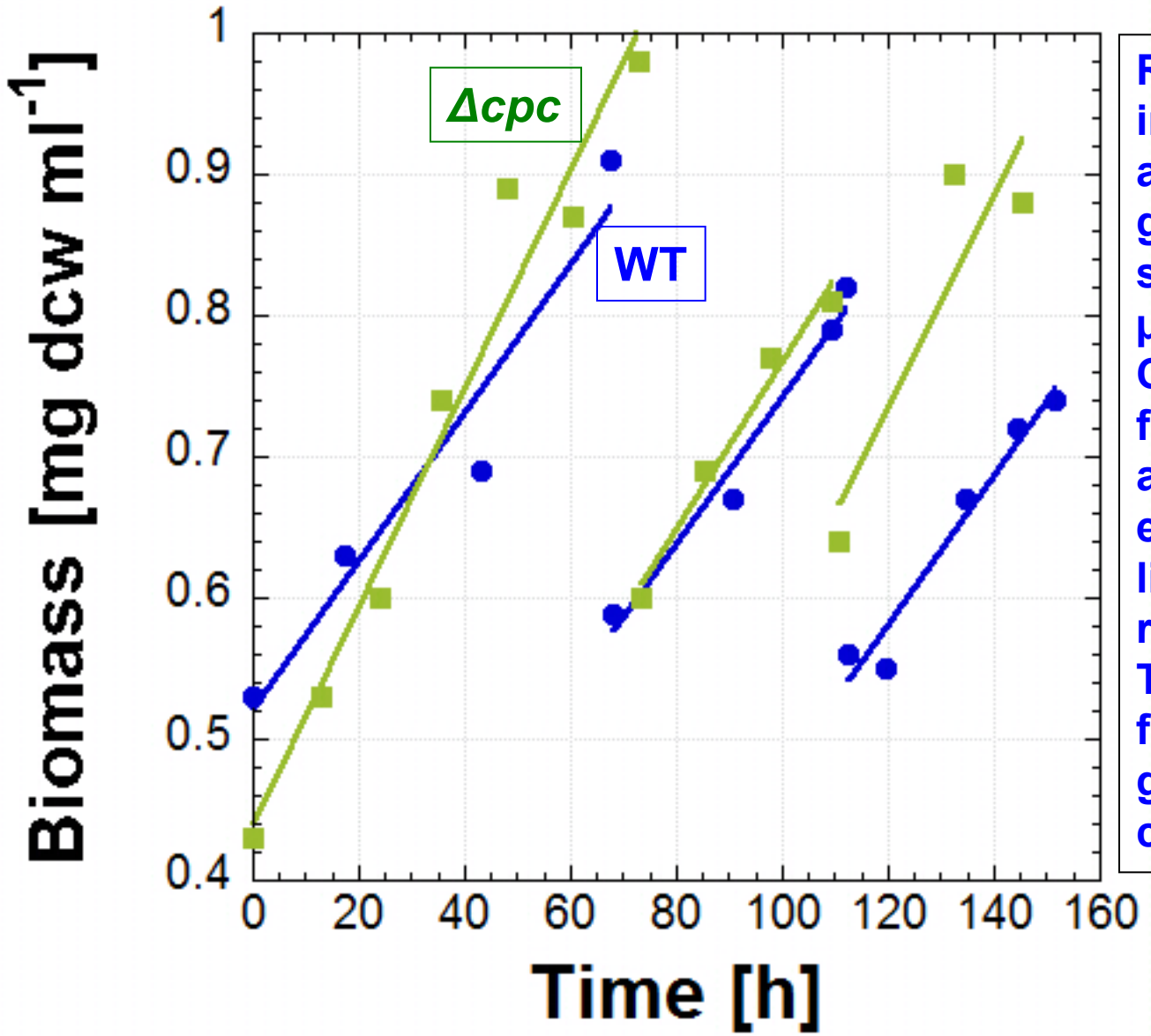
Light Saturation Curves of Photosynthesis of Wild Type and *Δcpc* Strains



Light intensity
[µmol photons m^{-2} s^{-1}]

