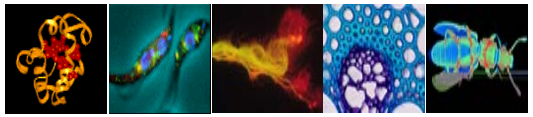


Imperial College London

Prohibitin homologues in *Synechocystis* sp. PCC 6803

Marko Boehm

Wolfson Biochemistry Building

Division of Biology

Imperial College London

South Kensington campus

SW7 2AZ, UK

Coral bleaching

Prohibitins

- Prohibitins are ubiquitously found and have been studied in yeast and humans, but very little about them is known in procayotes.
- Prohibitins belong to the Band 7 protein superfamily which share the SPFH domain as a common motif (SPFH = **S**tomatin, **P**rohibitin, **F**lotillin and **H**flK/C).
- The functions of prohibitin homologues are still unclear, although they have been linked to many important cellular processes, such as:
  - cellular signalling and transcriptional control
  - senescence and apoptosis
  - mitochondrial biogenesis
  - chaperone activity

The FtsH connection

- Chaperone activity of Prohibitins in yeast -

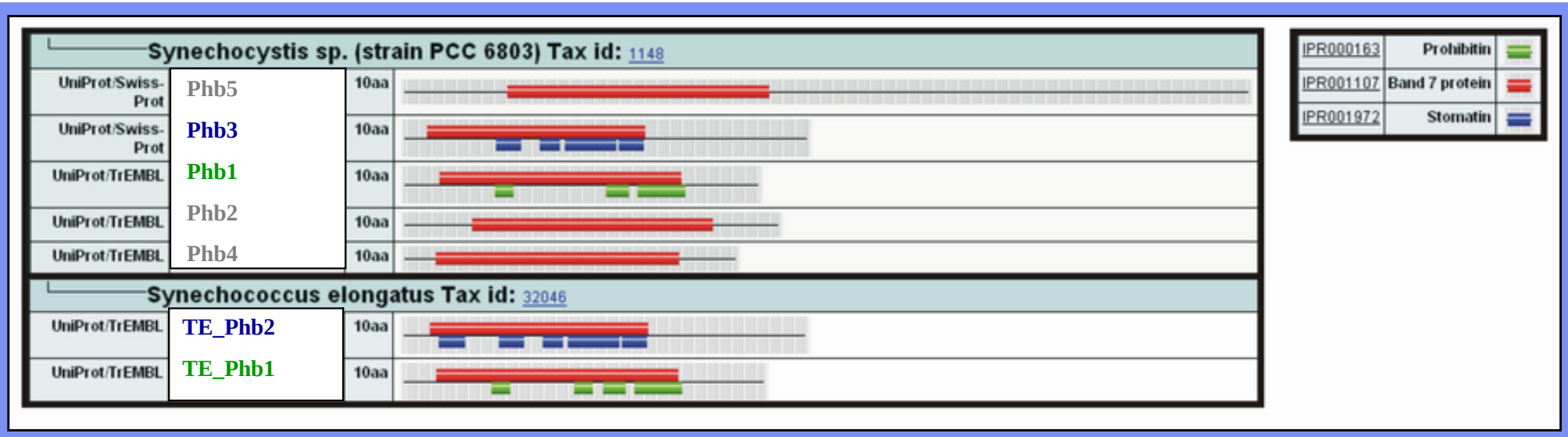
- In *S. cerevisiae* and *E. coli* prohibitin homologues have been found to form large, heteromultimeric complexes.
- In both organisms these complexes seem to be associated with FtsH proteases.
- In *S. cerevisiae* prohibitin complexes have been reported to negatively regulate an FtsH homologue by binding newly synthesised membrane proteins.
- In *Synechocystis* sp. PCC 6803 an FtsH homologue has been found to help protect the organism from the damaging effects of light.

Aims

- Identification of prohibitin homologues in *Synechocystis* sp. PCC 6803 and *Thermosynechococcus elongatus*.
- Bioinformatic analysis of identified prohibitin homologues.
- Identification and characterisation of possible prohibitin homologue complexes in *Synechocystis* sp. PCC 6803 *in vivo.*
- Testing the possible interactions between the prohibitin and FtsH homologues.
- Generation of prohibitin inactivation mutants in *Synechocystis* sp. PCC 6803 and elucidation of the potential roles of the respective proteins.

