The structure of the FtsH complex involved in Photosystem II repair in cyanobacteria

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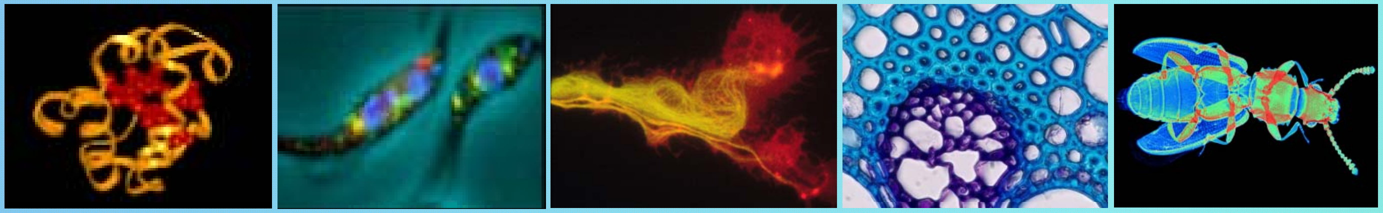
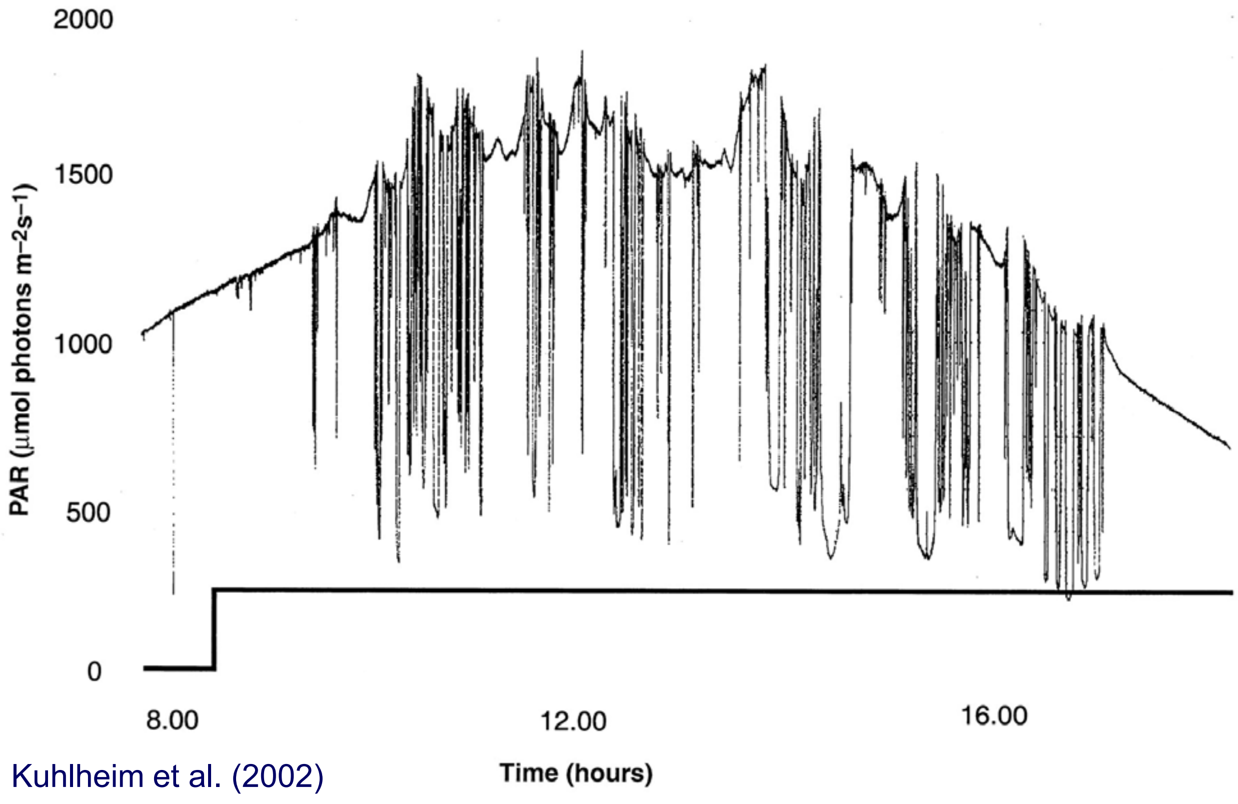


Overview

- Introduction
- The PSII repair cycle in cyanobacteria
- D1 protein removal and degradation
    - Proteases (DegP and FtsH)
    - Notes on the mechanism
    - Isolation of the FtsH protein complex
    - Structural analysis
- The FtsH-only model

Too much of a good thing?

- Light quantity and qualitiy in the field vs the lab -



Kuhlheim et al. (2002)

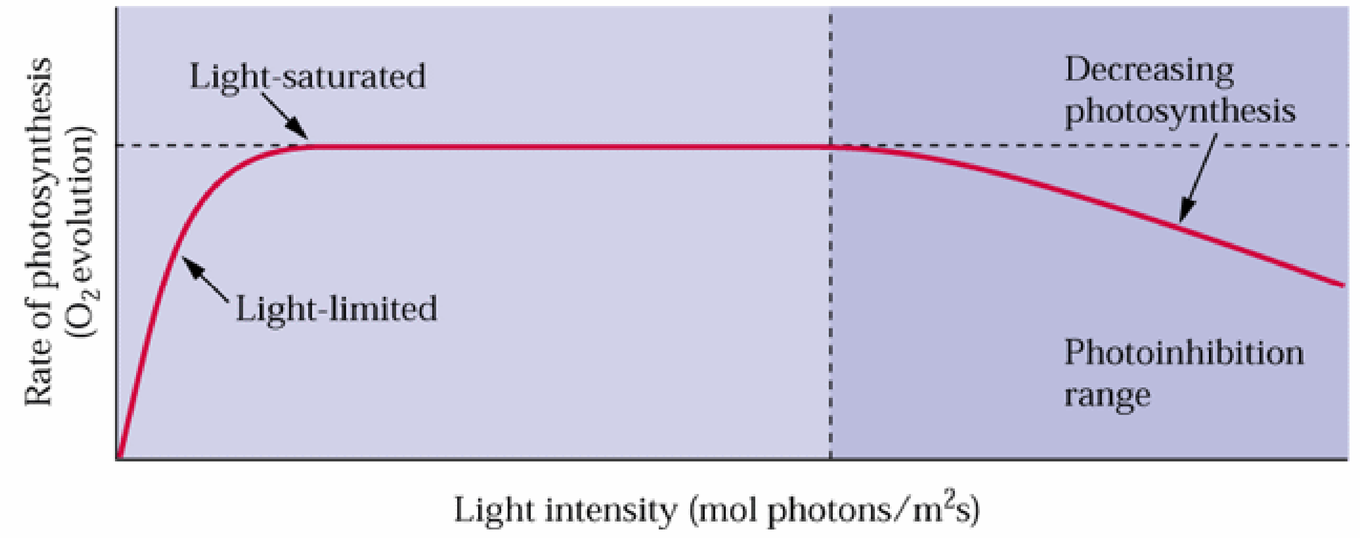
PAR – photosynthetically active radiation

in the field

controlled conditions

Too much of a good thing!