TLA Cyanobacterial Analysis

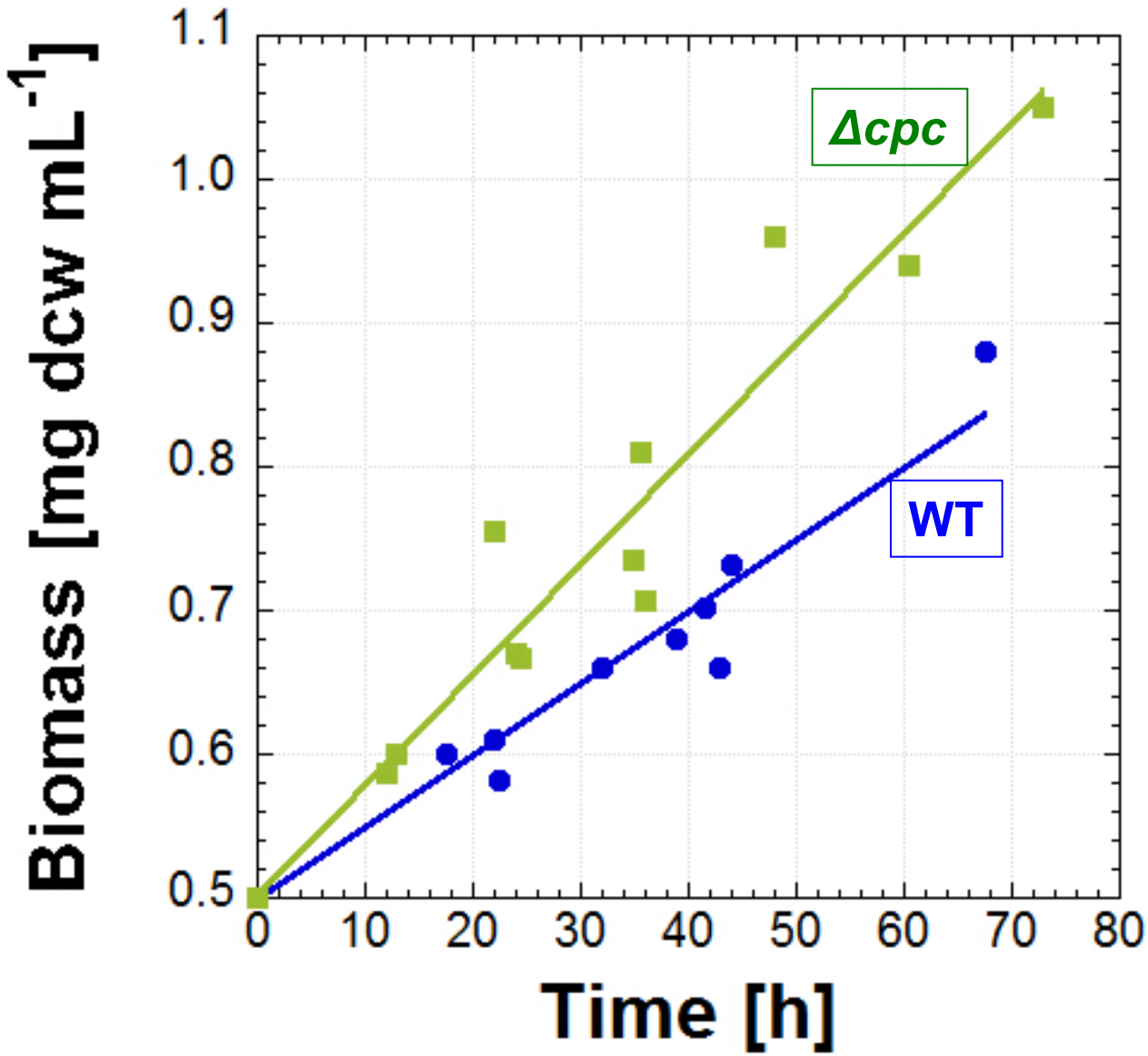
Productivity of Wild Type and *Δcpc* Cultures



Rate of biomass accumulation in *Synechocystis* wild type and *Δcpc* transformants, grown under simulated bright sunlight conditions (2,000 µmol photons m^{-2} s^{-1}). Cultures were diluted with fresh growth media at 60 h and 110 h into this growth experiment. The slope of the linear regressions defined the rate of biomass accumulation. This rate was always greater for the *Δcpc* transformants grown under these conditions compared to the wild type.

