

Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Project ID # PD036

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



Overview

Timeline

- Start: 01-Dec-2004
- End: 31-Jan-2014
- Completion: 95%

Budget

- **Total Project Funding**
- DOE: \$1.74M
- UCB: \$ 675K
- Swedish Res Council: \$55.2K (**FY11**)
- **Funding received in FY12: \$150K**
- **Planned funding for FY13: \$150K**

Barriers

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

Partners

- NREL
- Swedish Research Council



Relevance

The TLA concept

(TLA = Truncated Light-harvesting Antenna):

Minimize the light-harvesting antenna size of the photosystems to prevent the early light-saturation of photosynthesis and the associated wasteful dissipation of absorbed sunlight.

Relevance

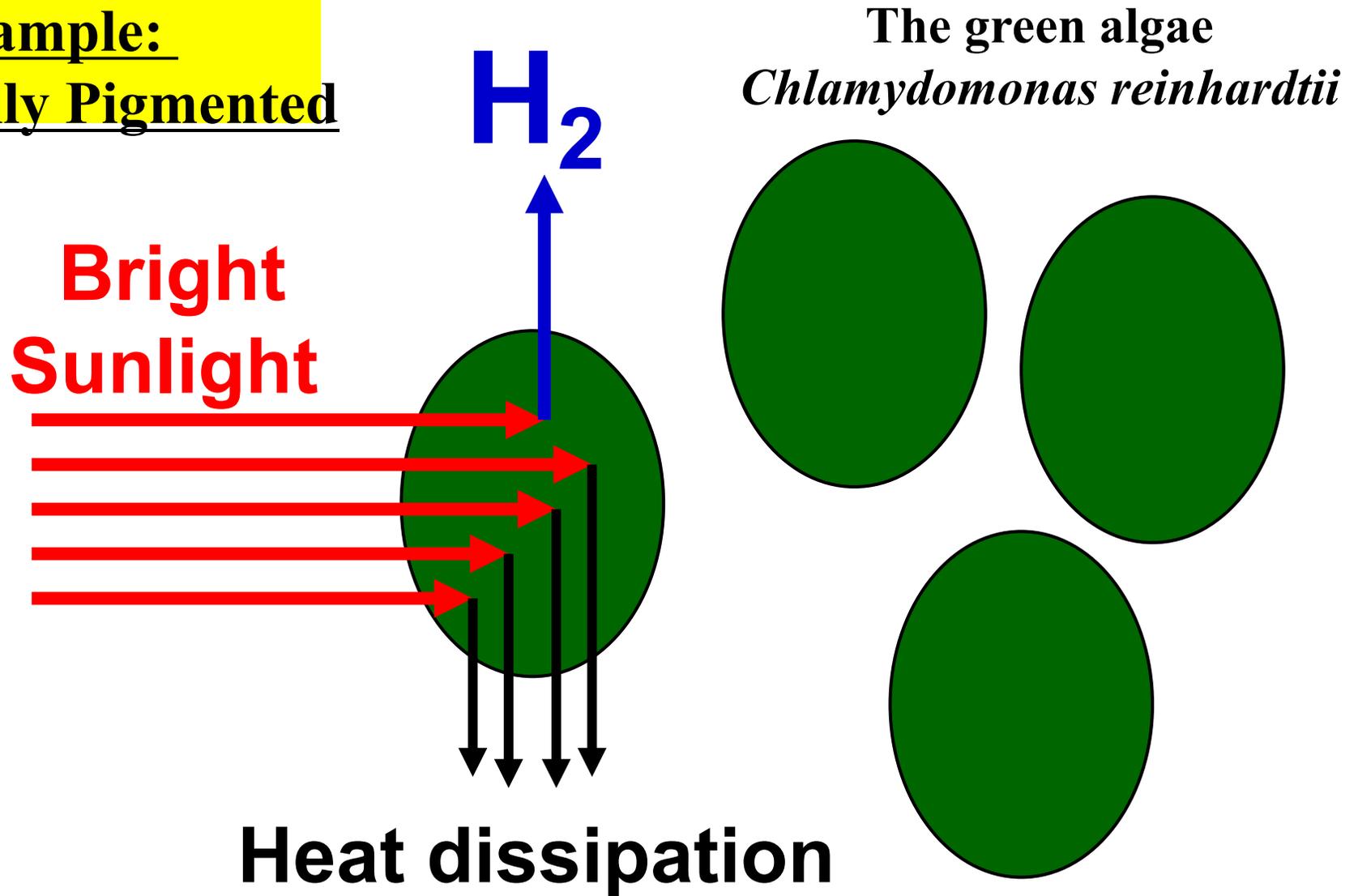
Improve, the sunlight-utilization efficiency of photosynthesis in microalgae by up to 300%, which will improve H₂ or fuels production in microalgae and cyanobacteria by about the same percentage.

The work links with effort both at NREL and at the J. Craig Venter Institute, where H₂-production technologies in microalgae and cyanobacteria are being developed.