- Photoinhibition: light-induced reduction of photosynthetic activity -

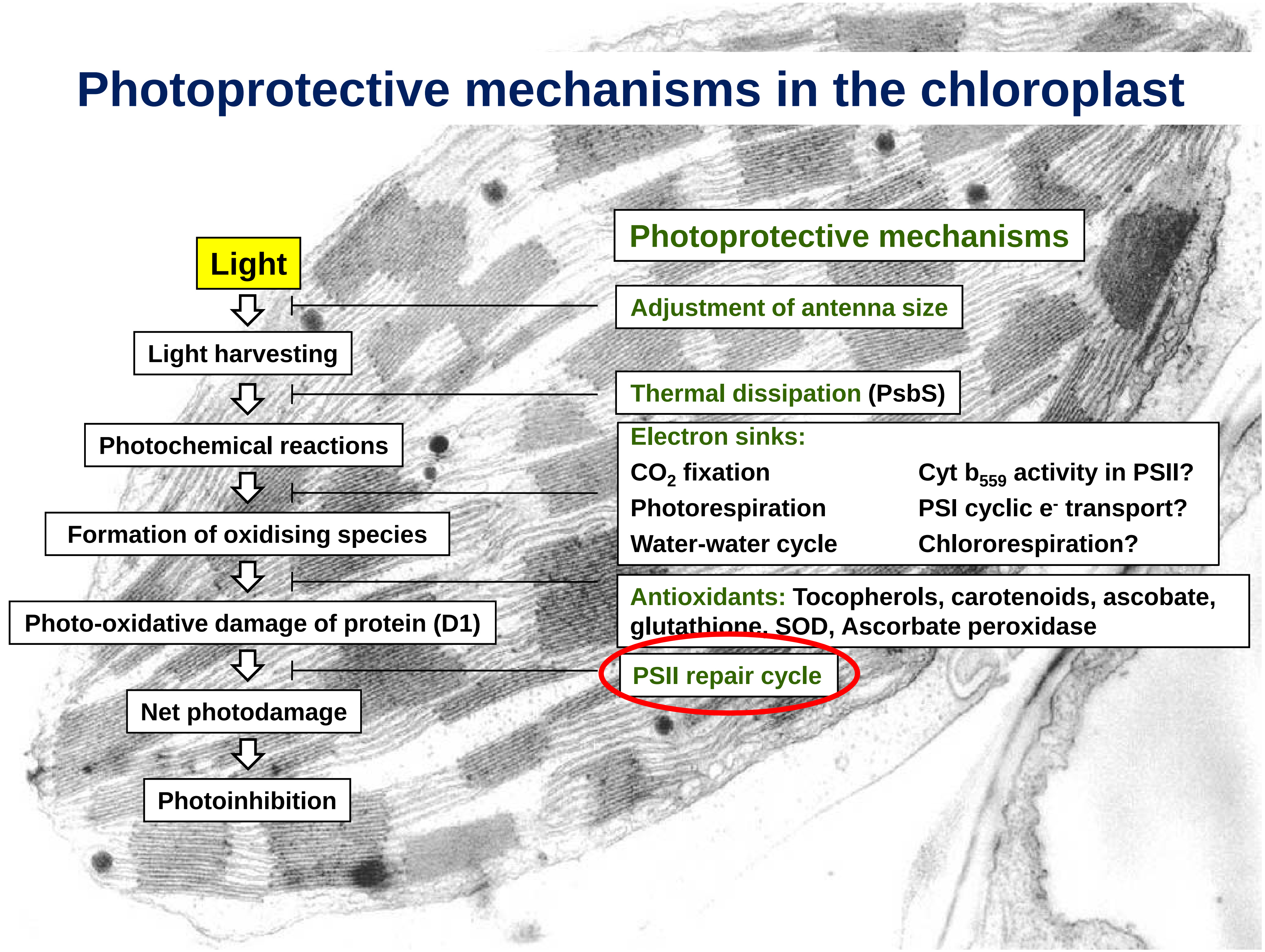


Biochemistry & Molecular Biology of Plants
(Buchanan, Gruissem and Jones eds)

Ifuku et al. (2005)

Coral bleaching

Photoprotective mechanisms in the chloroplast



Light

Light harvesting

Photochemical reactions

Formation of oxidising species

Photo-oxidative damage of protein (D1)

Net photodamage

Photoinhibition

Photoprotective mechanisms

Adjustment of antenna size

Thermal dissipation (PsbS)

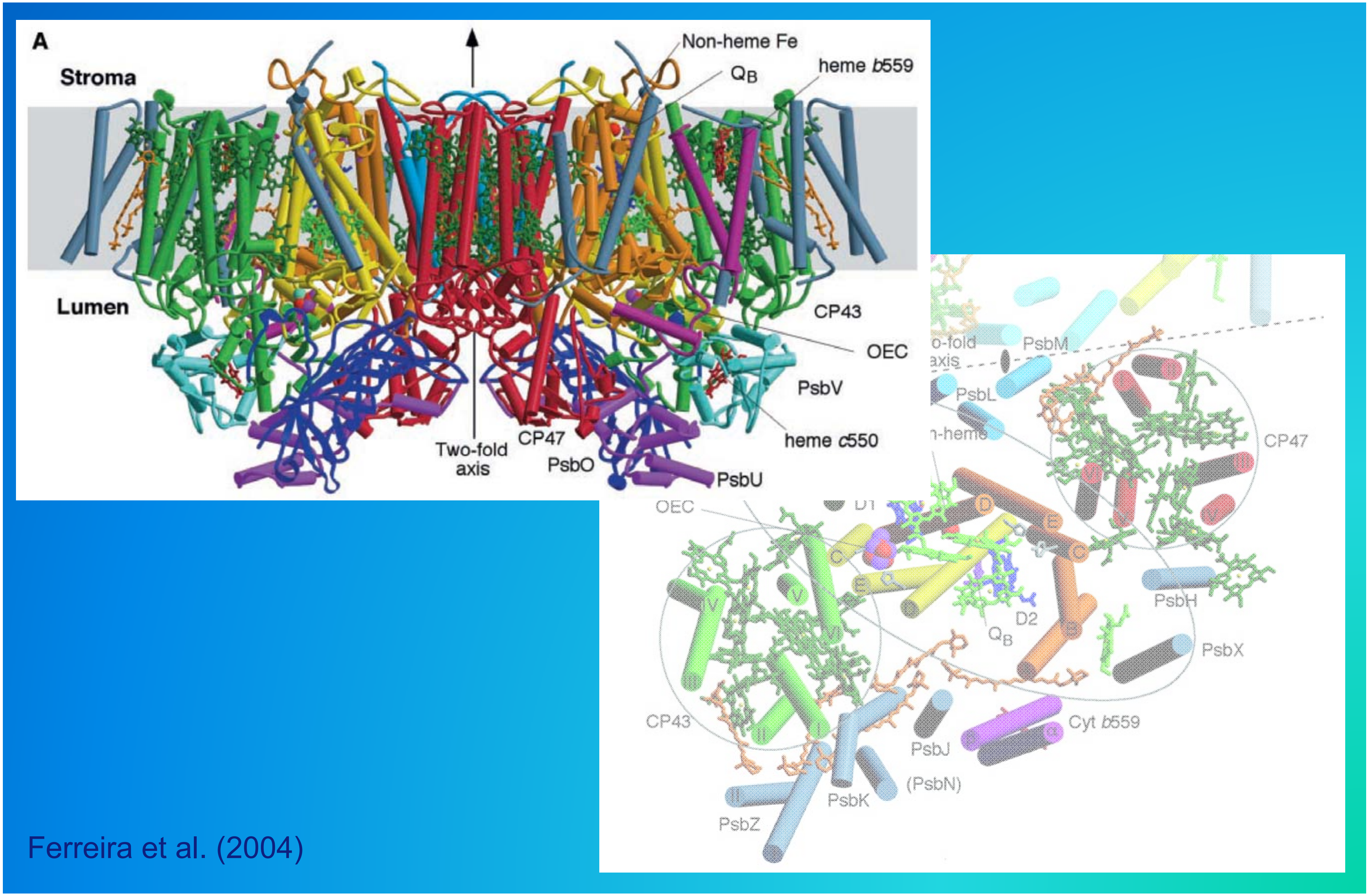
Electron sinks:

CO_2 fixation	Cyt b_{559} activity in PSII?
Photorespiration	PSI cyclic e^- transport?
Water-water cycle	Chlororespiration?