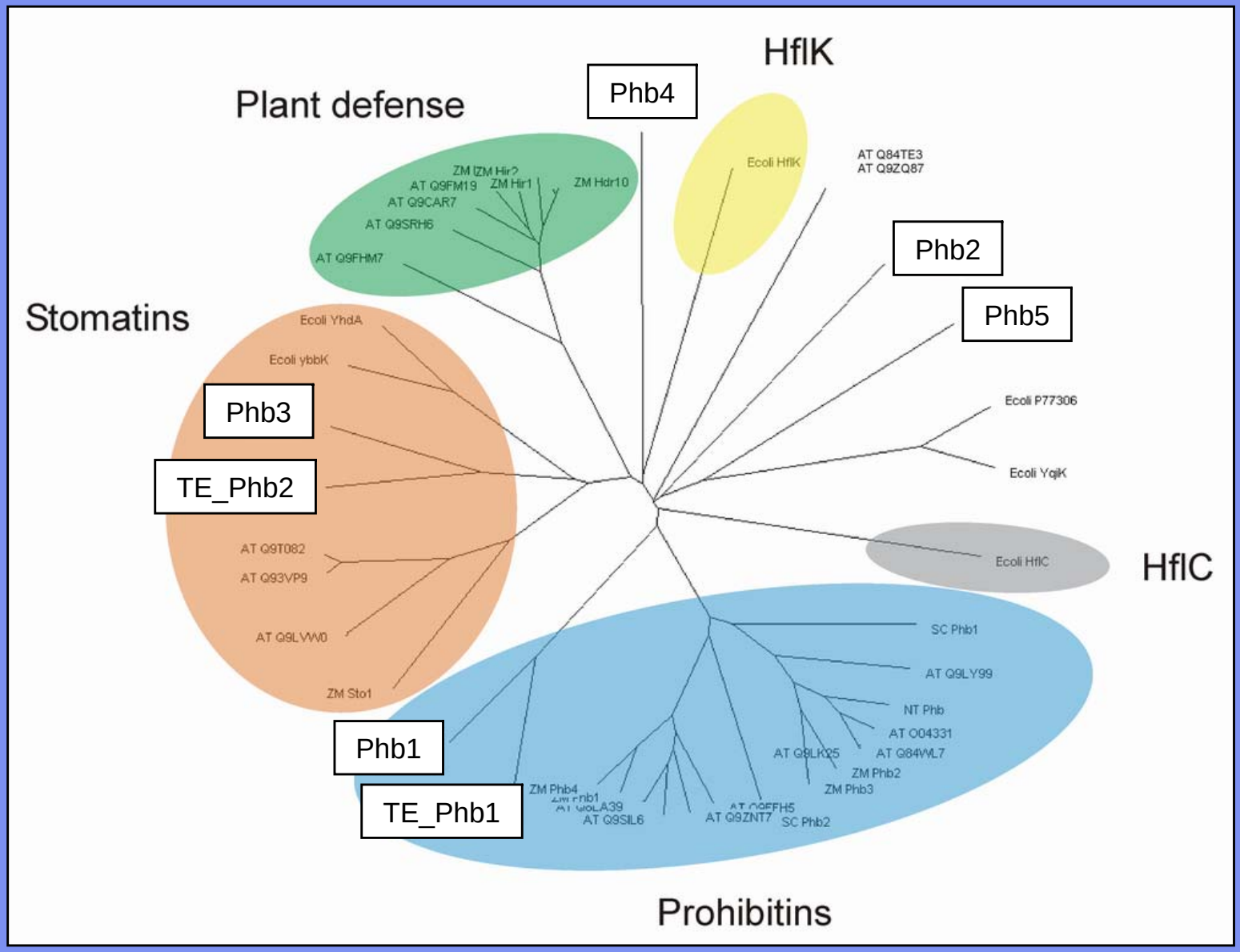
BIOINFORMATICS

5 Prohibitin homologues are found in Synechocystis sp. PCC 6803



Protein	UniProt accession #	bps	aas	Calculated MW	pI	TMs
Phb1	P72754	849	282	30.57	5.21	1
Phb2	P73049	897	298	32.83	5.58	2
Phb3	P72655	966	321	35.73	5.55	1
Phb4	P74042	795	264	30.37	8.36	0
Phb5	P72929	2022	673	74.42	5.08	1
TE_Phb1	Q8DI32	864	287	31.55	5.32	1
TE_Phb2	Q8DGX8	963	320	35.68	5.55	1

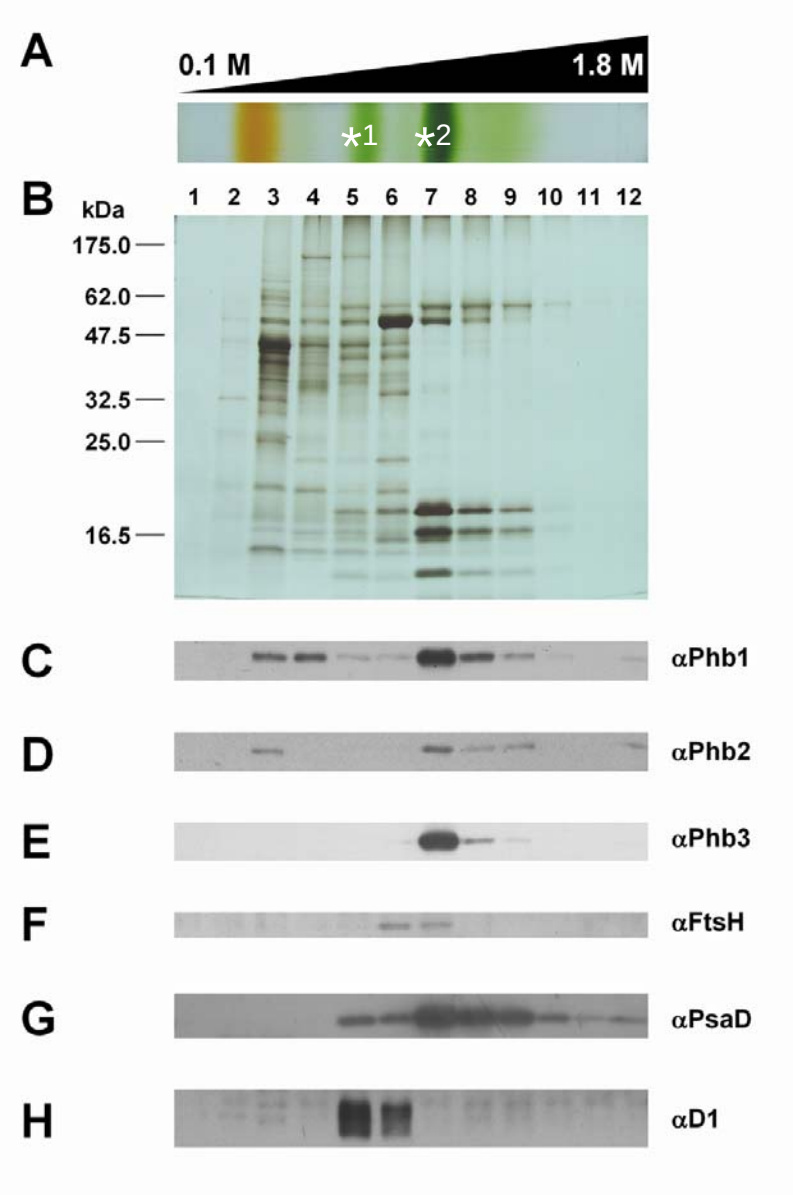
Prohibitin homologues are only distantly related



(based on Nadimpalli et al., 2000)