Relevance

Example:
Fully Pigmented

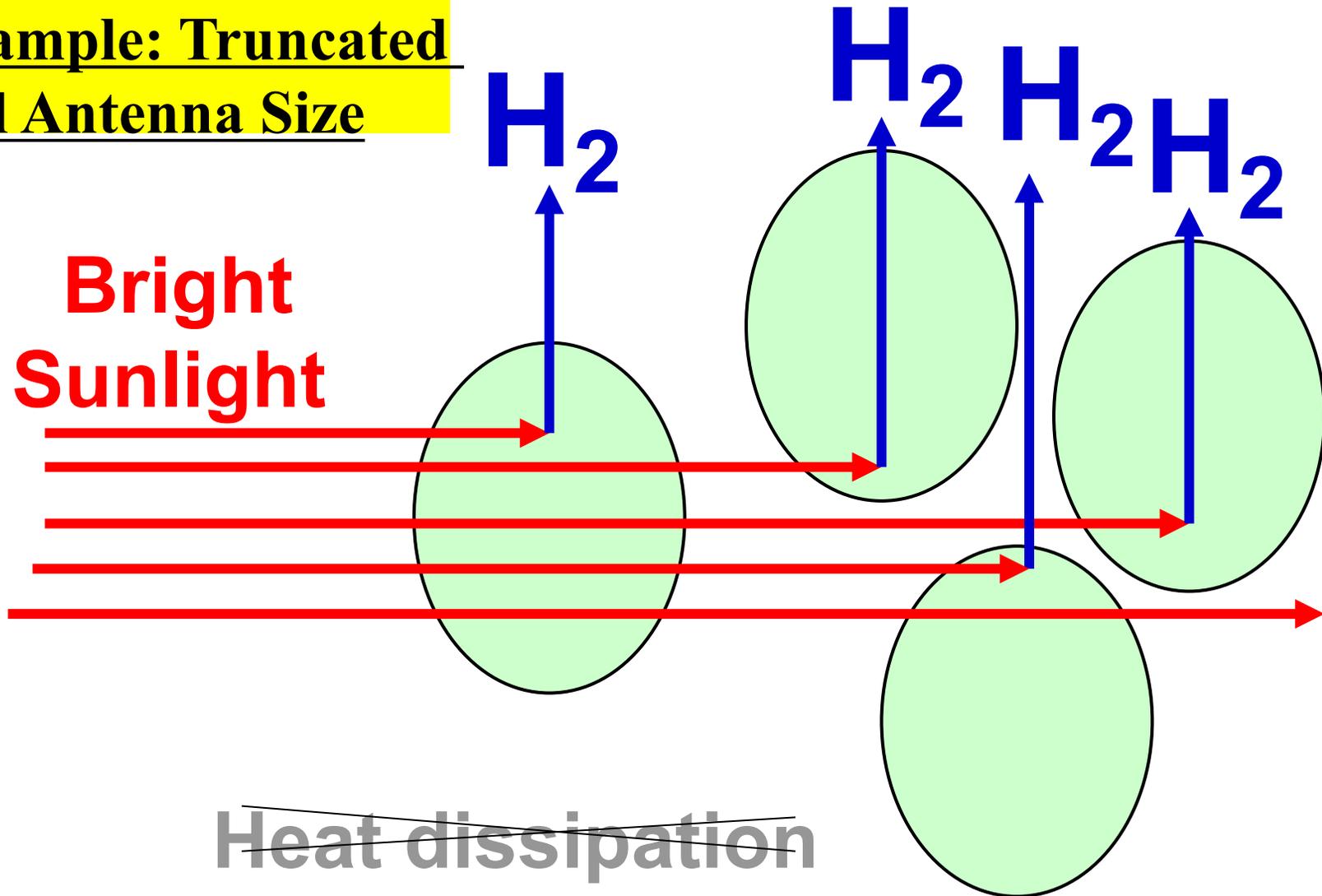


Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.



Relevance

Example: Truncated Chl Antenna Size

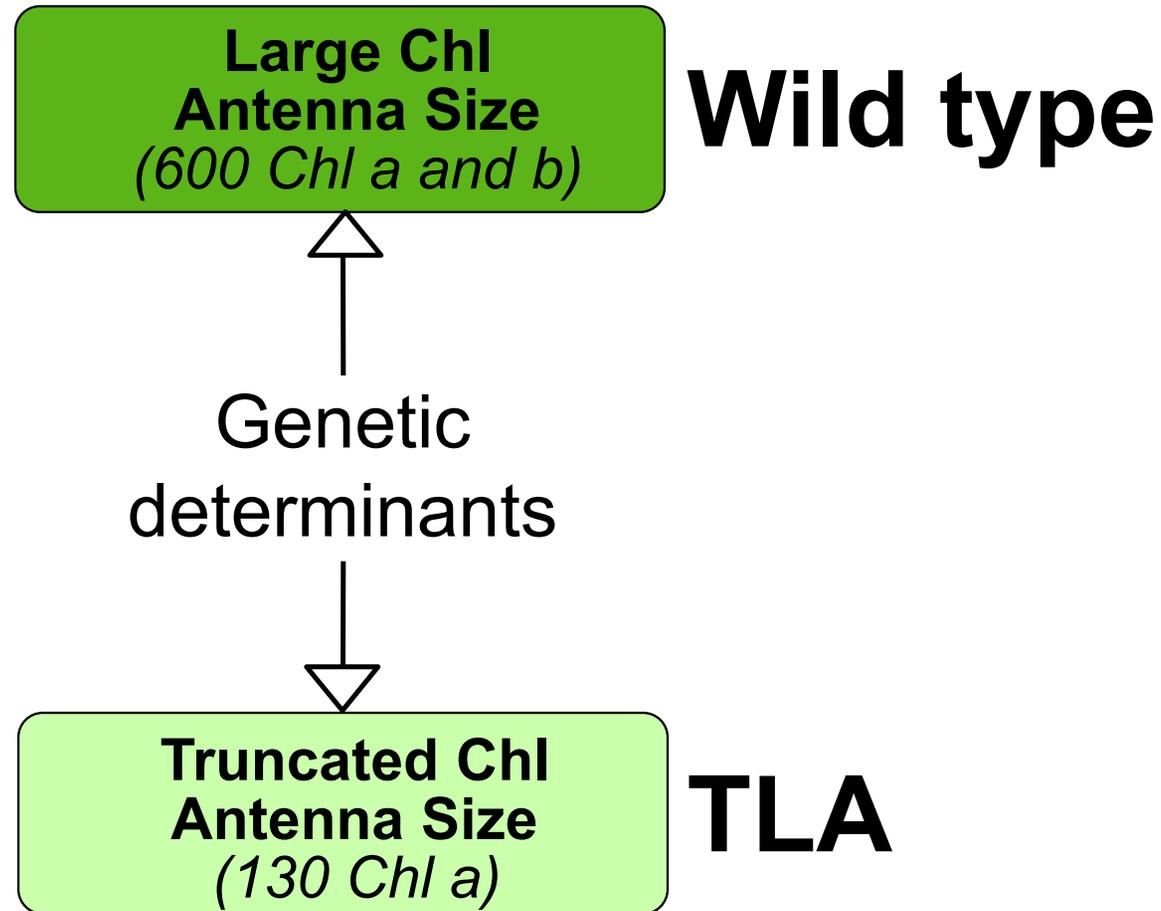


Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.



Objectives and Approach

Chlorophyll *a* and *b* antenna size in microalgae



Identify genes and molecular mechanisms to enable a truncated antenna size in microalgae and cyanobacteria.



Objectives and Approach

Objectives:

Identify genes and associated molecular mechanisms that confer a Truncated Light-harvesting Antenna (TLA property) in the *tla3* strains of *Chlamydomonas reinhardtii*.

Develop protocols for the targeted truncation of the light-harvesting antenna size in cyanobacteria.

Approach:

(a) Cloning of the genes responsible for the **TLA3** phenotype in *Chlamydomonas reinhardtii*. (b) Identification of genes to be interrupted or deleted in cyanobacteria. (c) Functional analysis of the transformants ([Berkeley expertise](#)).