Antioxidants: Tocopherols, carotenoids, ascobate, glutathione, SOD, Ascorbate peroxidase

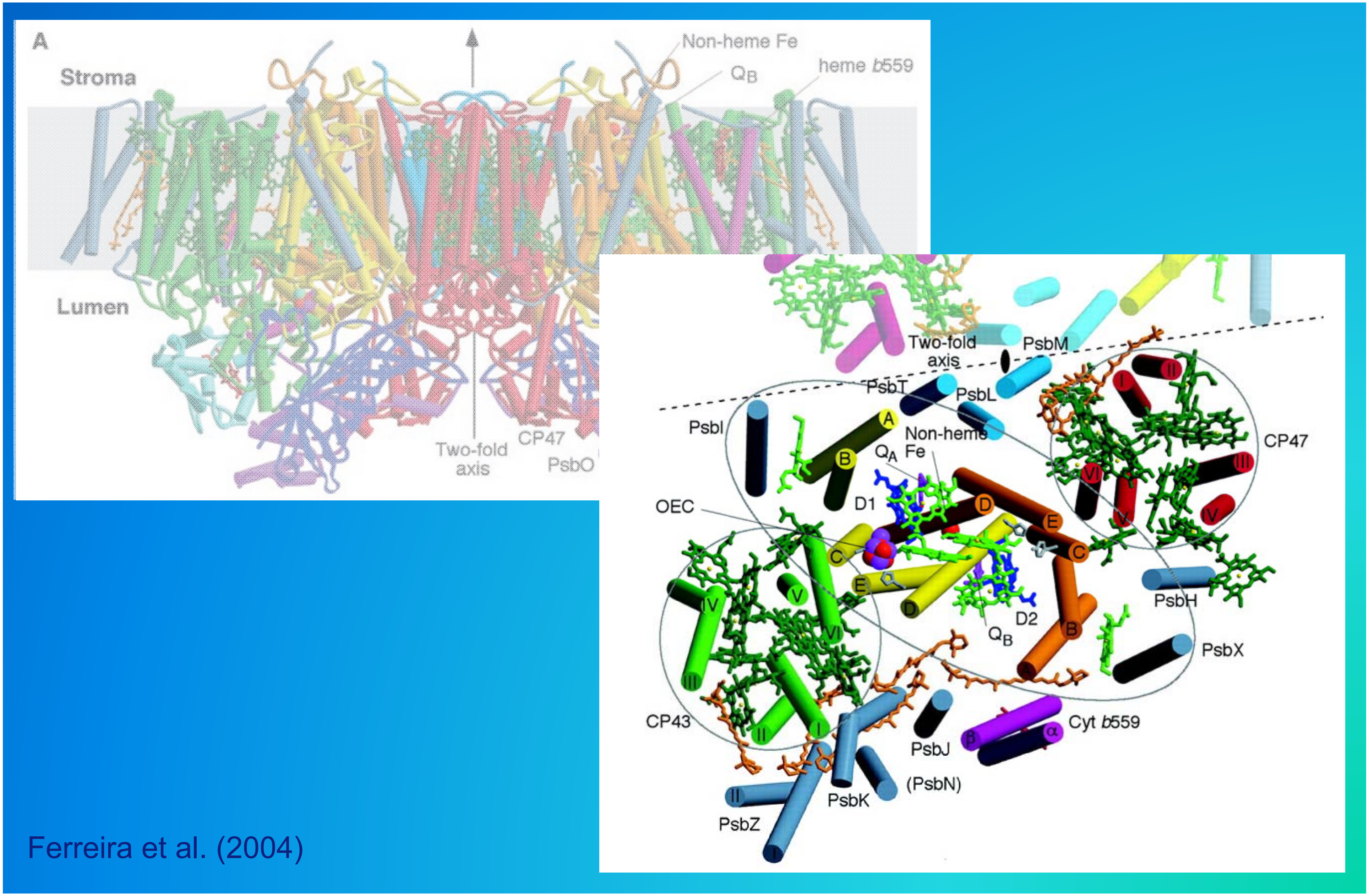
PSII repair cycle

The cyanobacterial PS II complex



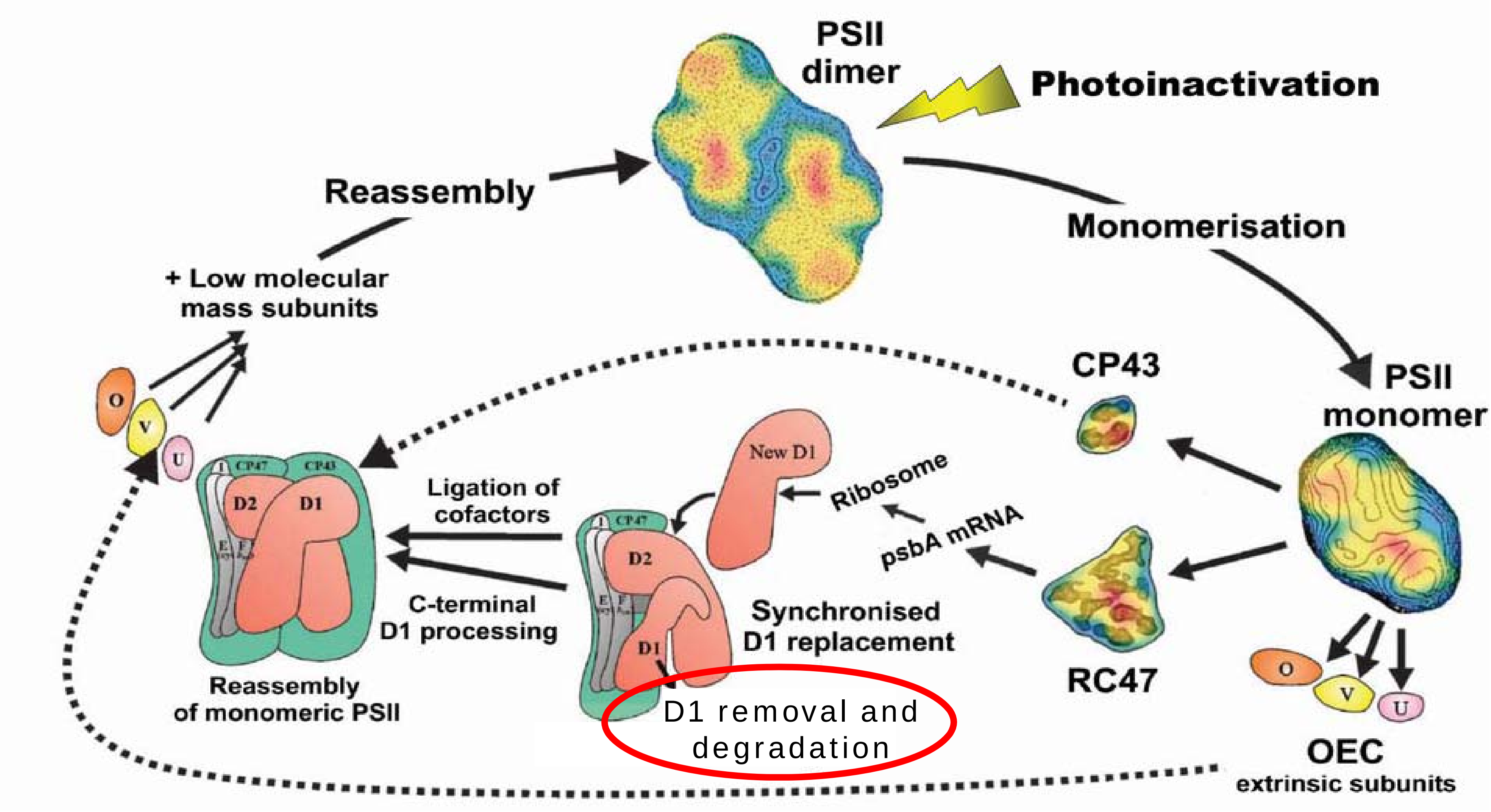
Ferreira et al. (2004)

The cyanobacterial PS II complex



Ferreira et al. (2004)