COMPLEX CHARACTERISATION

I - Prohibitin homologues form large complexes

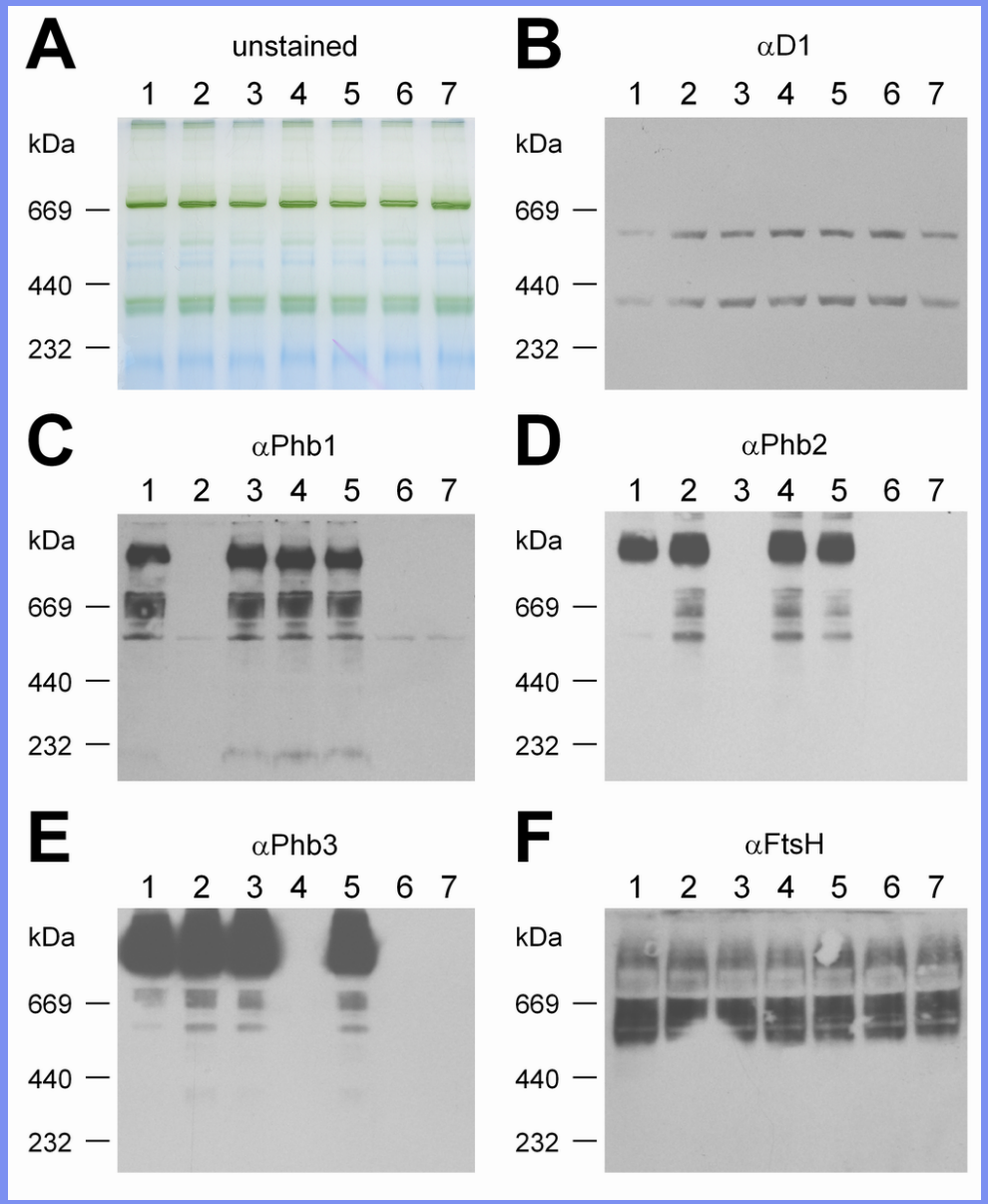


*1 = monomeric PSI and PSII (~300 kDa)
*2 = trimeric PSI (~900 kDa)

The prohibitin homologues 1, 2 & 3 form large, multimeric complexes (>900 kDa).

An interaction with FtsH homologues is possible.

II - Prohibitin homologues form large complexes



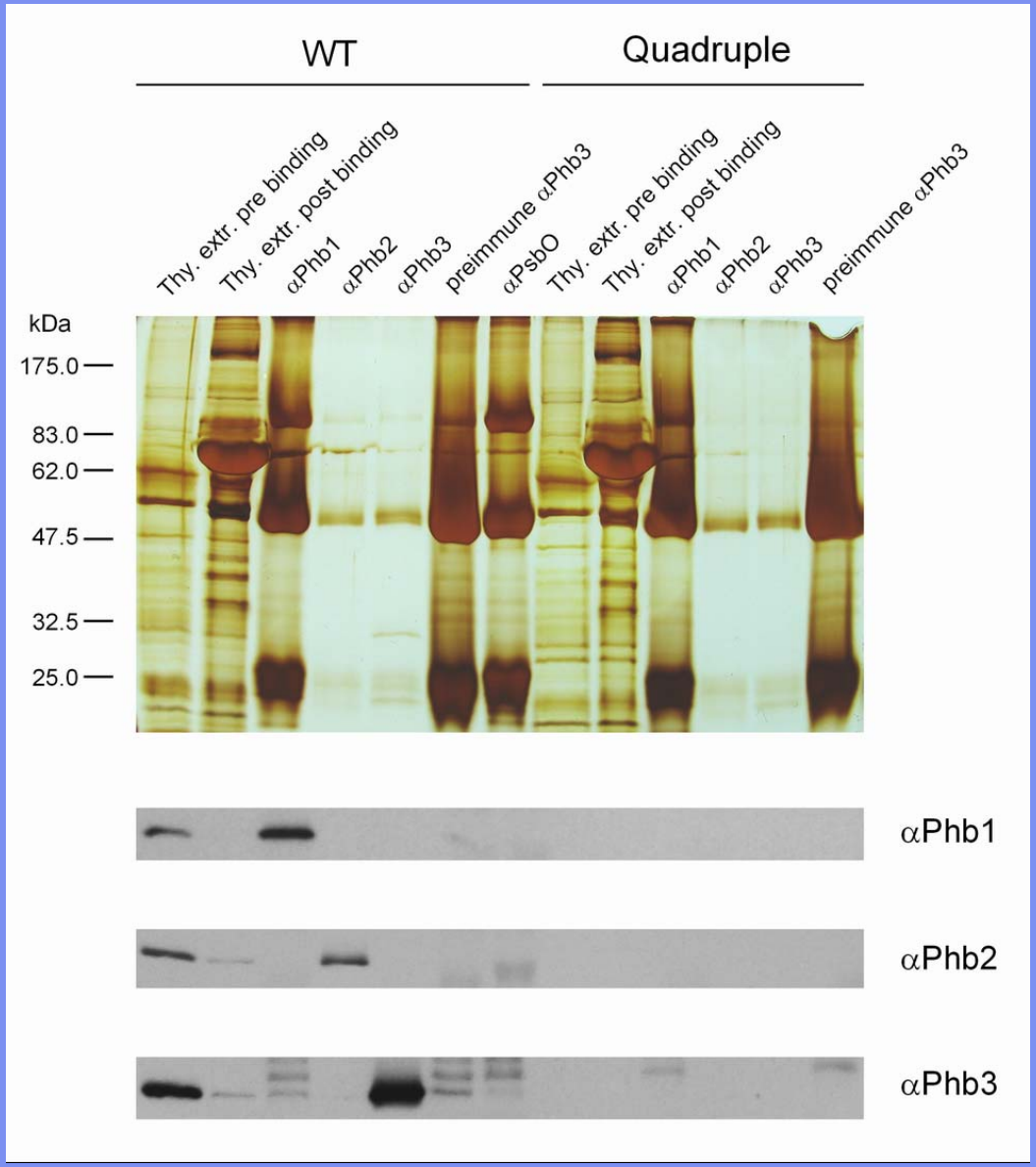
1 = WT
2 = ΔPhb1
3 = ΔPhb2
4 = ΔPhb3
5 = ΔPhb4
6 = ΔPhb1 ΔPhb2 ΔPhb3 (triple mutant)
7= ΔPhb1 ΔPhb2 ΔPhb2 ΔPhb4 (quadruple mutant)

The prohibitin homologues 1, 2 & 3 form large multimeric complexes (>900 kDa).