PART 1

Green Microalgae



Microalgae Accomplishments and Progress

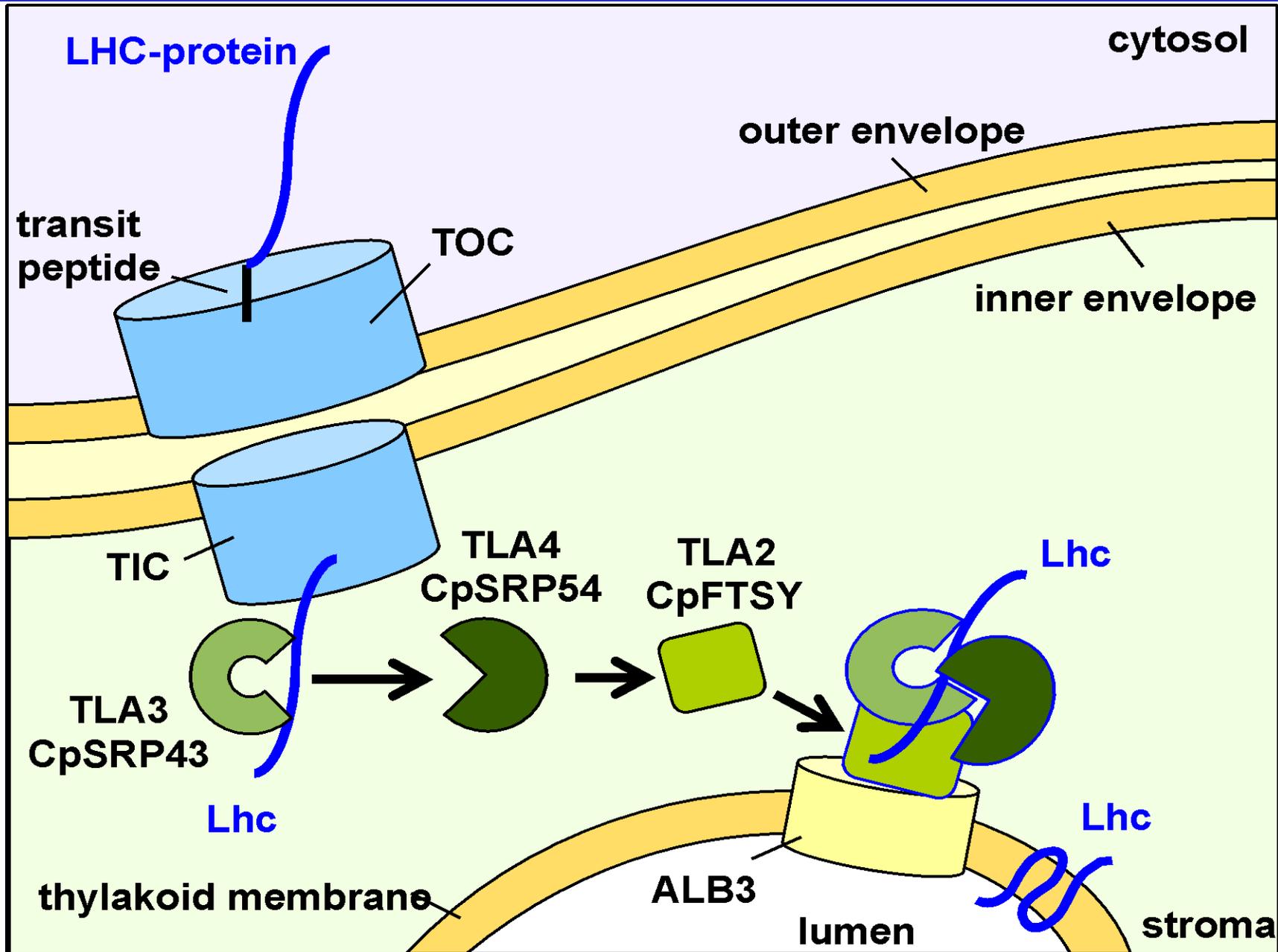
Sunlight Utilization Efficiency, % of Incident Solar Energy (maximum possible = 30%)

	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Light utilization efficiency)	3%	10%				15%			20%
Tla strain with the highest efficiency identified	3% (WT)	10% <i>TLA1</i>	15% <i>TLA2</i>		25% <i>TLA3</i>				
Gene cloning from the TLA strains				TLA1: Mov34 MPN			TLA2: FTSY	TLA3: SRP43	