TLA Cyanobacterial Analysis

Productivity of Wild Type and *Δcpc* Cultures



Summary of biomass accumulation measurements in *Synechocystis* from several cultures under simulated bright sunlight conditions (2,000 µmol photons m^{-2} s^{-1}). The linear regression of the points represents the average rate of biomass accumulation of wild type (WT) and *Δcpc* transformants. Results showed a 55-60% greater yield of biomass in *Δcpc* strains, compared to WT.

Accomplishments and Progress

- **The TLA concept is applicable to cyanobacteria:**
  - → **Photosynthesis in *Δcpc* (TLA) transformants is saturated at about twice the light intensity for the saturation of the wild type.** **(Fewer absorbed photons are wasted in the *Δcpc* (TLA) than in the wild type).**
  - → **Rate of biomass accumulation in *Δcpc* (TLA) transformants is about 55-60% greater than that of the wild type.**

Evidence showed a substantially greater yield of biomass accumulation in *Synechocystis Δcpc* TLA strains, as compared to the wild type.

Accomplishments and Progress

Peer reviewed publications:

Kirst H, Melis A (2014) The chloroplast *Signal Recognition Particle* pathway (CpSRP) as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity. Biotechnology Advances 32: 66–72

Kirst H, Formighieri C, Melis A (2014) Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. Manuscript submitted for publication.

Invention disclosure:

Submitted to the UC Berkeley Office of Technology Licensing. Provisional patent application approved by the UCB OTL.

Application and Impact of the TLA R&D

→ **TLA strains were requested from the *Chlamydomonas* library and acquired, as follows:**

Universities (29), Research Institutions (10), Industry (x16), Government labs (x6), High schools (x6).

→ **A total of 67 *tla* strains were shipped since 2010, most of the requests were for the *tla1* strain.**

→ **Consultation was offered in the form of advising some of the above groups in the proper use of TLA strains.**

Proposed Future Work

→ **Wrapping up of this project with efforts to focus on the publication of the submitted paper, preparation and submission of the *Δcpc* patent application, and the submission of quarterly, annual, and closing reports to the DOE, Fuel Cell Technologies Office.**

Response to Reviewers

→ **A slide showing *Δcpc* transformants indicates some change has already occurred. More quantitative information as to the extent of the effect would be appreciated in next year's presentation.**

Response: Full quantitative information as to the extent of the *Δcpc* effect is provided.

→ **Cyanobacteria with reduced antenna size were first produced about 10 years ago, so the general accomplishment is not new.**

Response: The applicability of TLA cyanobacteria to improving culture productivity was not addressed until this work.

→ **There is no indication of collaboration on the use of bioreactors to determine a more real-life trial on its stability and production capability.**