The complexes still form, even if another prohibitin homologue is inactivated.

FtsH complexes do not seem to be affected by prohibitin homologue inactivation.

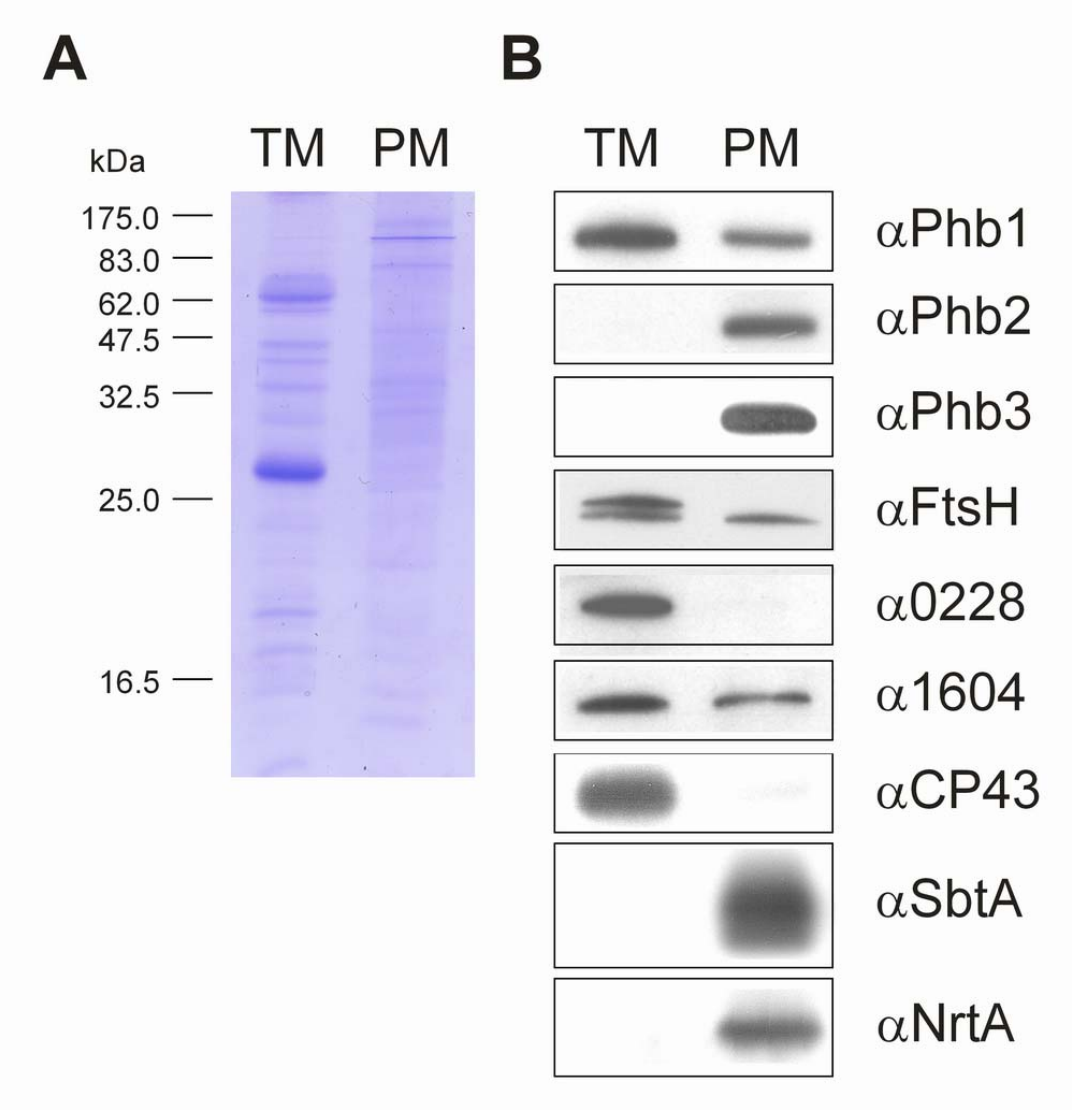
Prohibitins do not seem to interact

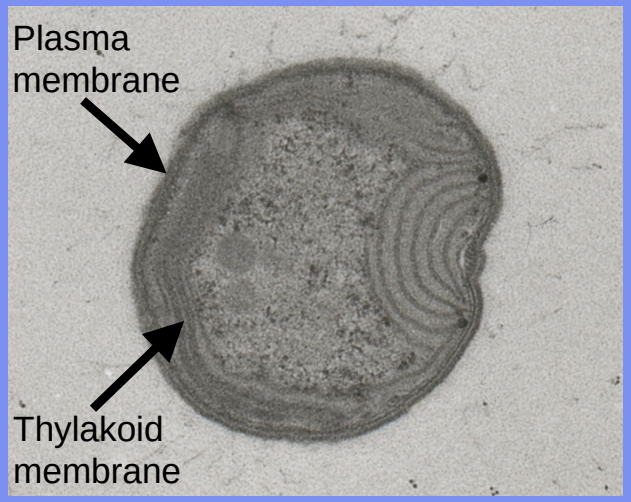


The prohibitin homologues 1, 2 & 3 do not seem to interact with one another.

Whether there is an interaction with an FtsH homologue needs to be tested.