Microalgae Accomplishments and Progress

Mechanism of TLA2 and TLA3 Function in LHC-protein assembly



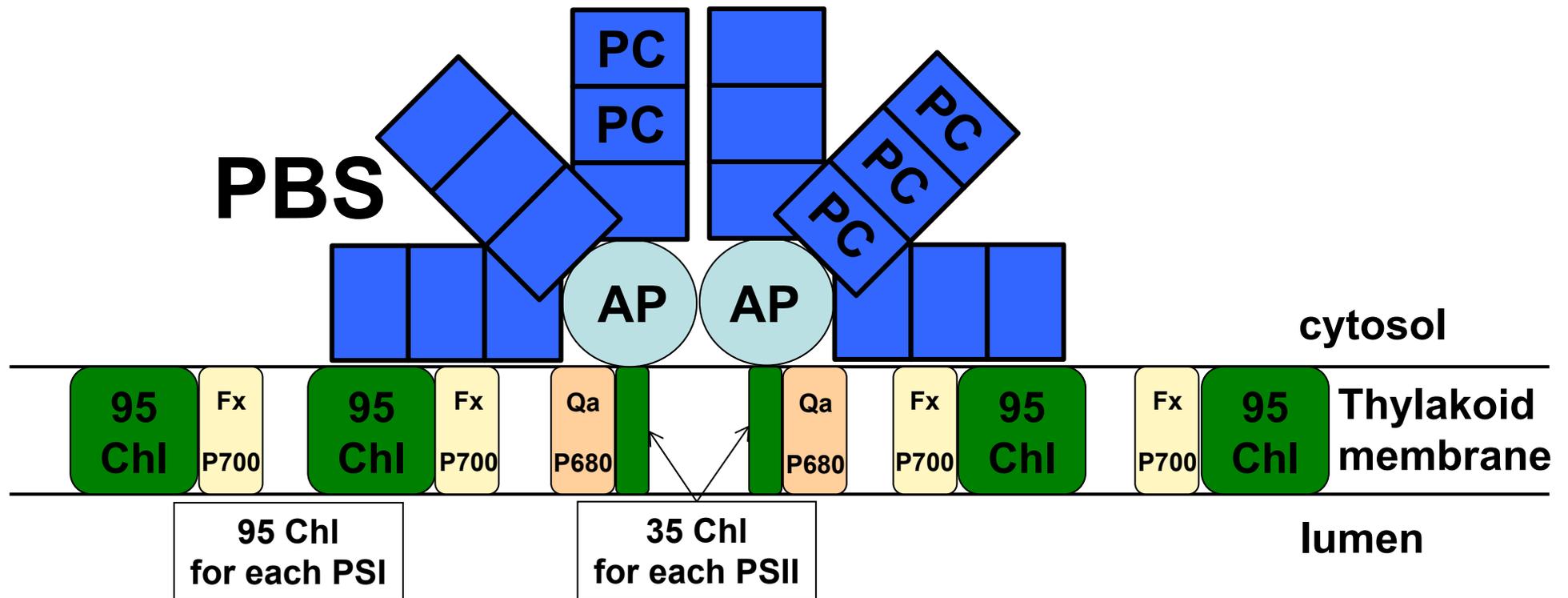
PART 2

Cyanobacteria



Cyanobacterial Approach

Unit photosynthetic apparatus and cyanobacterial antenna organization

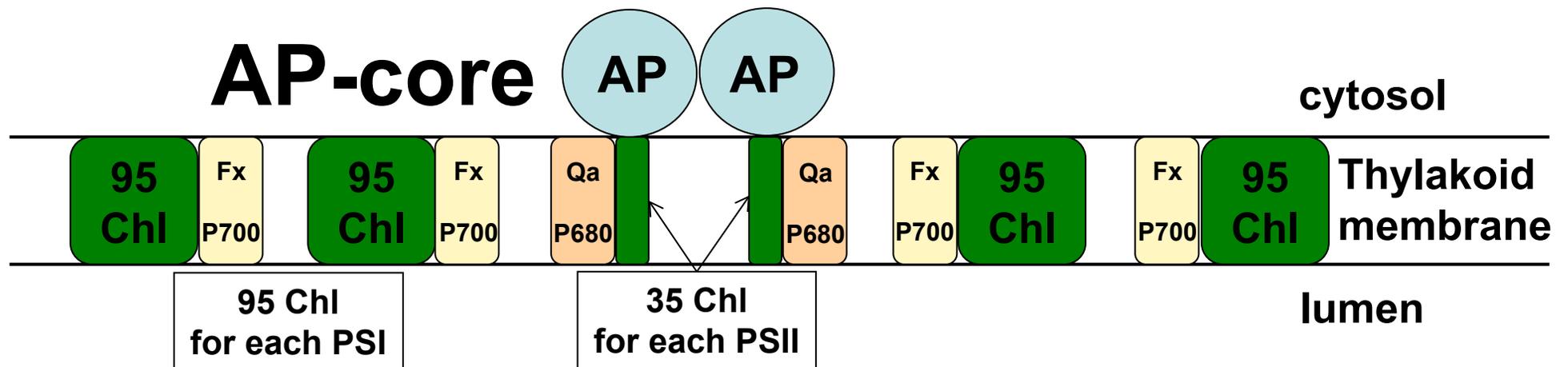


Photosystem stoichiometry and phycobilisome-chlorophyll antenna organization in the thylakoid of cyanobacteria. Cyanobacteria may possess up to **850** phycocyanin (PC), allophycocyanin (AP), and chlorophyll (Chl) molecules per unit photosynthetic apparatus. *Phycobilisome (PBS) schematic adapted from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



Cyanobacterial Approach

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

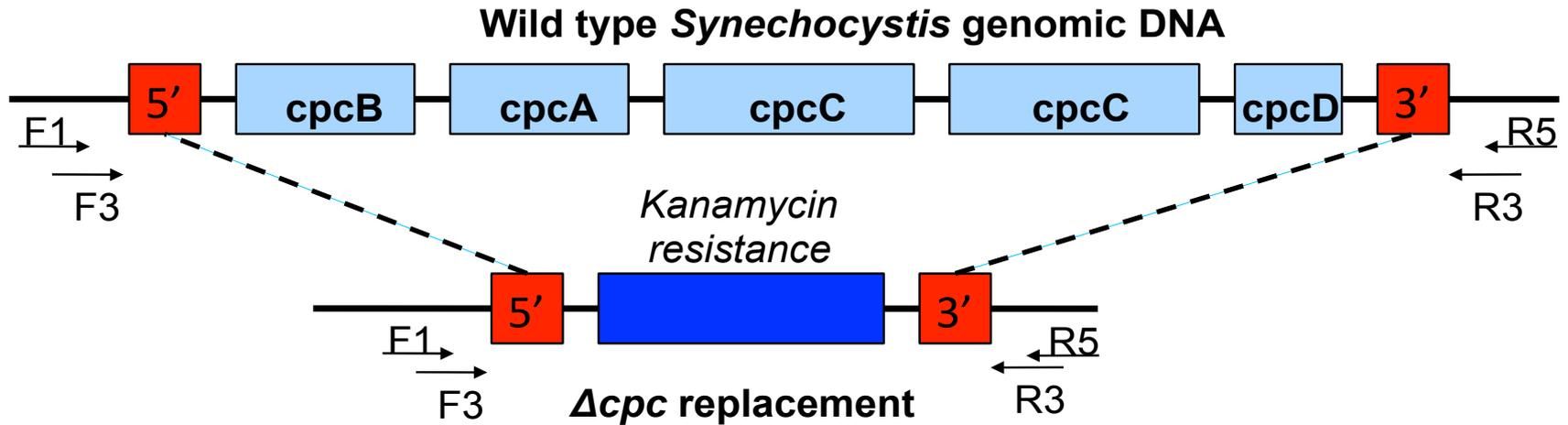


Go/No-Go Decision: Present evidence at the **molecular** and **biochemical** levels to demonstrate proof-of concept and capability of gene replacement in cyanobacteria, as the method of choice for the generation of TLA mutants in these photosynthetic microorganisms.