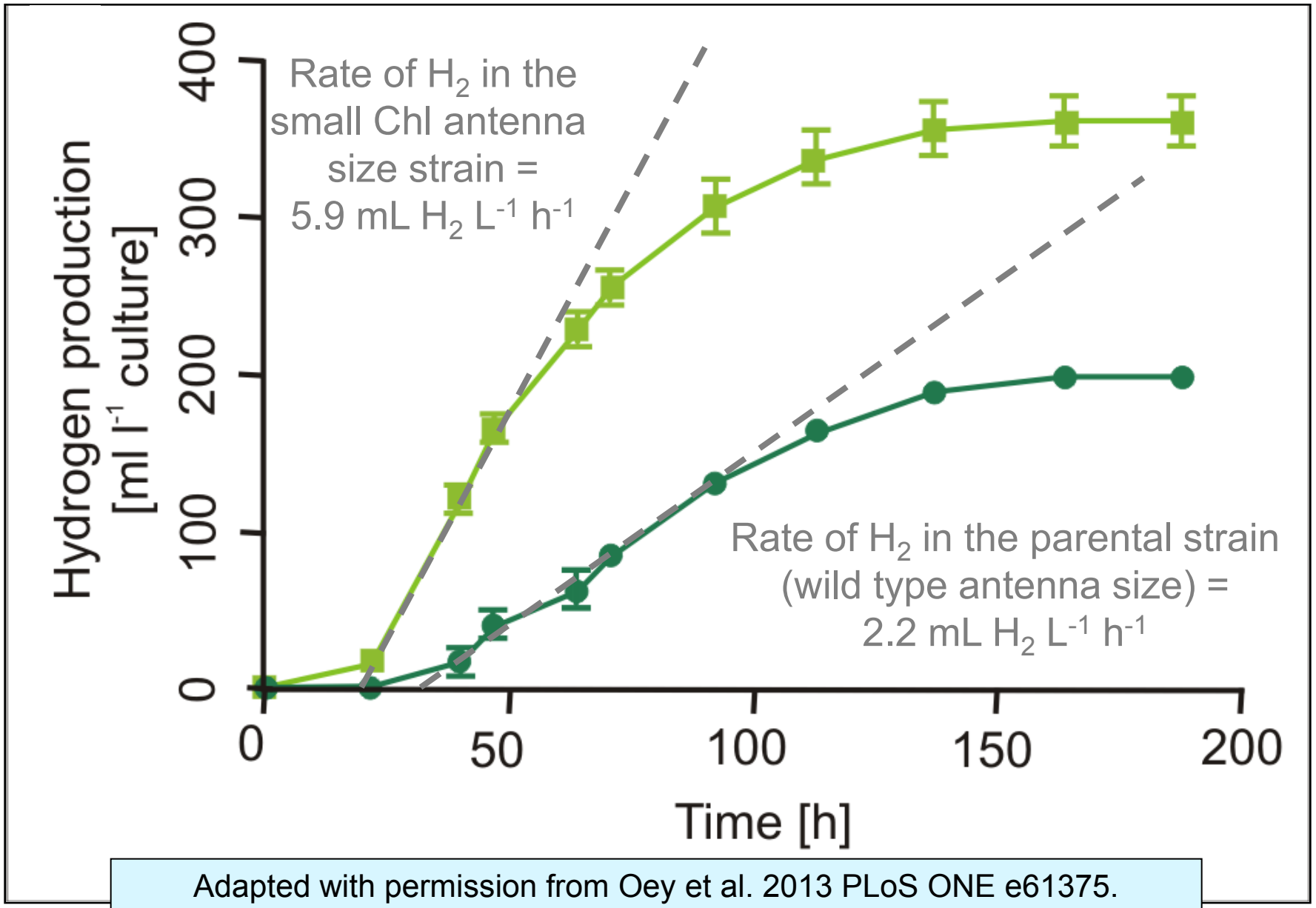
Response: Interested parties have "consulted" with us on the proper use of TLA strains for productivity measurements, including cell-density for different photobioreactor geometries. We helped them with technical information.

→ **The technique has general applicability and is not limited to hydrogen-producing organisms. However, this technique must be combined with significant advances in other aspects of cell modification (to improve the yield of H_2-production) in order to achieve DOE Hydrogen production goals.**

Response: Others are investigating the task of improving the yield of H_2-production photosynthetic microorganisms, and of testing the yield of H_2-production in TLA strains. Example is recent work at NREL. Also see the recently-published paper by Oey et al., demonstrating how TLA technology improves hydrogen production in microalgal cultures. (Please see slide # 19 below).