Localisation of prohibitin and FtsH homologues





Electron micrograph of *Synechocystis* sp. PCC 6803; provided by Dr Uwe Kahmann

0228 and 1604 = FtsH homologues

CP43 = chlorophyll binding protein; subunit of PSII; thylakoid marker

SbtA = sodium-dependent bicarbonate transporter; plasma membrane marker

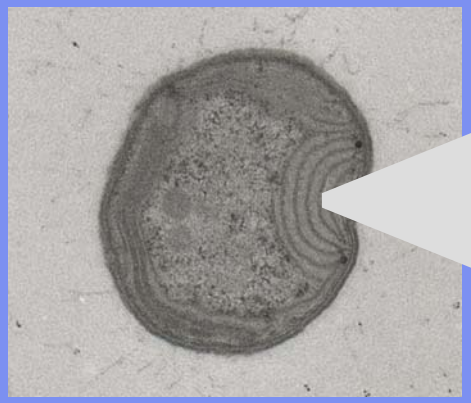
NrtA = nitrate/nitrite transport system substrate-binding protein ; plasma membrane marker

PHYSIOLOGICAL RELEVANCE

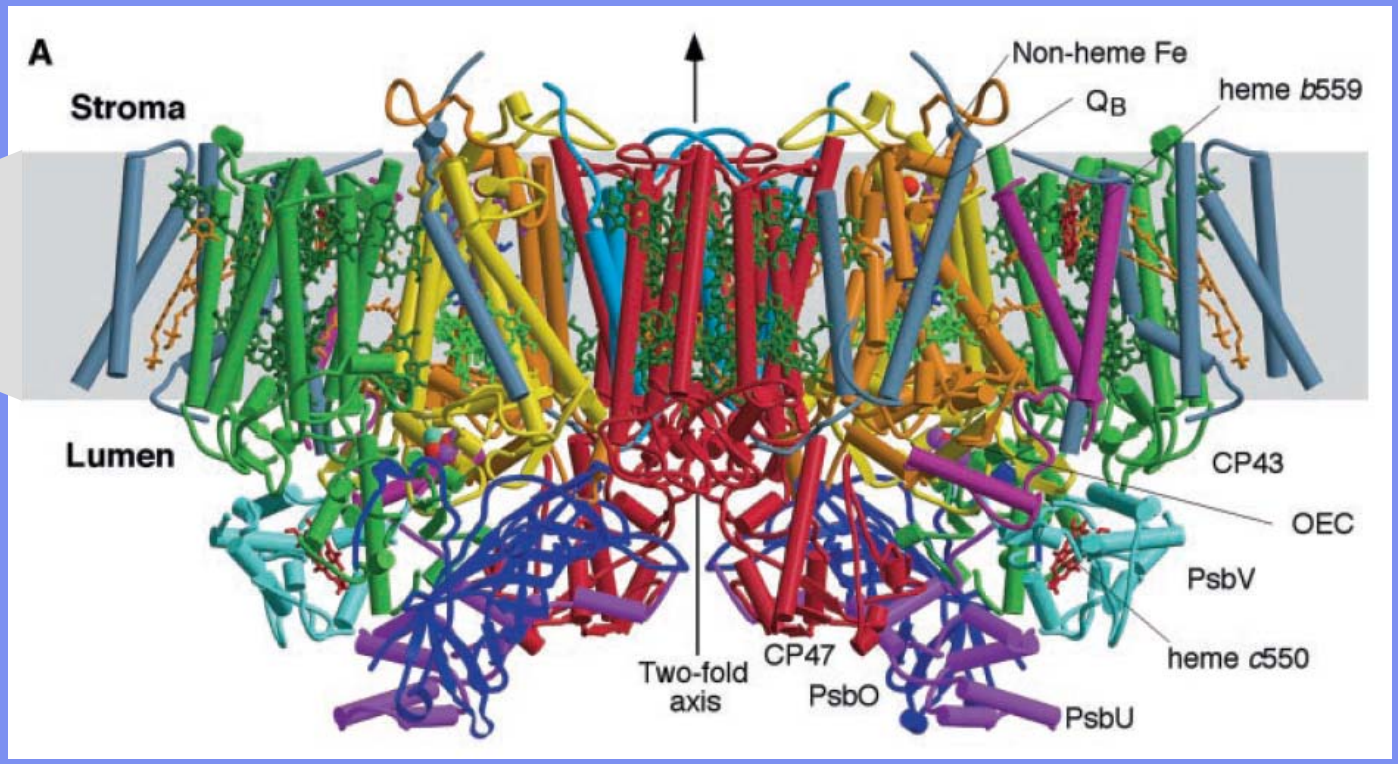
Synechocystis sp. PCC 6803

performs oxygenic photosynthesis

$$6\,H_2O + 6\,CO_2 \xrightarrow{\text{light}} C_6H_{12}O_6 + 6\,O_2$$



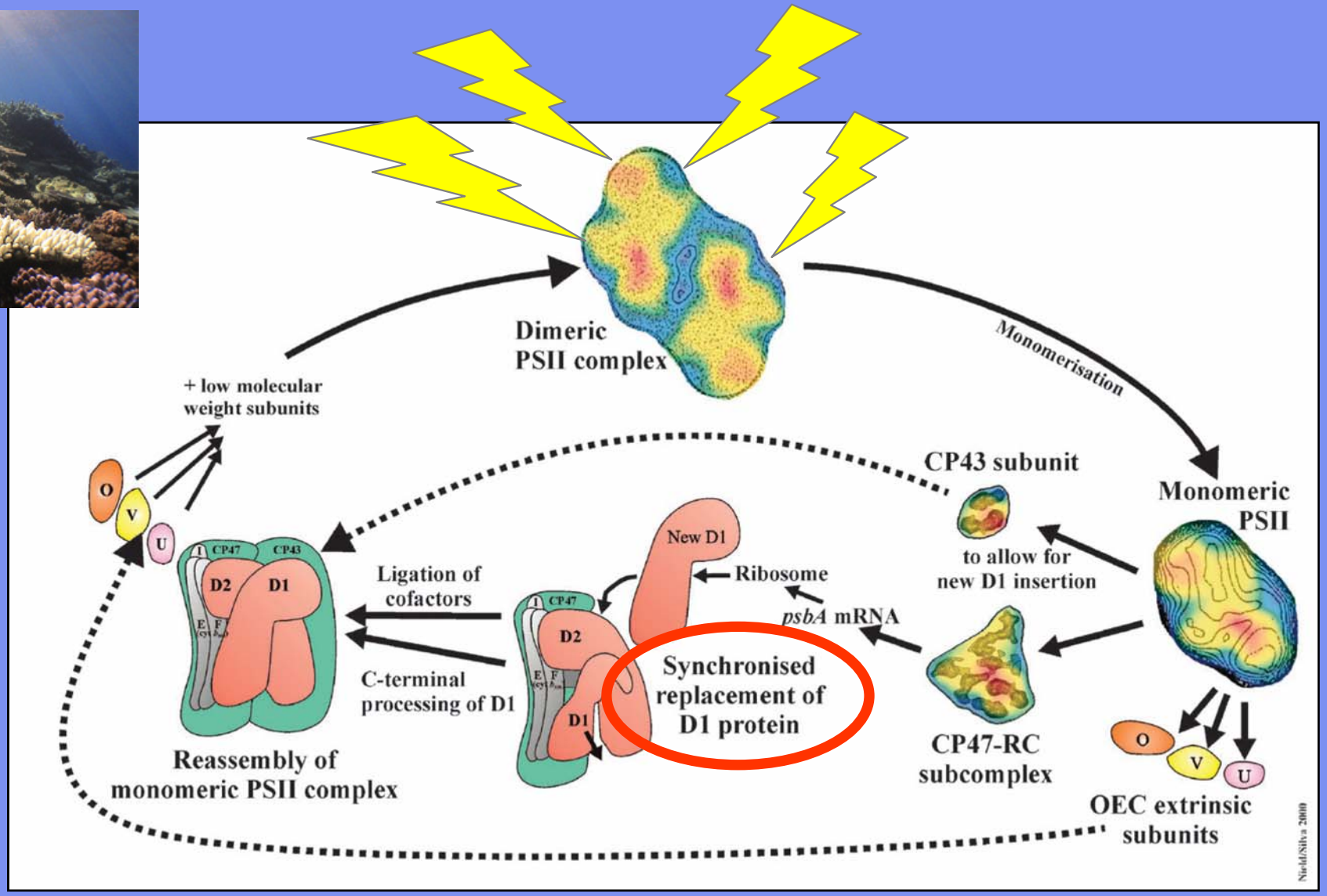
Electron micrograph of *Synechocystis* sp. PCC 6803; provided by Dr Uwe Kahmann