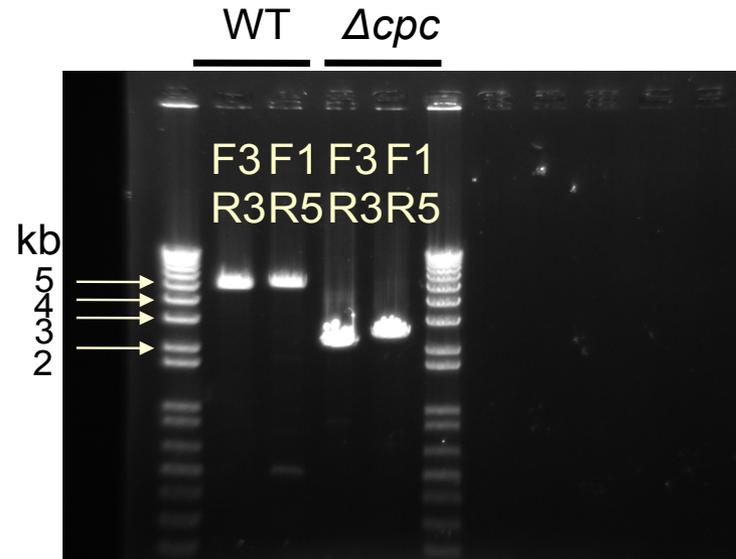
Cyanobacterial Approach

Replacement of the phycocyanin-encoding operon (**Go/No-Go Decision**)



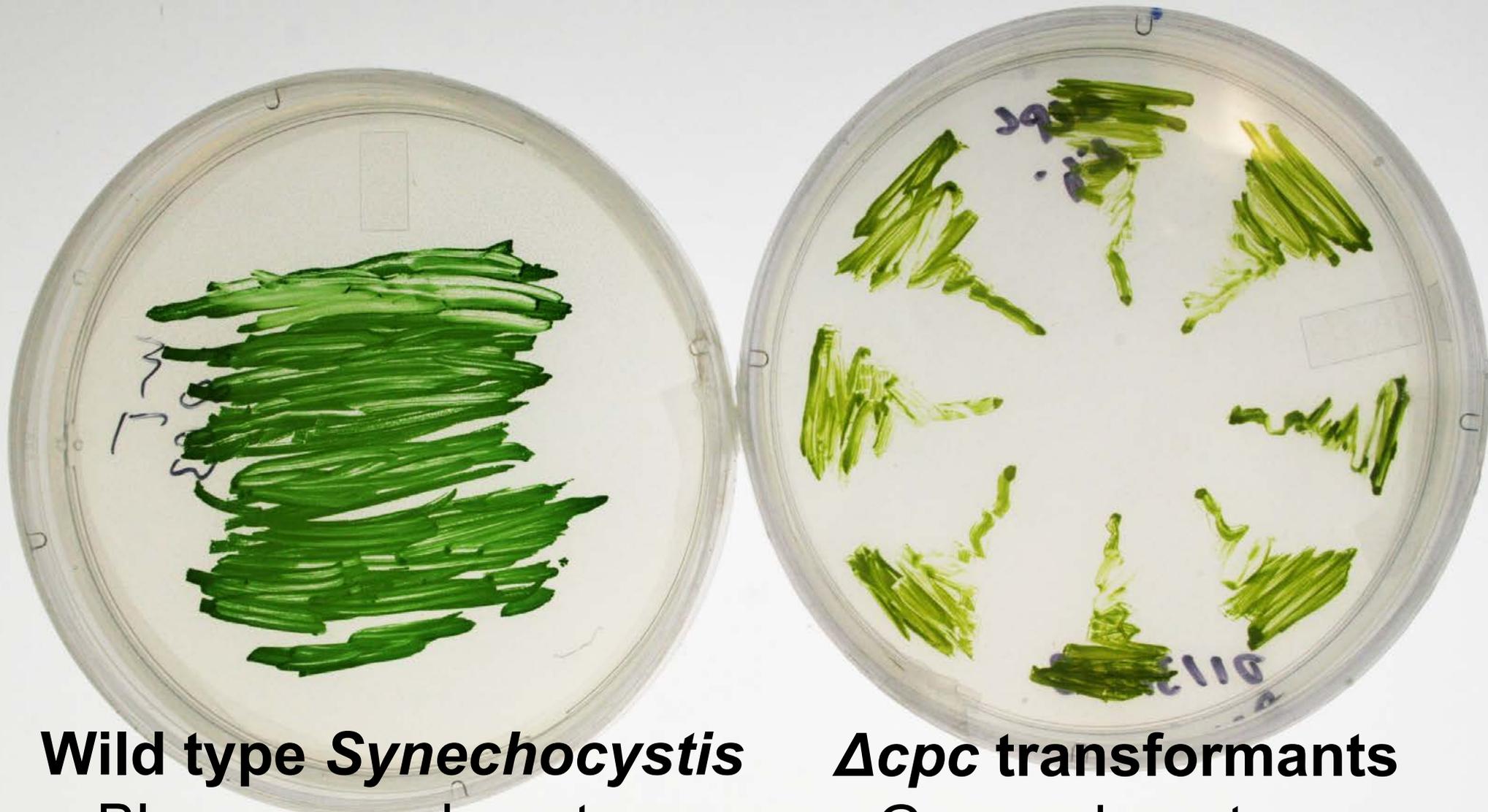
Expected PCR product size:

	WT [bp]	Δcpc [bp]
F3R3	4683	2126
F1R5	4973	2416



Cyanobacterial Approach

Molecular and Physiological Evidence of Transformation (**Go/No-Go Decision**)