The cyanobacterial PS II repair cycle

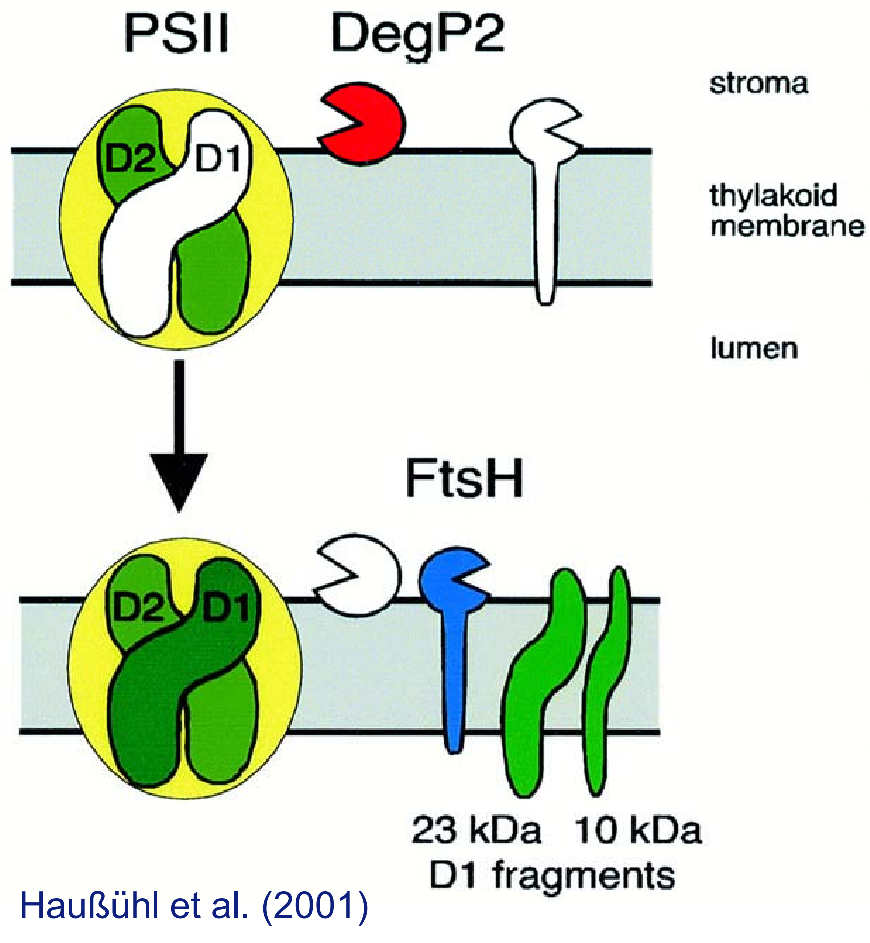


modified from Nield et al. (2005)

Some longstanding questions concerning light-induced D1 degradation and PSII repair

- What are the proteases involved *in vivo*?
- How is damaged D1 recognised?
- What is the mechanism of degradation?
- What is the physiological relevance of reactive oxygen species in D1 degradation?
- Are there different degradation pathways depending on the type of damage (donor-side versus acceptor-side damage)?

The DegP2/FtsH model for D1 degradation in chloroplasts

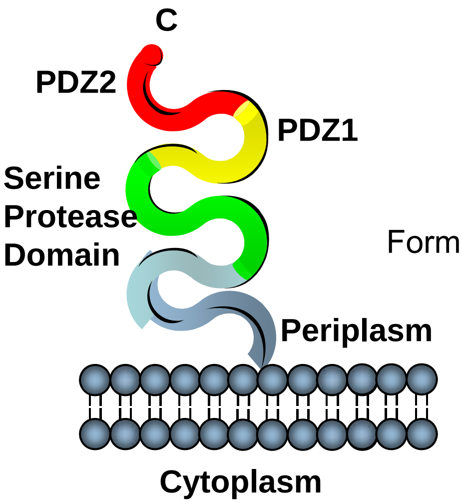


Haußühl et al. (2001)

Model based on *in vitro* experiments!

DegP and FtsH proteases
in Synechocystis sp. PCC 6803

DegP/HtrA proteases

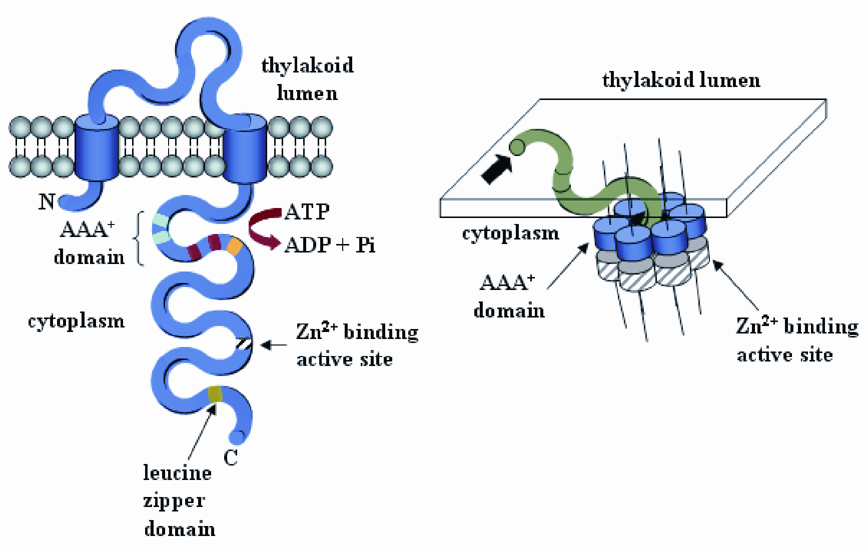


Forms dimer of trimers

3 homologues in *Synechocystis* sp. PCC 6803

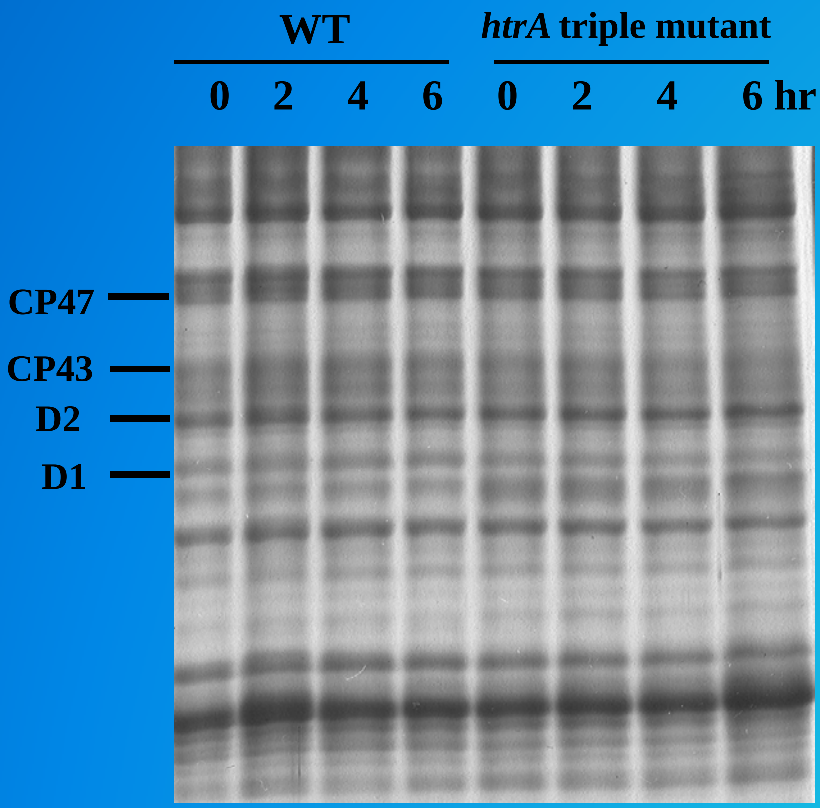
(HtrA, HhoA and HhoB)

FtsH proteases

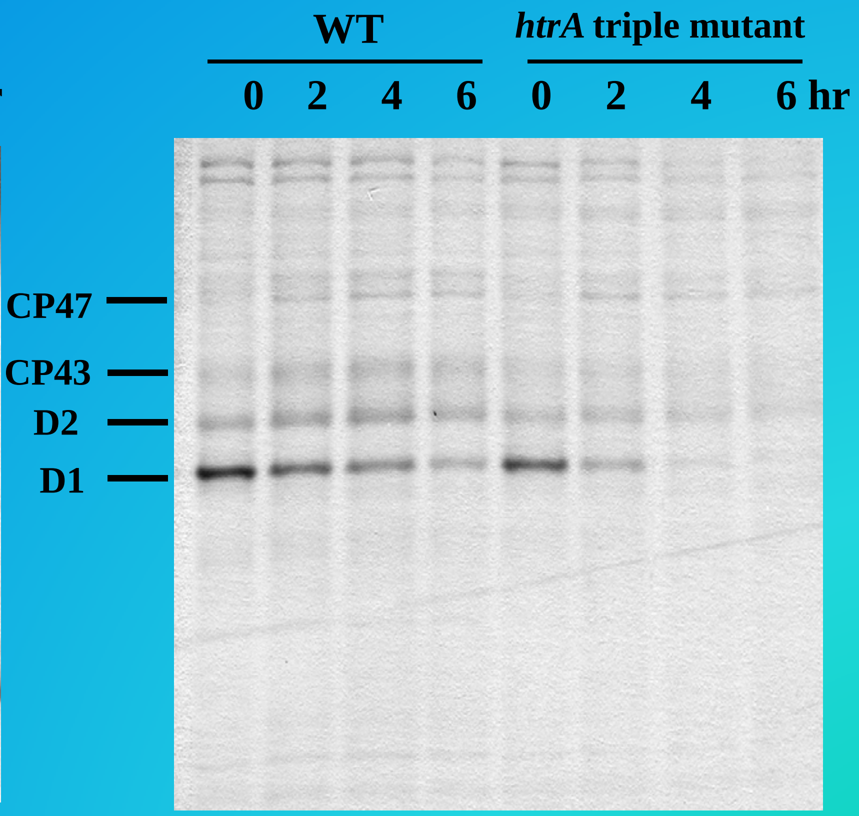


4 homologues in *Synechocystis* sp. PCC 6803
slr1390 and slr1604 vital
slr0228 and sll1463 dispensible
(Mann et al. 2000)