Structure of photosystem II; (Ferreira et al., 2004)

The PSII repair cycle

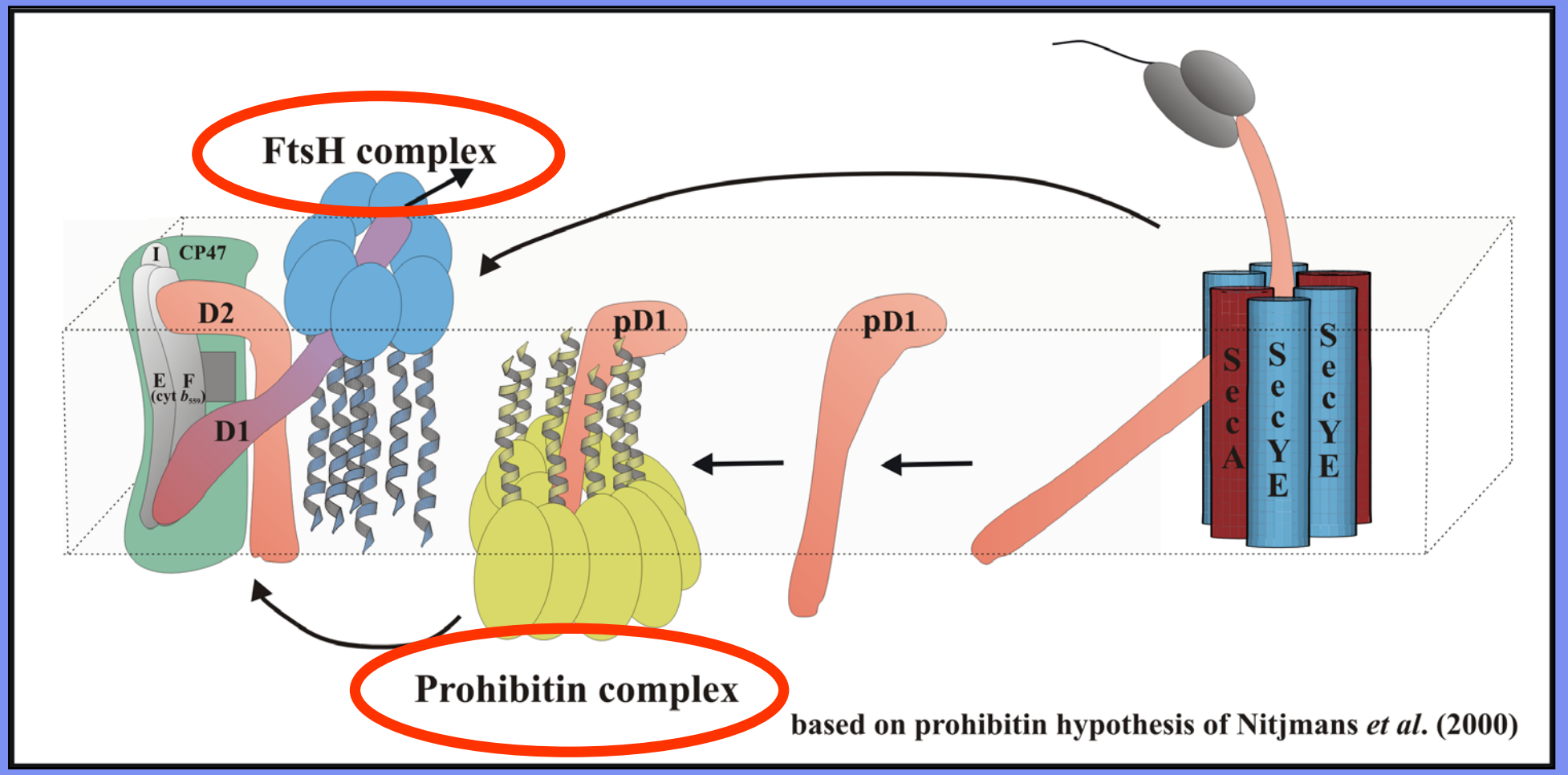
Coral bleaching



PSII repair cycle ;provided by Dr Paulo Silva and Dr Jon Nield

Hypothesis and working model

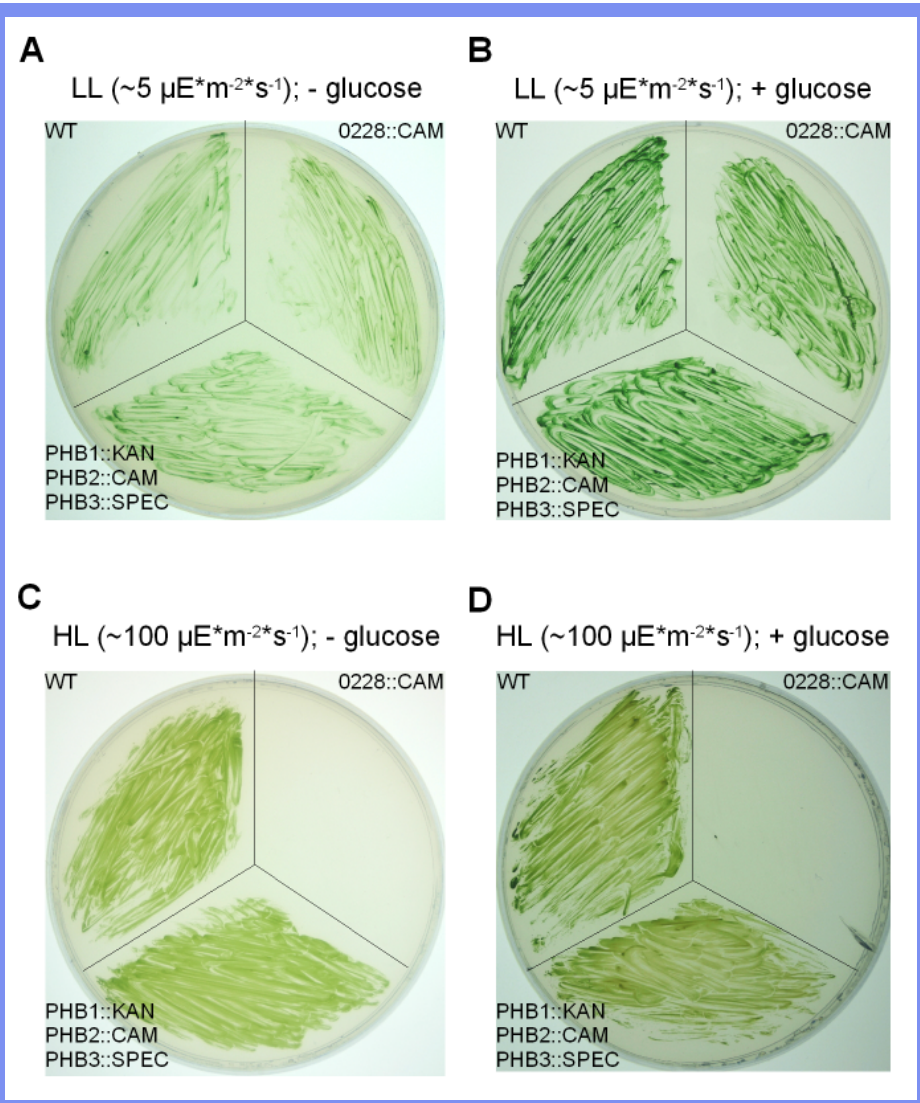
- for synchronised replacement of the D1 protein -