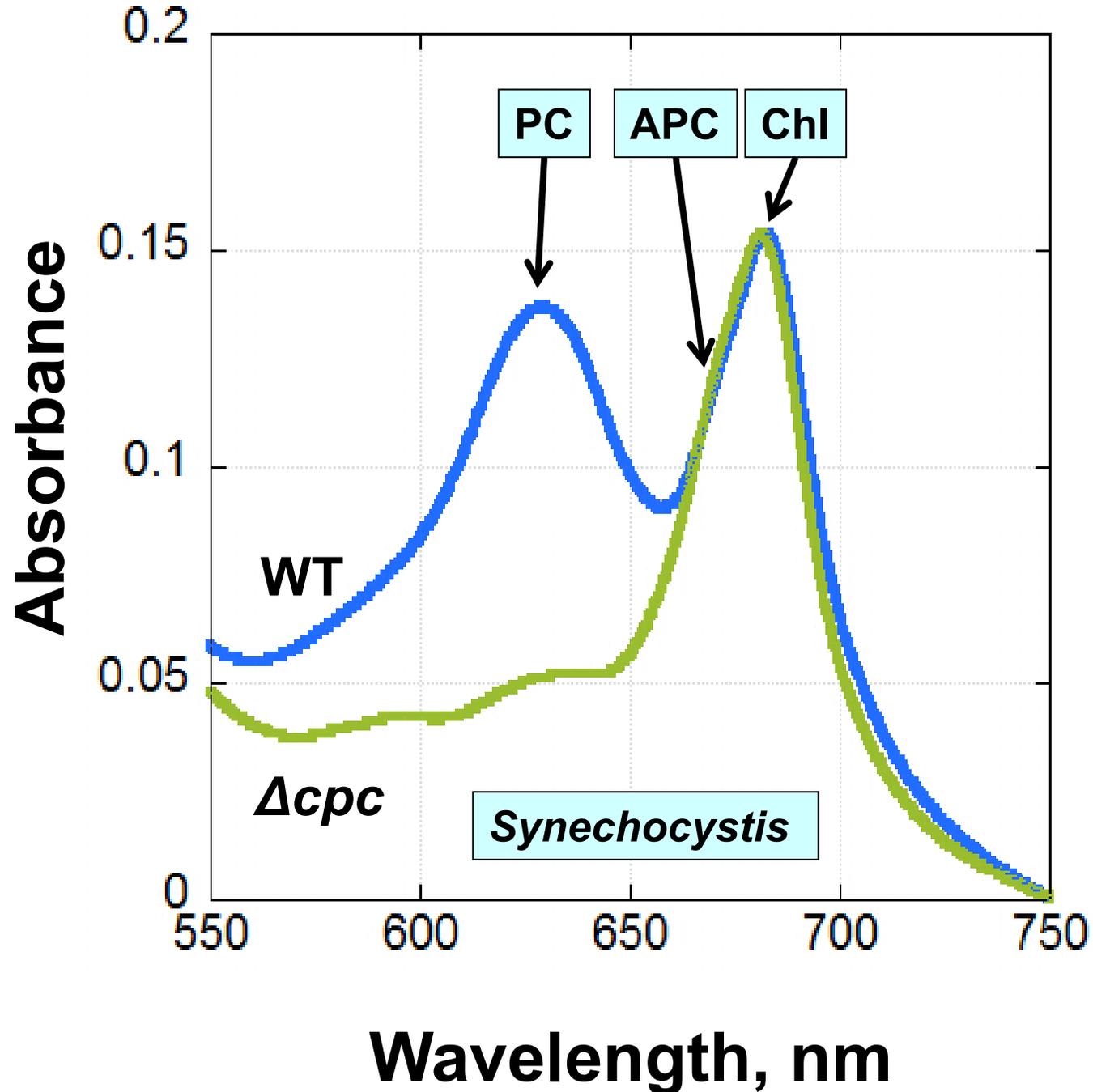


Wild type *Synechocystis*
Blue-green phenotype
due to PC and Chl pigments

Δcpc transformants
Green phenotype
indicating loss of PC

Cyanobacterial Approach

Biochemical Evidence of Transformation (**Go/No-Go Decision**)



Accomplishments and Progress

Evidence is presented at the genetic and molecular levels to demonstrate successful double homologous recombination for replacement of the ***cpc*** DNA operon in cyanobacteria.

Further evidence is presented at the biochemical level to show the ensuing absence of phycocyanin light-harvesting pigments from cyanobacteria (Δ ***cpc*** strains), seen by the green coloration of the latter, as opposed to the blue-green coloration of the wild type and by the absence of phycocyanin from the absorbance spectra of the Δ ***cpc*** strains.



Accomplishments and Progress

Peer reviewed publications:

- Mitra M, Dewez D, García-Cerdán JG, Melis A (2012) Polyclonal antibodies against the TLA1 protein also recognize with high specificity the D2 reaction center protein of PSII in the green alga *Chlamydomonas reinhardtii*. *Photosynth Res* 112:39-47
- Kirst H, Garcia-Cerdan JG, Zurbriggen A, Ruehle T, Melis A (2012) Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the *TLA3-CpSRP43* gene. *Plant Physiol.* 160(4):2251-2260
- Mitra M, Kirst H, Dewez D, Melis A (2012) Modulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii* by *TLA1* gene over-expression and RNA interference. *Phil. Trans. R. Soc. B* 367:3430-3443



Accomplishments and Progress

- **Successfully completed the TLA3 project, deposited strains in the *Chlamydomonas* library**
- **Successfully applied the TLA concept to cyanobacteria.**
- **Potential of enhancing photosynthetic efficiencies and hydrogen production of cyanobacteria under mass culture conditions.**



Collaborations, Applications, and Impact of the R&D

- The *TLA* concept is applied at:
NREL and by the Brisbane (Australia)-Bielefeld (Germany) groups in *Chlamydomonas* for H₂ production and by the U-Wageningen (The Netherlands) for biomass production, and by two companies in the private sector for commercial production of polyunsaturated fatty acids (PUFAs) in *Nannochloropsis*.
- TLA strains were requested from the *Chlamydomonas* library and acquired by universities (x29), industry (x14), government labs (x4), research institutions (5), and high schools (x6).
- (A total of 58 strains were shipped since 2010, most of them in 2012.)
- Collaboration was offered in the form of advising some of the above groups in the use of the TLA strains.



Proposed Future Work

Compete the analysis of the TLA cyanobacteria, including:

- (i) Assessment of stability and fitness of the Δcpc transformants;**
- (i) Organization of the photosynthetic apparatus (measurement of PS stoichiometry and functional antenna size in the Δcpc transformants);**
- (ii) Efficiency and productivity of photosynthesis measurements from the light-saturation curve); and**
- (iii) Measurements of Δcpc productivity under mass culture conditions. (Currently in progress.)**