Response to Reviewers

TLA Technology Improves Hydrogen Production in Microalgal Cultures



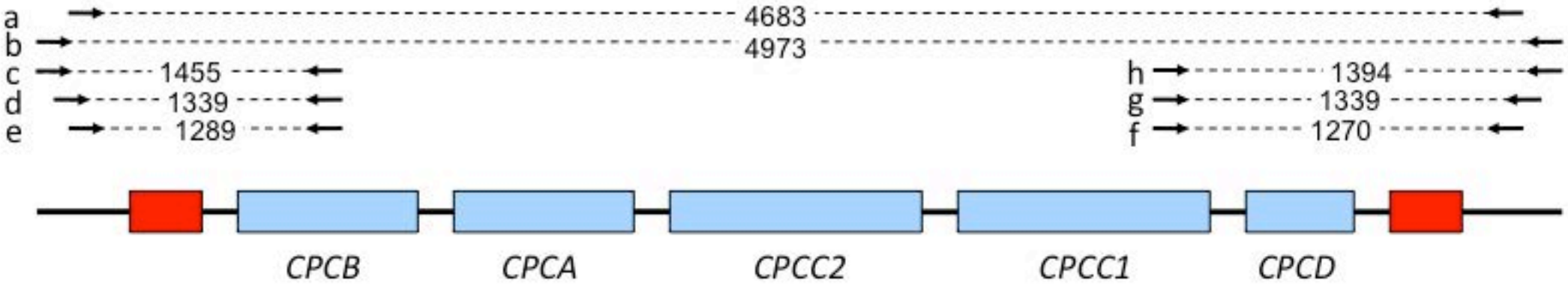
Adapted with permission from Oey et al. 2013 PLoS ONE e61375.
(Validation of the TLA Concept from the Hankamer and Kruse labs)