D1 turnover in the DegP/HtrA triple mutant is unimpaired



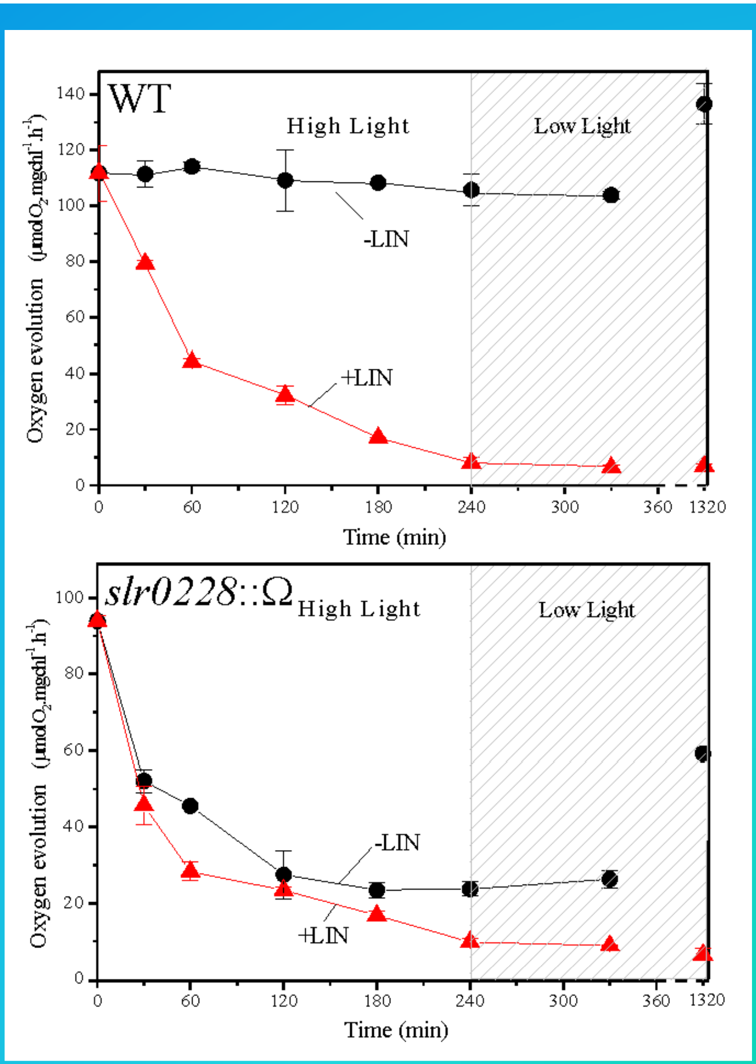
Coomassie stain



Autorad

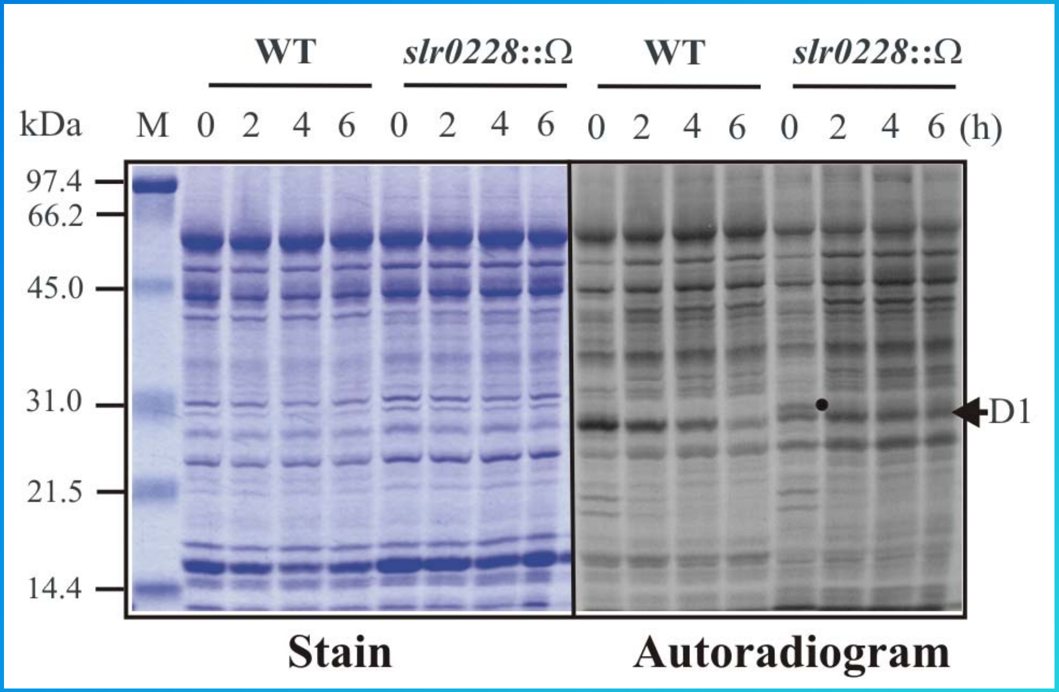
Barker et al. (2006)

The Δ0228 (FtsH2) insertion mutant is light sensitive and the PSII repair cycle is impaired



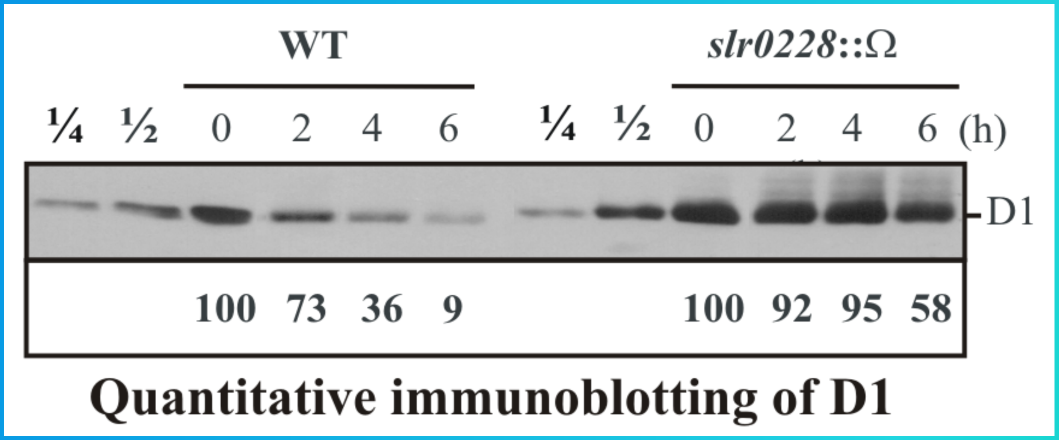
Silva et al. (2003)