Working model; (Silva et al., 2002)

Prohibitin inactivation mutants

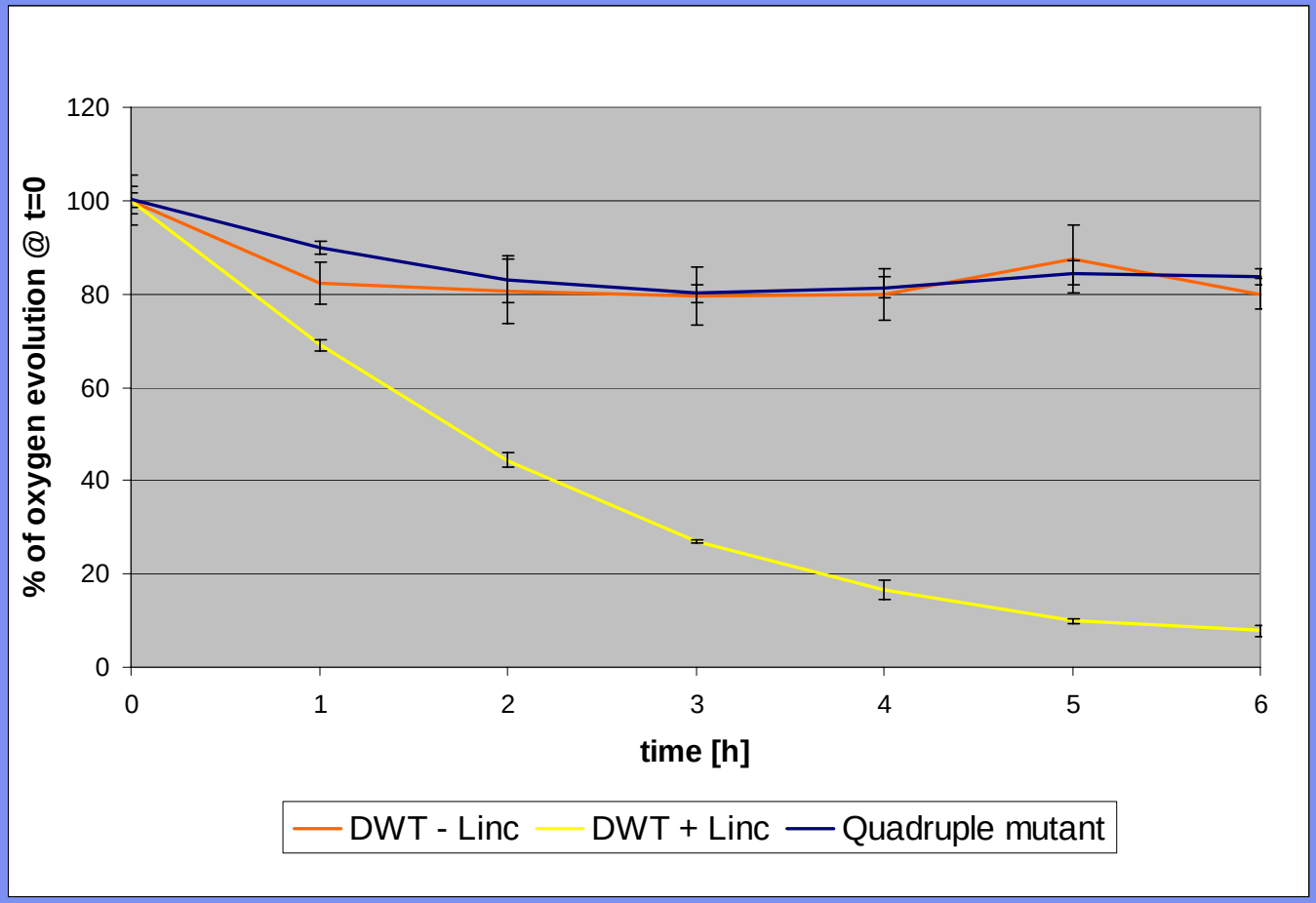
are viable under “high light“ intensities



The prohibitin homologue 1, 2 and 3 triple inactivation mutant is viable under elevated light intensities.

The FtsH inactivation mutant dies under the same conditions.