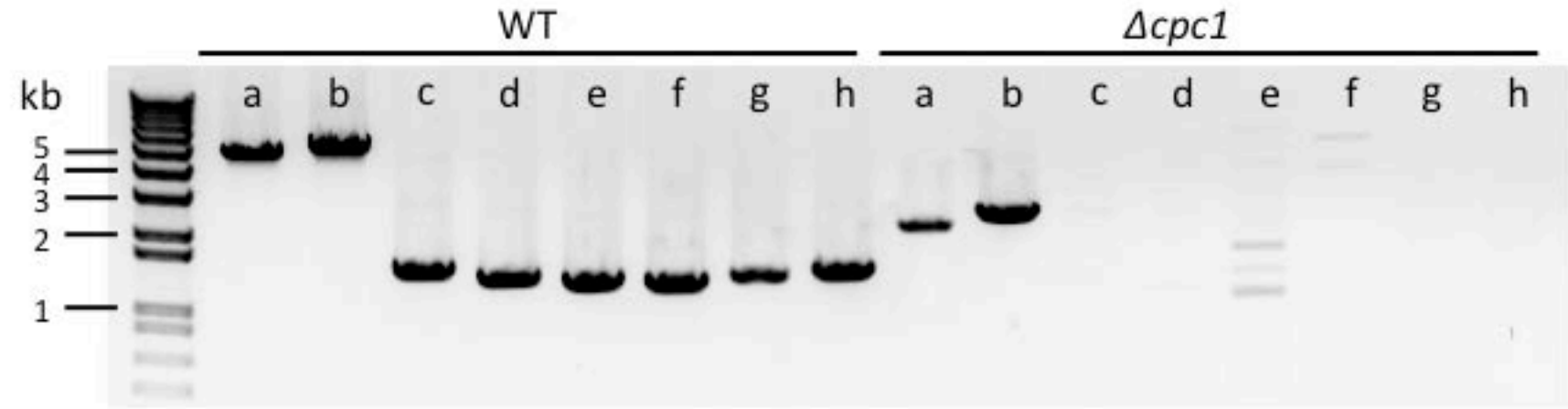
Closing Project Summary

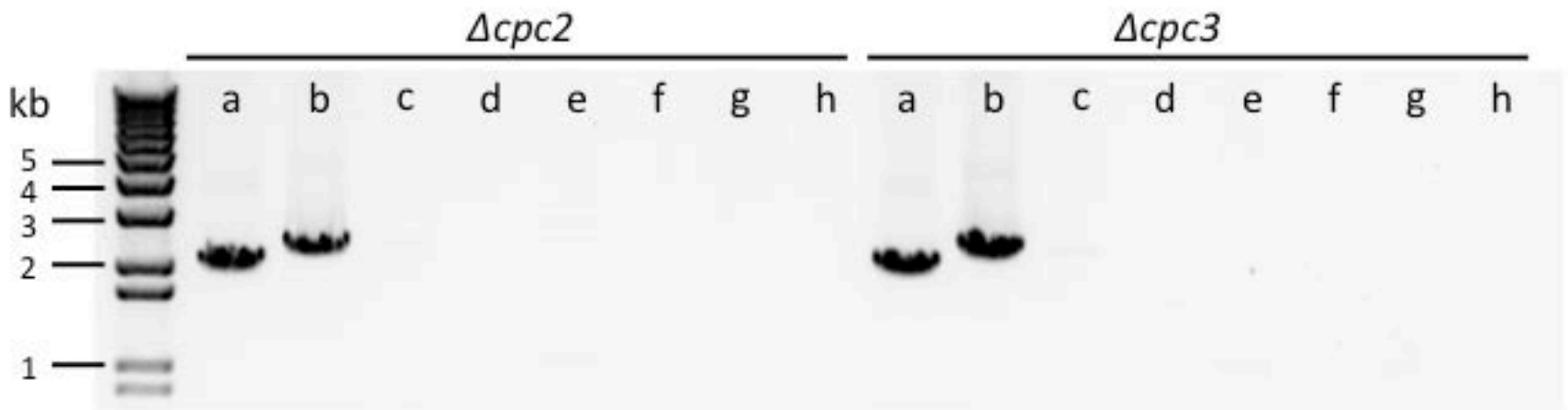
- → A new field of science and technology was created from the execution of this project. The ***TLA technology*** promises to enhance the photosynthetic productivity of microalgae, plants, and cyanobacteria by up to 300% over currently achieved yields.
- → Four novel genes were identified in green microalgae and plants, as determinants of the light-harvesting antenna size, manipulation of which resulted in the generation of TLA strains.
- → Twenty four highly-cited peer-reviewed papers were published.
- → Three different pieces of intellectual property and associated patents resulted from this work.
- → TLA strains of green microalgae were deposited in a national library (The *Chlamydomonas* Center) and are available to the field.