D1 degradation is slower in the Δ0228 insertion mutant



Stain

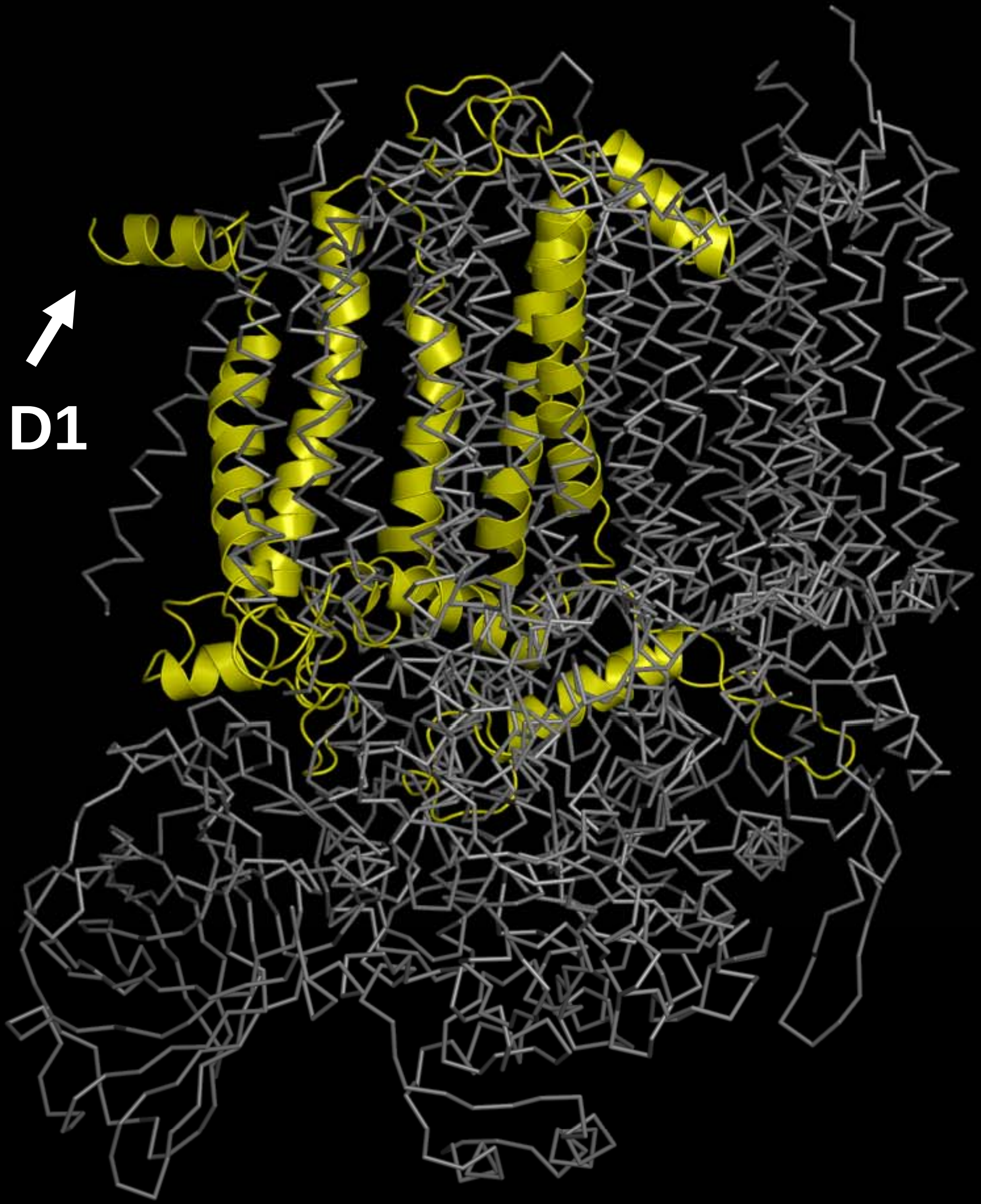
Autoradiogram



Quantitative immunoblotting of D1

No D1 fragments!

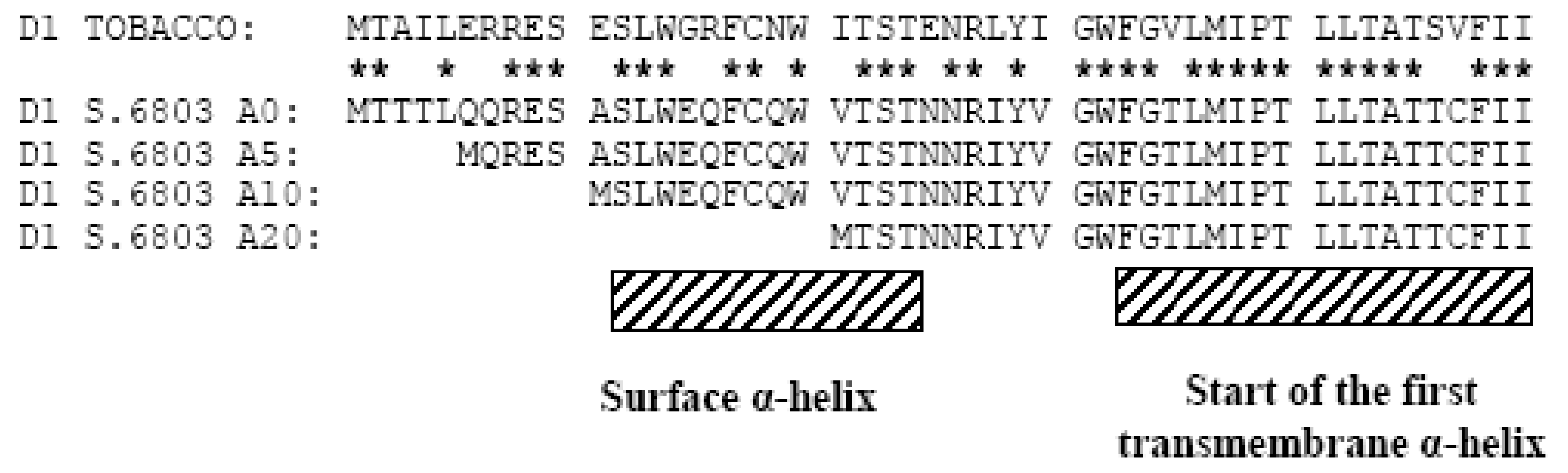
Silva et al. (2003)



N-terminus of D1

Structure of PSII from *T. elongatus* (provided by James Murray and Jim Barber)