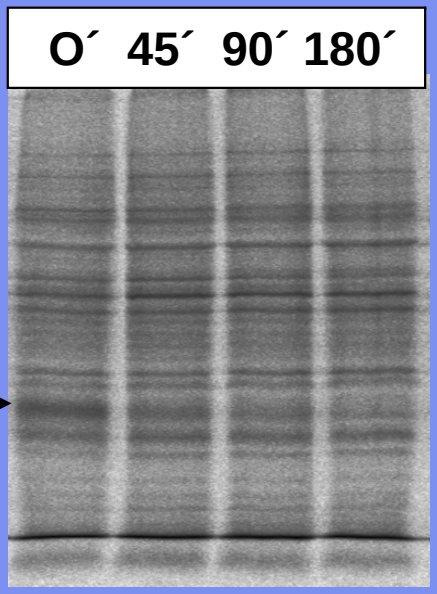
The PS II repair cycle is not impaired in the quadruple inactivation mutant

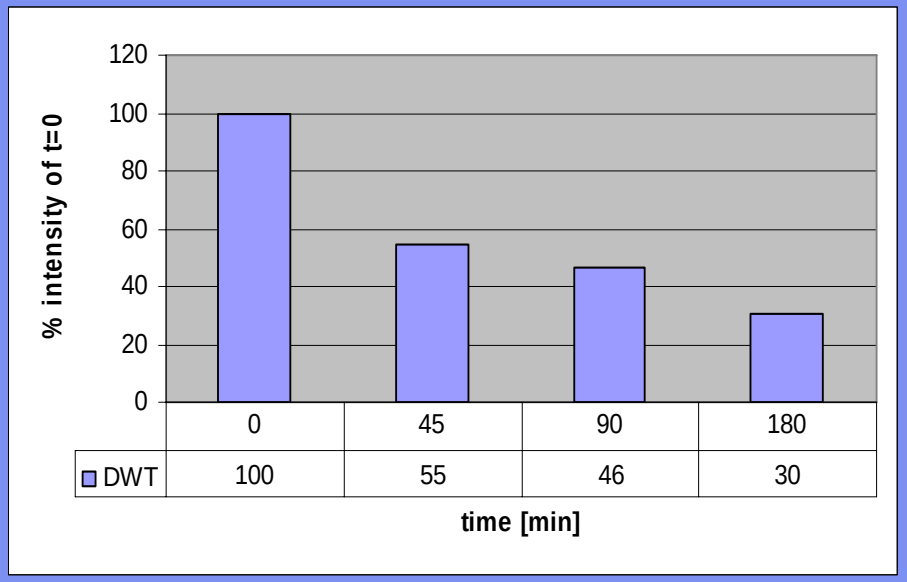


- Wildtype cells maintain their PSII activity (oxygen evolution).
- PSII activity decreases in wildtype cells in the presence of a protein synthesis inhibitor.
- The quadruple mutant also maintains PSII activity.

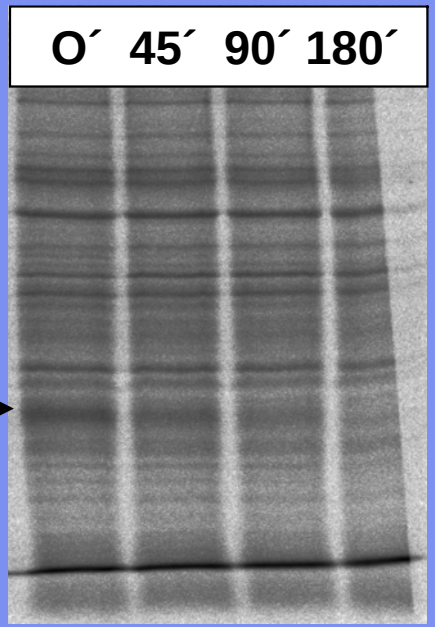
The D1 protein is turned over at a similar rate

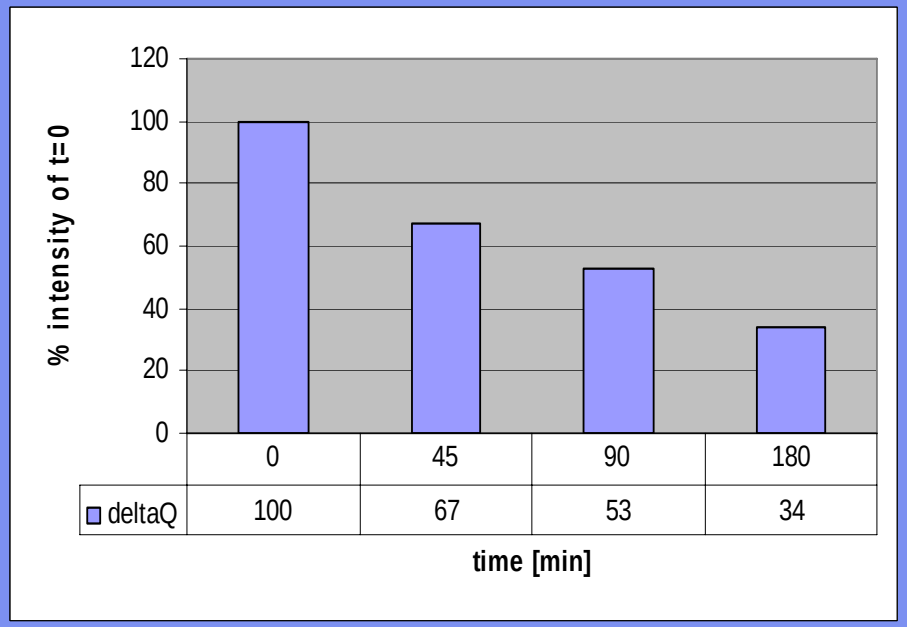
WT





Quadruple mutant





Conclusions

- ✓ The identified cyanobacterial proteins are only distantly related to other known, eukaryotic prohibitin homologues and amongst themselves.
- ✓ The prohibitin homologues Phb1, Phb2 and Phb3 form large and possibly homomultimeric protein complexes.
- ✓ An interaction with an FtsH homologue has not yet been observed.
- ✓ Prohibitin 1 is localised in the thylakoid and plasma membrane, whereas the Prohibitin homologues 2 and 3 are localised in the plasma membrane.
- ✓ The prohibitin homologues Phb1, 2, 3, 4 & 5 are not essential for cell viability under the conditions tested so far.
- ✓ The prohibitin homologues Phb1, Phb2, Phb3 and Phb4 seem not to be involved in the PSII repair cycle or affect the rate of D1 protein turnover in the performed experiments.

Future Work

- Immunoprecipitation experiments with purified antisera and testing for a direct interaction with an FtsH homologue.

- Affinity purification of prohibitin homologue complexes.
- Purification of His tagged prohibitin homologue complexes.
- Single particle analysis of purified protein complexes.

- Testing further stress conditions to find the physiological relevance of prohibitin homologues.

Aknowledgements

Imperial College

Prof. Peter Nixon

Myles Barker

Remco de Vries

Franck Michoux

Dr. Bart Feyes (Imperial College London)

Dr. Huw Williams (Imperial College London)

University of Turku

Prof Eva Maria Aro

Pengpeng Zhang

Universität Bielefeld

Dr Uwe Kahmann