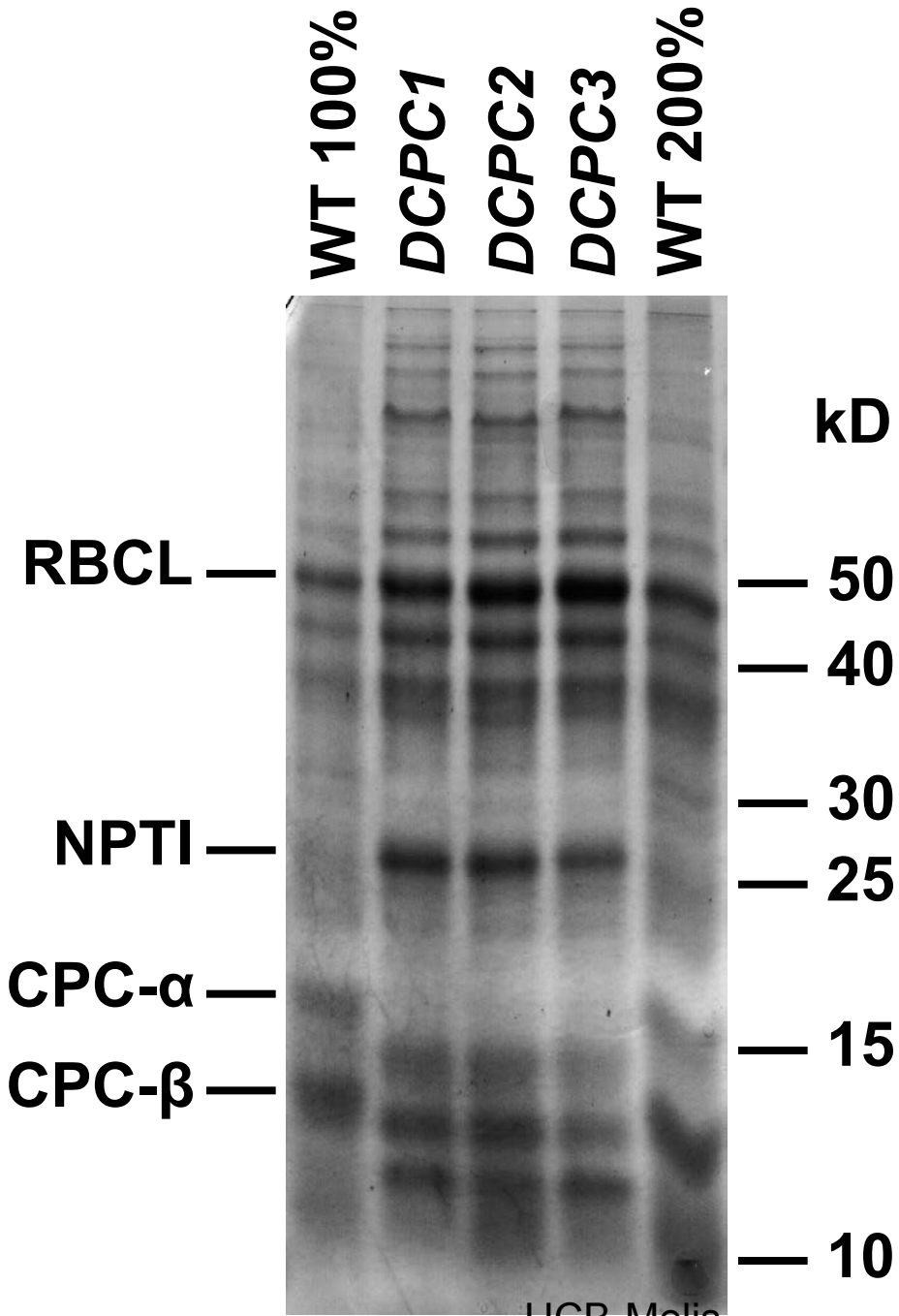
Technical Backup Slides

Additional Exploratory PCR Analyses Undertaken for Wild type and *Δcpc* transformants


a
b
c
d
e
4683
4973
1455
1339
1289
h
g
f
1394
1339
1270
CPCB
CPCA
CPCC2
CPCC1
CPCD