The A20 mutant of *Synechocystis* sp. PCC 6803 lacks 20 aa residues from the exposed N-terminal tail



```
D1 TOBACCO:      MTAILERRES ESLWGRFCNW ITSTENRLYI GWFGVLMIPT LLTATSVFII
                 **  *  ***  ***  ** *  *** ** *  **** ***** *****  ***
D1 S.6803 A0:    MTTTLQQRES ASLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A5:          MQRES ASLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A10:              MSLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A20:                         MTSTNNRIYV GWFGTLMIPT LLTATTCFII
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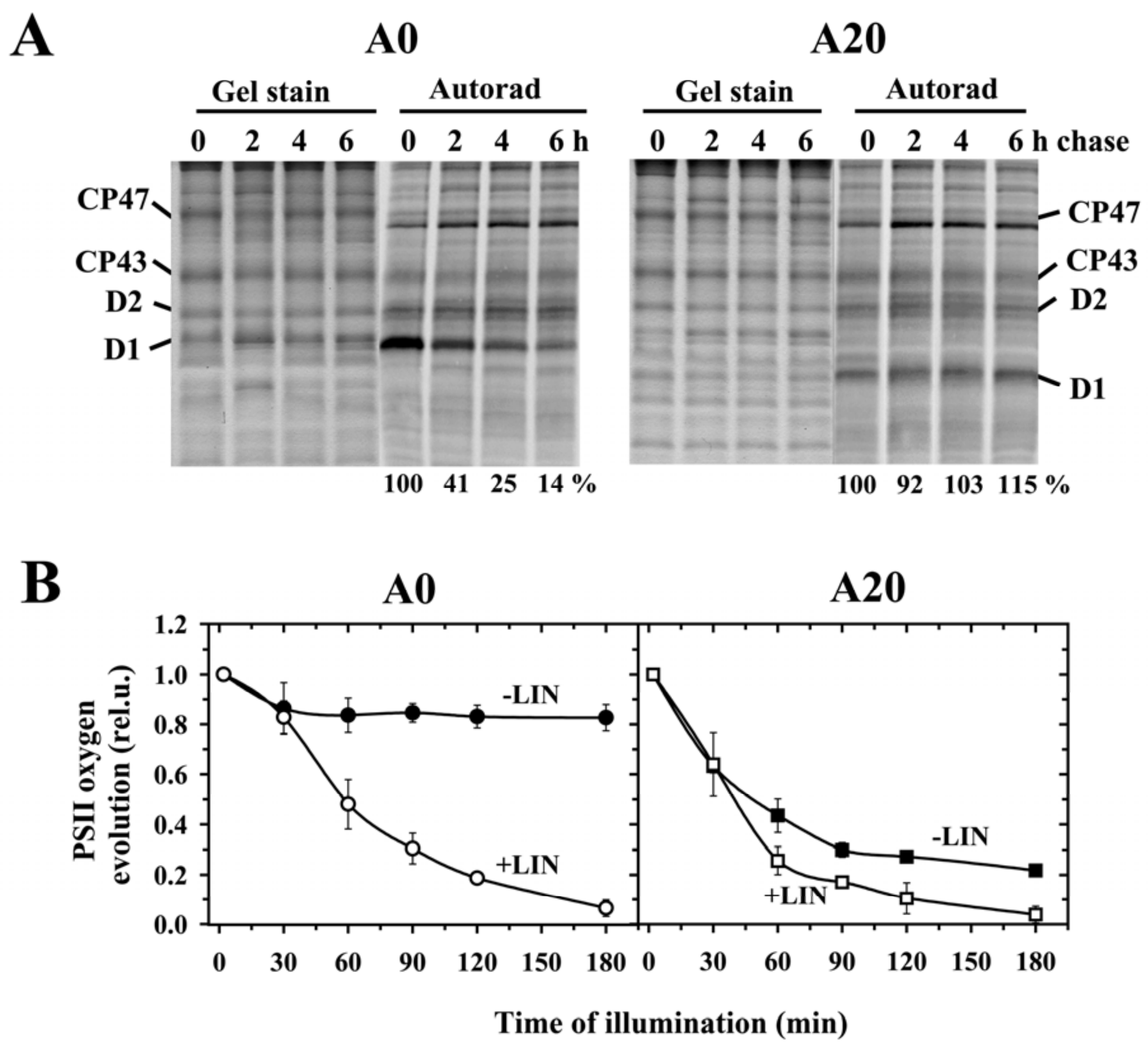
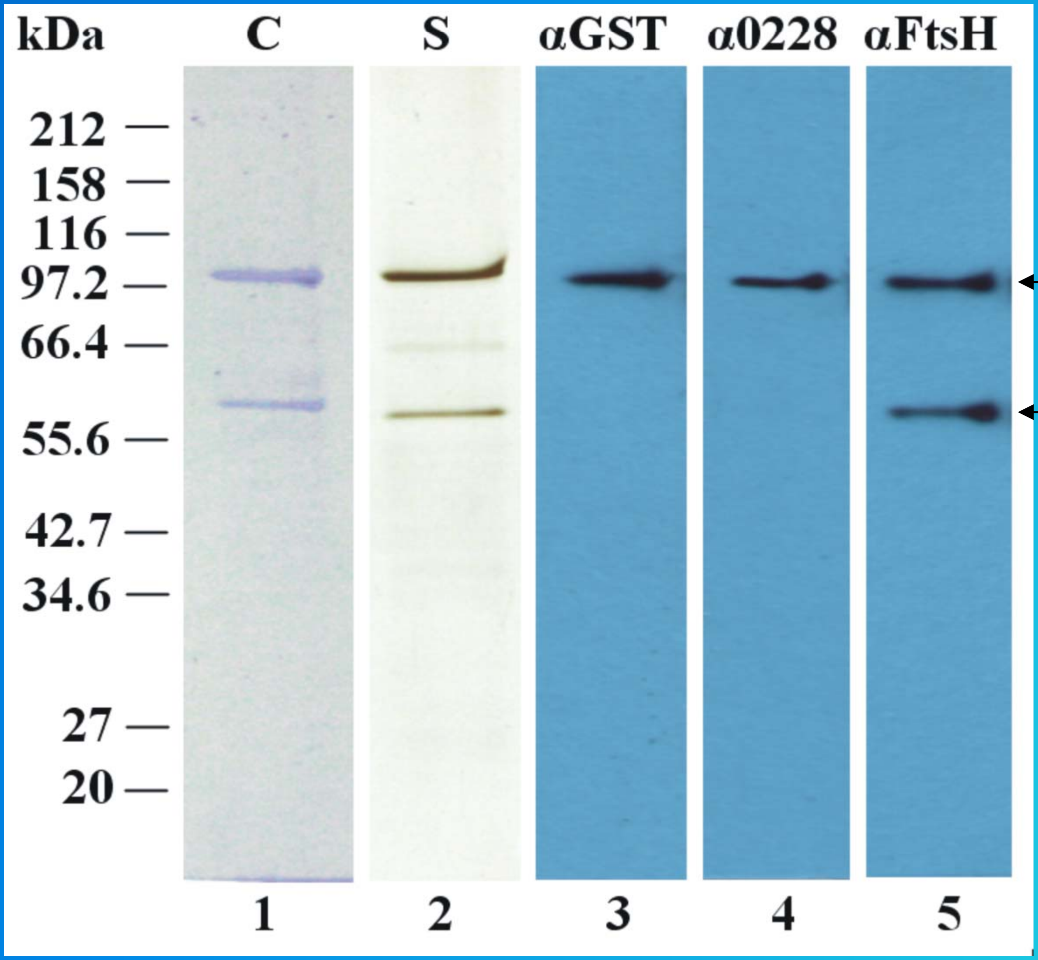
The A20 D1 mutant is capable of assembling monomeric and dimeric PSII complexes at WT levels.

D1 degradation and PSII repair are inhibited in A20 at high light intensities



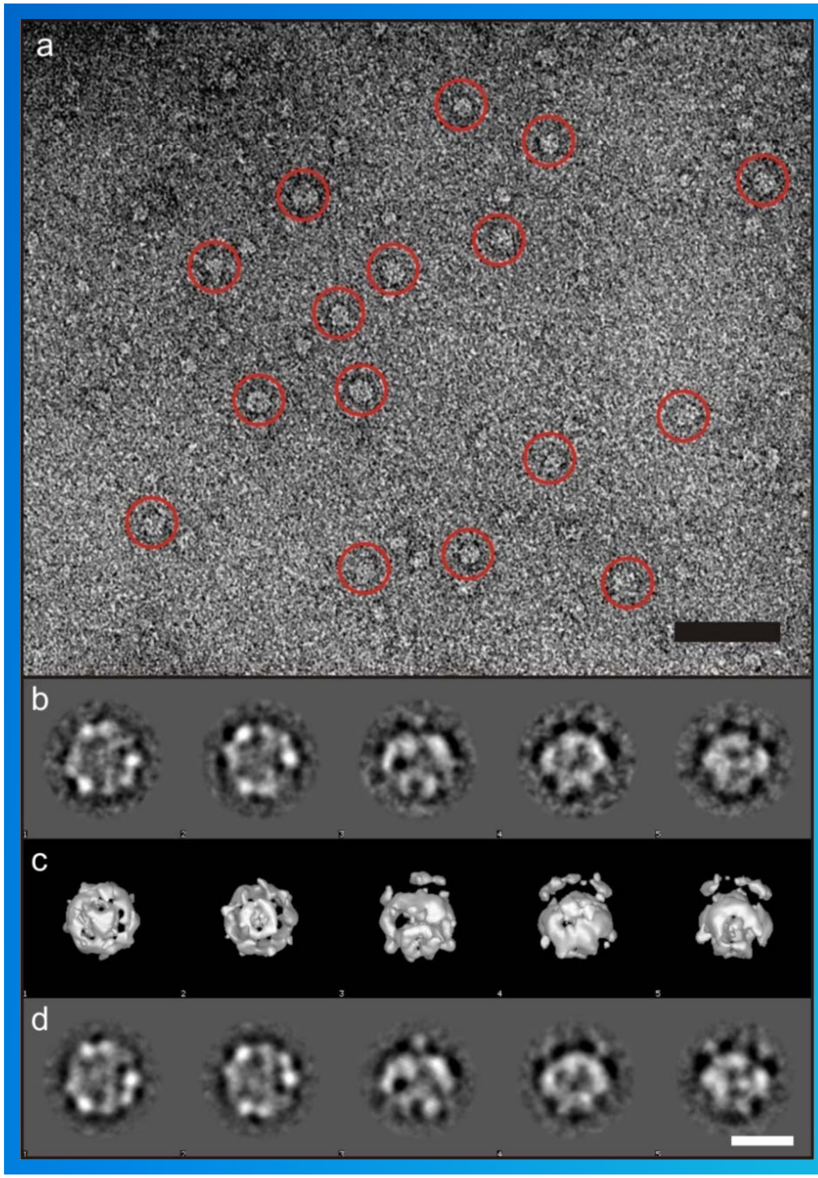
Isolation of *in vivo* FtsH2(Slr0228)-GST fusion protein from Synechocystis sp. PCC 6803



FtsH2(Slr0228)-GST fusion
MKFSXXXALL N-terminal sequence
MKFSWRTALL FtsH2 (Slr0228)

SKNNKKXXNA N-terminal sequence
MSKNNKKWRNA FtsH3 (Slr1604)

Electron microscopy and single particle analysis of FtsH2-GST/FtsH3 complex



a: negatively stained protein complexes

b: selection of five characteristic 2-D views