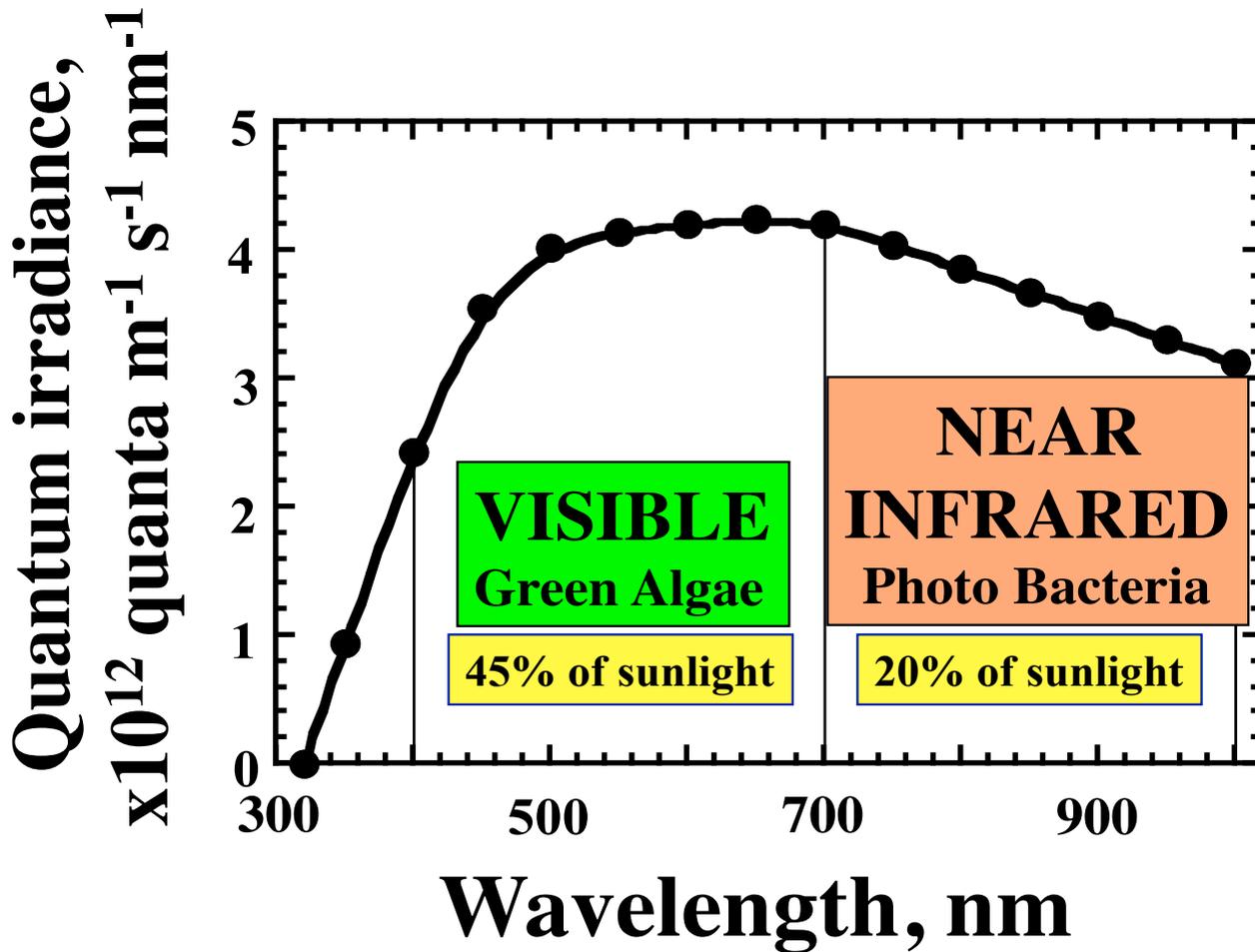
Advance exploration of the “*extended photosynthetically active radiation*” (ePAR) concept.

(Proprietary molecular genetic design not disclosed. Please see next slide for a concept explanation, to be further discussed during the presentation.)