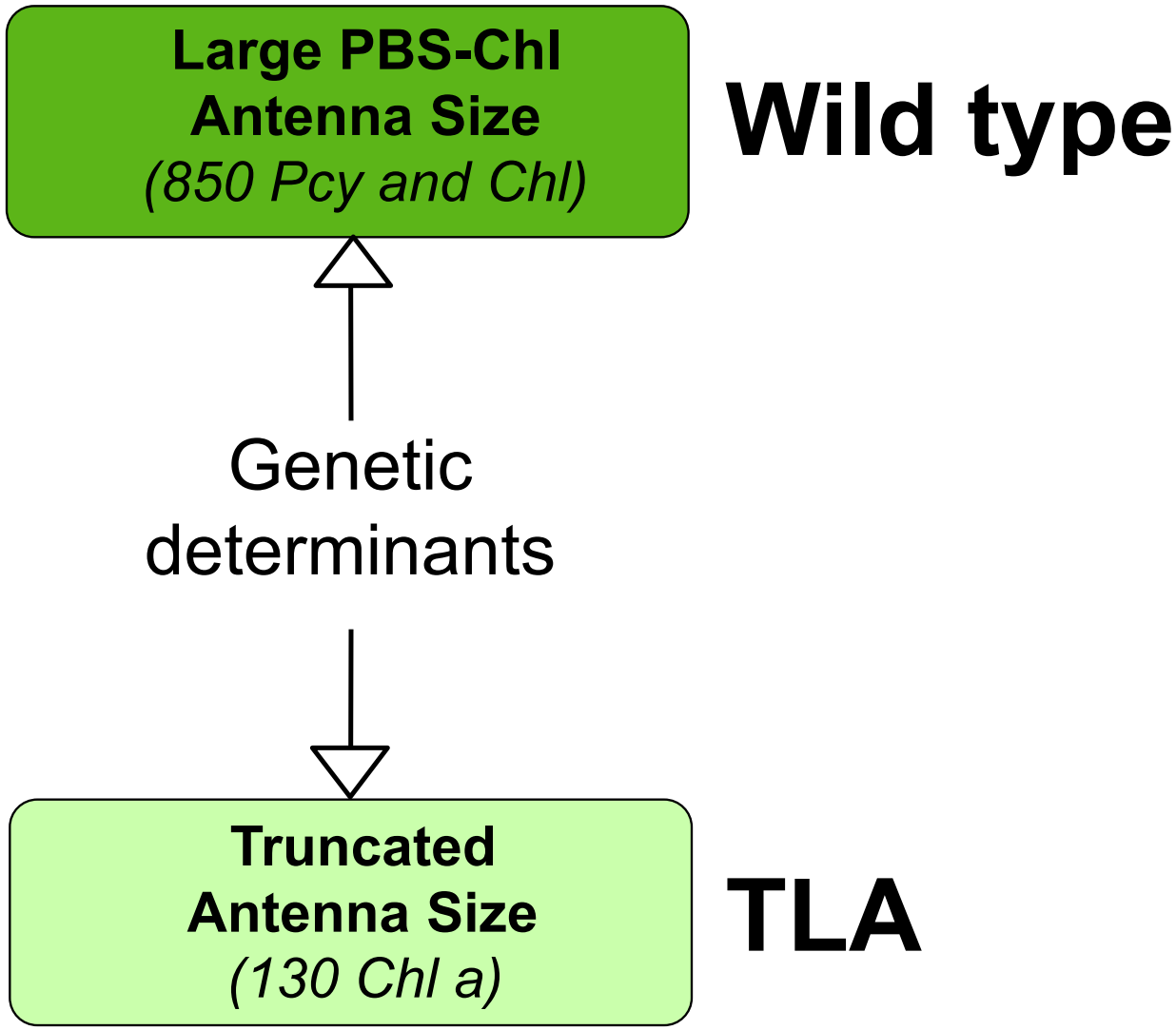

WT
Δcpc1
kb
a b c d e f g h a b c d e f g h
5
4
3
2
1


Δcpc2
Δcpc3
kb
a b c d e f g h a b c d e f g h
5
4
3
2
1


WT 100%
DCPC1
DCPC2
DCPC3
WT 200%
kD
RBCL
NPTI
CPC-α
CPC-β
50
40
30
25
15
10

UCB-Melis

Phycobilisome-Chlorophyll antenna size In Cyanobacteria



PBS Chromophores

Chl-PSI = 95x4 = 380 Chl
Chl-PSII = 35x2 = 70 Chl

AP-core cylinder = 6 per disc x 4 discs =
24 bilins per core cylinder
x 3 core cylinders = 72 bilins

Peripheral rods = 18 bilins per dimer disc
x 3 dimer dics per rod = 54 bilins per rod
x 6 rods per PBS = 324 bilins

Chl Antenna Size vs Light Utilization Efficiency
Utilization Efficiency of Absorbed Light Energy

- **Wild type antenna size = 470 Chl molecules (100%)**
  (PSII=230; PSI=240)
  Photon use efficiency of WT photosynthesis = ~6-10%
  Utilization Efficiency of Absorbed Light Energy by WT: ~3-5%

- ***tla1* antenna size = 275 Chl molecules (59% of control)**
  (PSII=115; PSI=160)
  Photon use efficiency of *tla1* photosynthesis = ~20%
  Utilization Efficiency of Absorbed Light Energy by *tla1*: ~10%

- ***tla2* antenna size = 195 Chl molecules (42% of control)**
  (PSII=80; PSI=115)
  Photon use efficiency of *tla2* photosynthesis = ~30%
  Utilization Efficiency of Absorbed Light Energy by *tla2*: ~15%

- **Long-term goal: 132 Chl molecules (28% of control)**
  (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis *goal* = ~60%
  Utilization Efficiency of Absorbed Light Energy *goal*: ~30%

Critical Assumptions and Issues

- Genes *TLA1*, *TLA2*, and *TLA3* are highly conserved in all eukaryotic photosynthetic organisms. However, application of these genes to other than *Chlamydomonas* microalgae would depend on the ability to transform them. This is now happening with *Nannochloropsis.*

- The rate and yield of H_2-production in all systems needs to be improved before the full benefit of the TLA technology in H_2-production can be materialized.