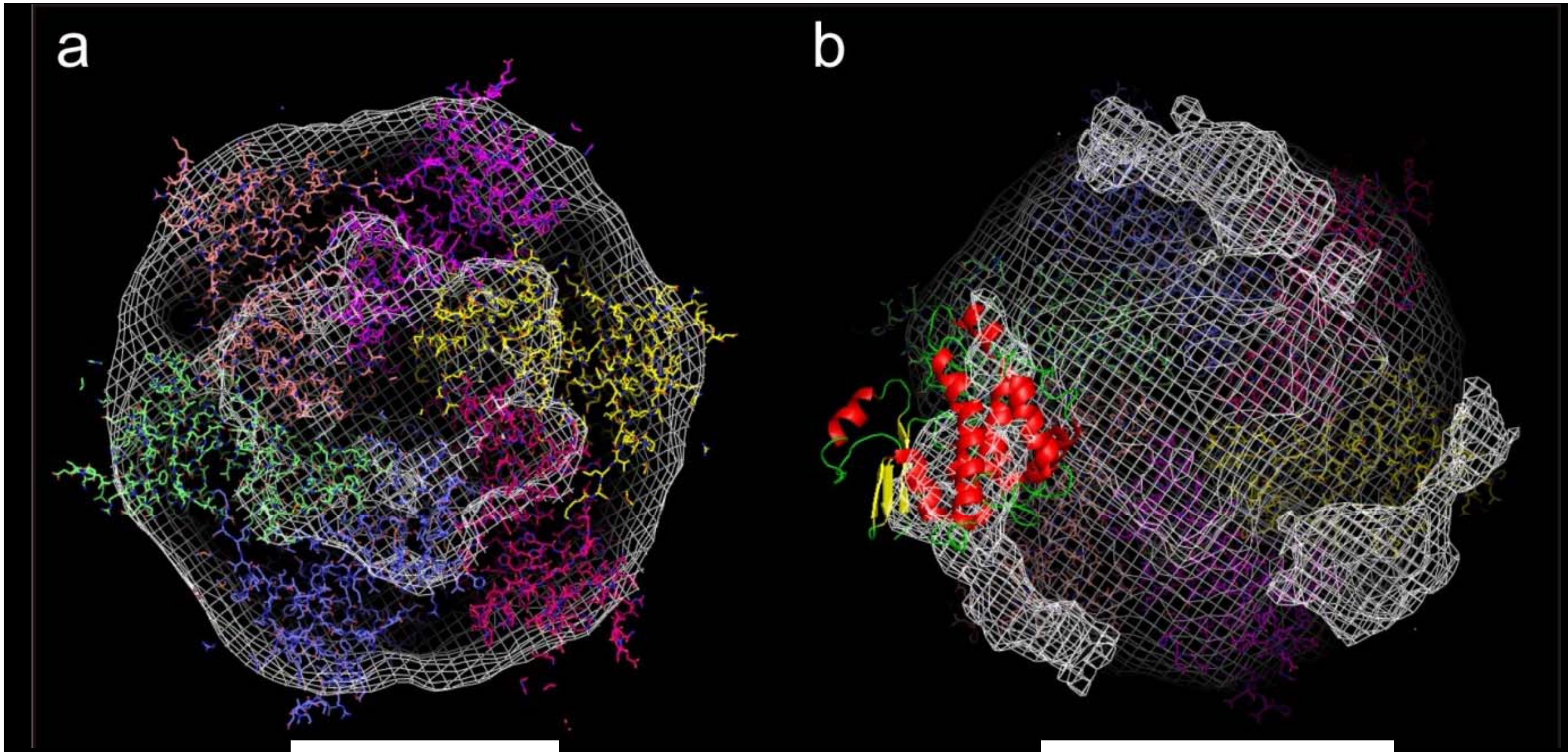
c: 3-D reconstruction of protein complex using 263 different class averages of 2,964 particles (at approx 26Å resolution)

d: surface-rendered views of final 3-D map calculated by angular reconstitution

Black bar 50 nm

White bar 10 nm

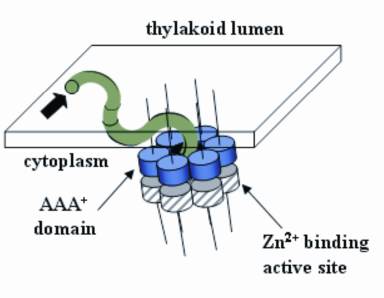
3-D-reconstitution suggests hexameric complex with alternating FtsH2-GST and FtsH3 subunits



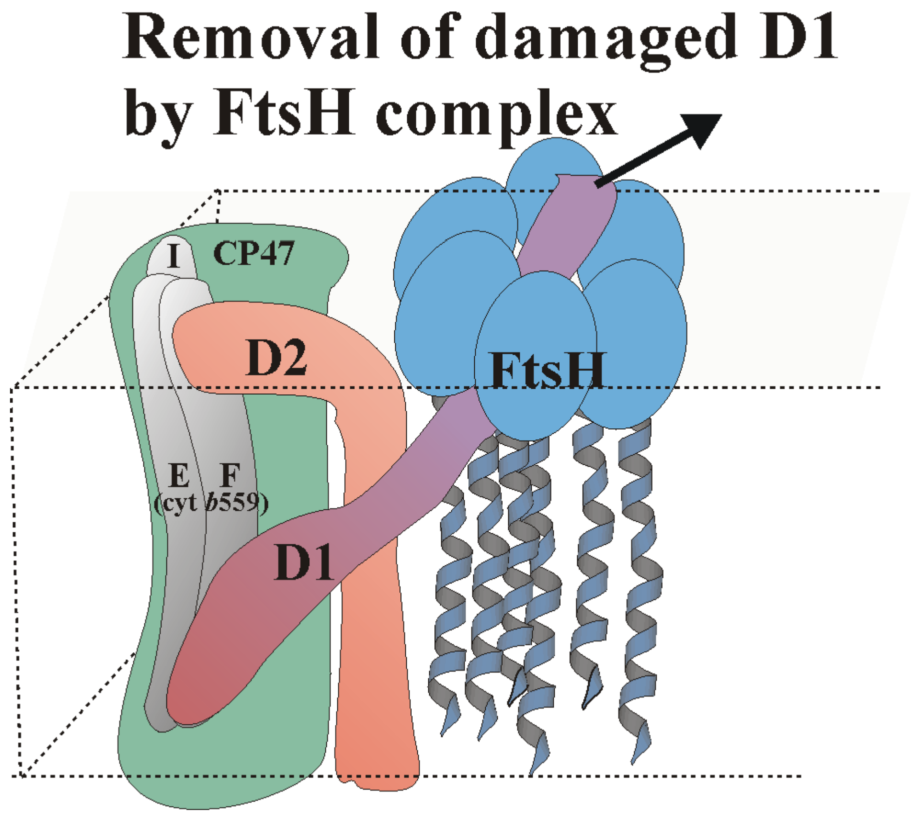
a: top view

b: bottom view

Structure of hexameric cytosolic region from *T. thermophilus* (Suno et al., 2006) + GST inserted into surface rendered model



The FtsH-only model for D1 degradation in cyanobacteria



Nixon et al., (2005)

- DegP proteases are not needed for D1 degradation.
- D1 degradation can be initiated at N-terminus (as long as greater than 20 residues).
- The FtsH2 complex is probably hexameric and might be homo- or hetero-oligomeric (FtsH3).
- Proteolysis is highly processive and is driven by ATP hydrolysis. No breakdown intermediates.
- D1 is triggered for degradation by destabilisation/partial unfolding. Common pathway for both donor-side and acceptor-side damage?
- Housekeeping role for FtsH. Degradation of unassembled PSII subunits and misassembled PSII complexes.

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