Proposed Future Work

Extended photosynthetically active radiation” (ePAR) concept

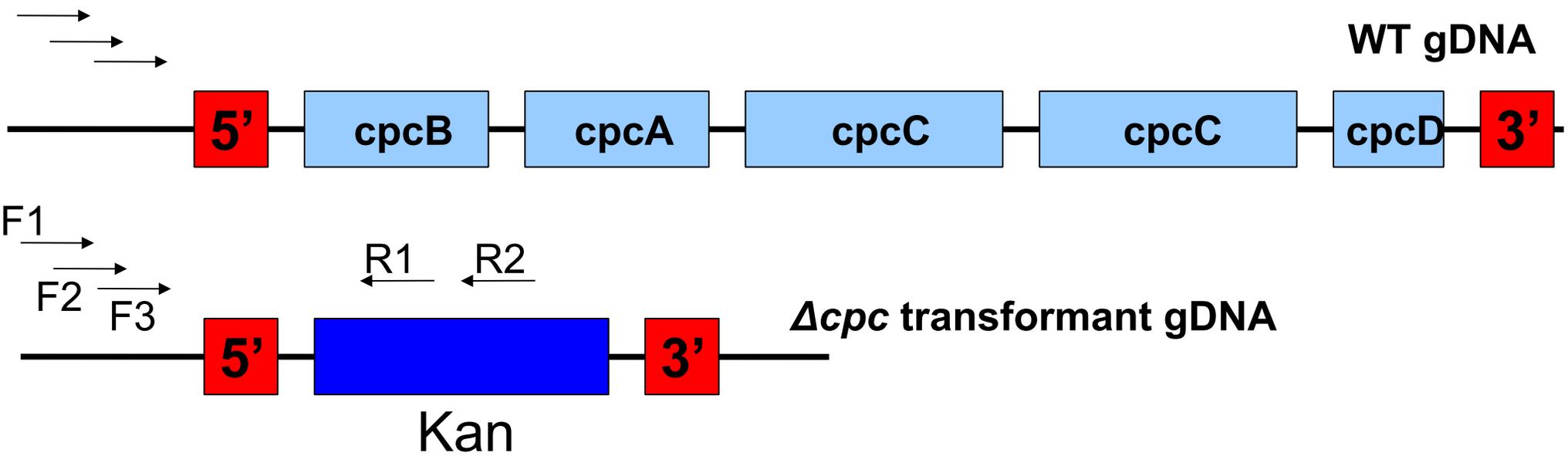


Technical Backup Slides



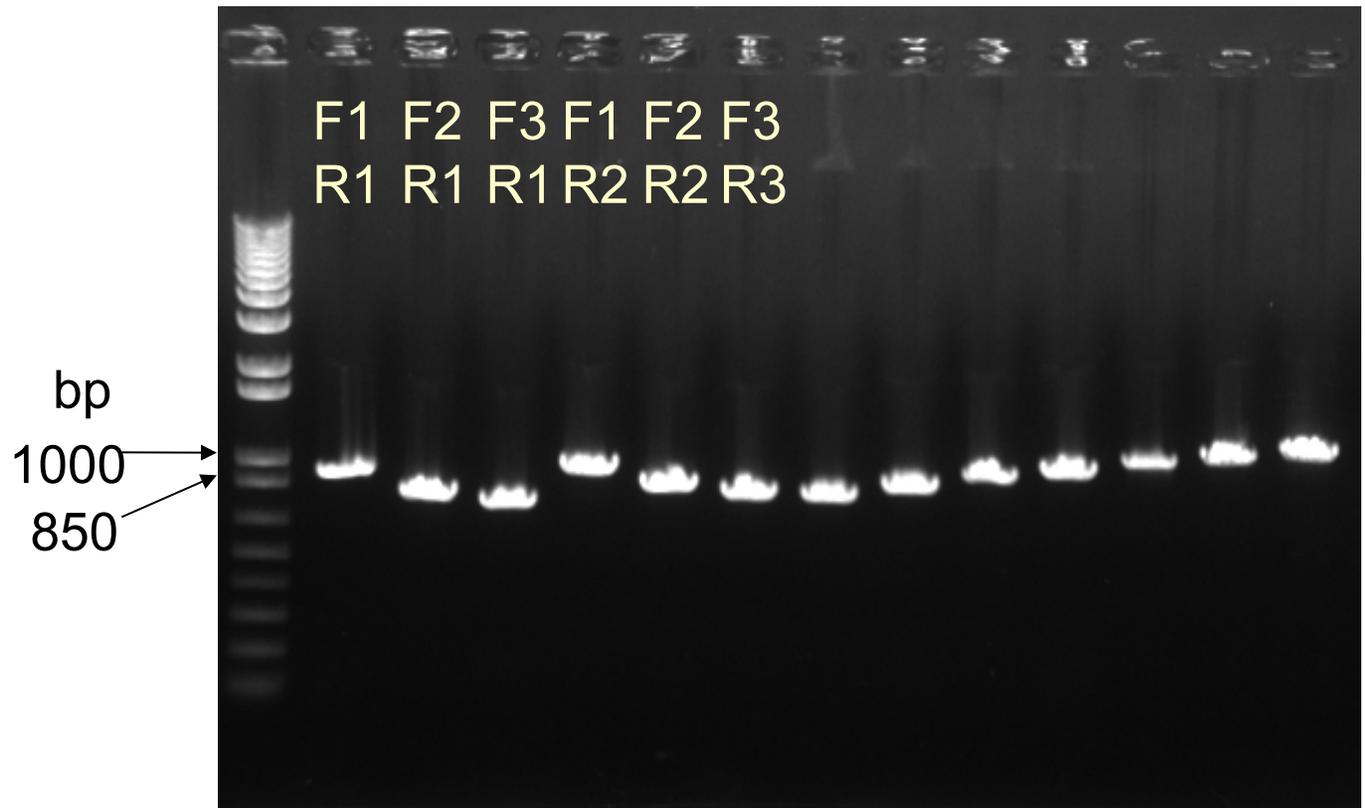
**Additional Exploratory PCR Analyses
Undertaken for
Wild type and Δcpc transformants**





Expected product size

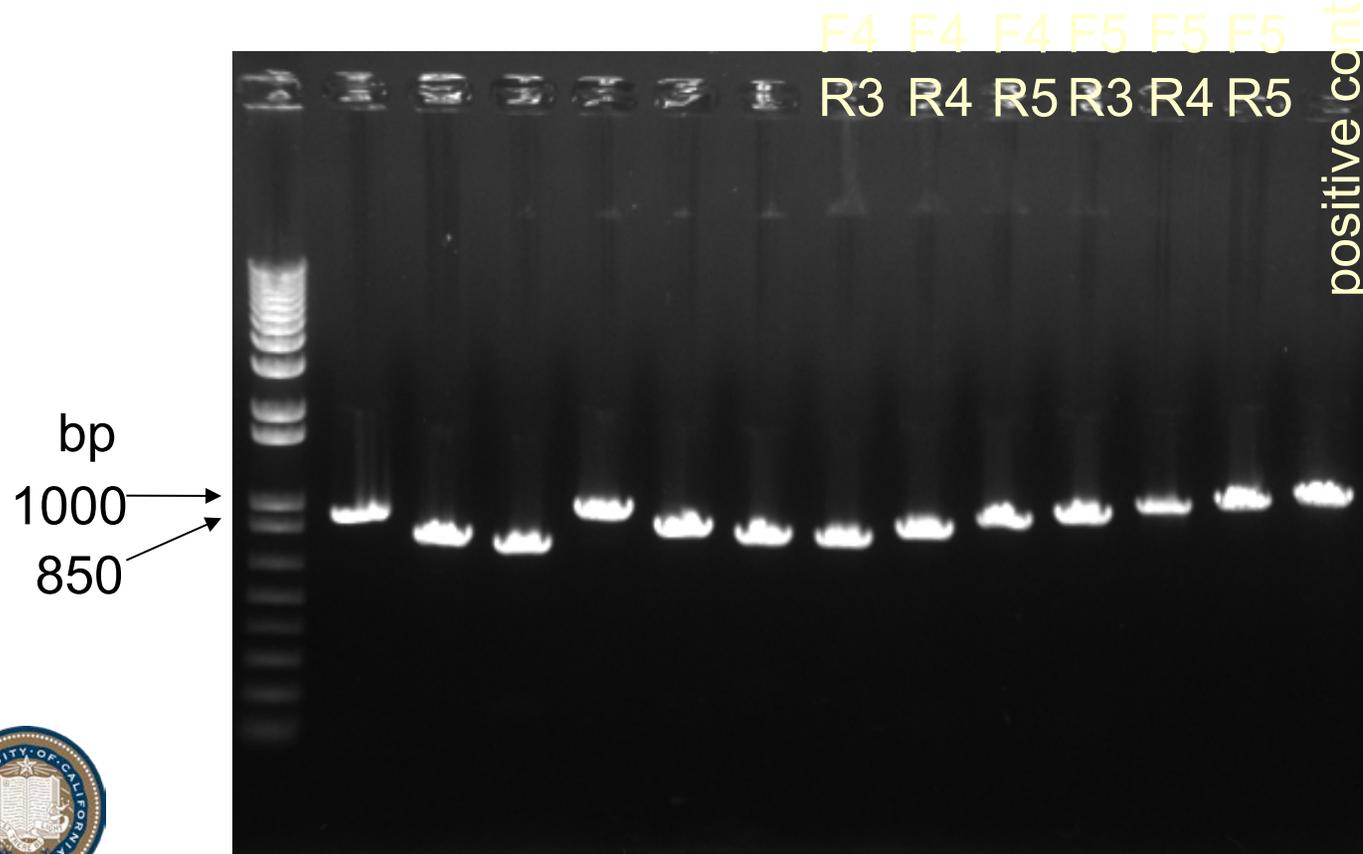
Primer set	Length [bp]
F1R1	935
F2R1	819
F3R1	769
F1R2	1003
F2R2	887
F3R2	837



WT gDNA



Δcpc transformant gDNA



Expected product size

Primer set	Length [bp]
F4R3	825
F4R4	894
F4R5	949
F5R3	1008
F5R4	1077
F5R5	1132



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%



Phycobilisome-Chlorophyll antenna size In Cyanobacteria

