Structural analysis of the FtsH2 homologue of *Synechocystis* sp. PCC 6803

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Introduction

The FtsH2 protease (Slr0228) of *Synechocystis* sp. PCC 6803 has been shown to be involved in the early stages of the cyanobacterial PSII repair cycle (see Figure 1; Silva et al., 2003) and to play an important role in the quality control of the Photosystem II (PSII) protein complex (Komenda et al., 2006). However, even though the functional roles of the FtsH2 protease are emerging, little is known about the structure of FtsH proteases in photoautotrophs.

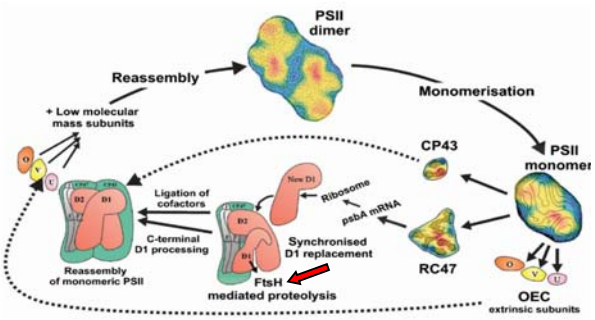

Figure 1: Model of the PSII repair cycle in *Synechocystis* sp. PCC6803. A functional dimeric PSII protein complex undergoes a series of disassembly steps to allow the synchronised replacement of a damaged D1 subunit by a newly synthesised copy. The PSII complex is then reassembled and the water-oxidizing Mn_4Ca cluster photoactivated. The red arrow indicates the stage at which the FtsH2 protease is involved in the PSII repair cycle.

AIMS

- **Determination of the *Synechocystis* sp. PCC 6803 membrane compartment in which the FtsH2 protease resides.**
- **Purification of the FtsH2 protease complex by affinity chromatography.**
- **Structural characterisation of the purified FtsH2 protease complex at low resolution in projection using transmission electron microscopy (TEM) and single particle analysis image-processing.**

Structure of FtsH proteases

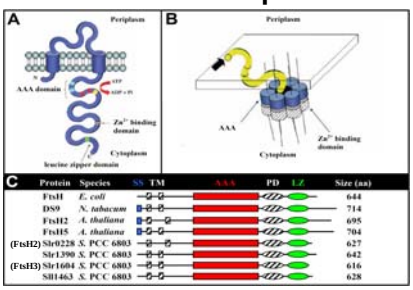

Figure 2: Structure of the FtsH protease family. (A) A cartoon depicting the cellular localisation and domain structure of a prototypal *E. coli* FtsH protease.

(B) Cartoon of an *E. coli* FtsH hexamer in the process of degrading a hypothetical substrate.

(C) Domain structure and size of certain FtsH proteases. Signal sequence (SS), transmembrane (TM) and ATPase (AAA) domains, zinc-binding active site (PD), predicted leucine zipper (LZ).

Localisation of FtsH2

FtsH proteases are typically found as membrane-bound proteins (Figure 1A). The cyanobacterium *Synechocystis* sp. PCC 6803, contains thylakoid membranes (TM; where PSII is located) and is surrounded by plasma membrane (PM). These two membrane types can be separated biochemically by the two-phase partitioning method (see Figure 3; Norling et al., 1998). From immunodetection, GST/Strep-tagged FtsH2 was found in the thylakoids.

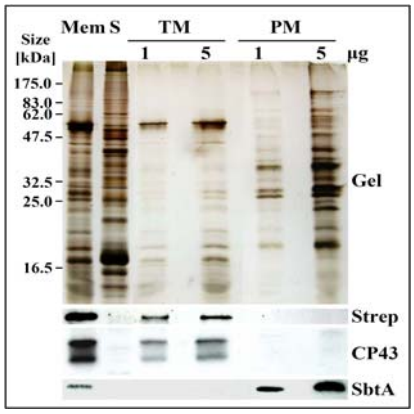

Figure 3: Immunodetection of FtsH2 in purified thylakoid membranes. SDS PAGE and immunoblotting analyses on thylakoid and plasma membranes purified by sucrose-density centrifugation and aqueous-polymer two-phase partitioning. Top panel: Silver-stained SDS PAGE gel. Crude membrane isolation (Mem), soluble proteins (S), thylakoid membranes (TM), plasma membranes (PM). Bottom 3 panels: Immunoblotting analyses with, as indicated, primary antibodies. The Strep/GST-tagged FtsH2 complex was identified in the thylakoids, as was the PSII subunit CP43. SbtA (sodium bicarbonate transporter) was used as a marker for the plasma membrane.

Purification and Structural analysis of FtsH2

An FtsH2-GST tagged strain of *Synechocystis* sp. PCC 6803 was constructed and used for the affinity-purification (Figure 4), followed by structural analysis using transmission electron microscopy and single particle analysis of the FtsH2 protease (Figure 5).

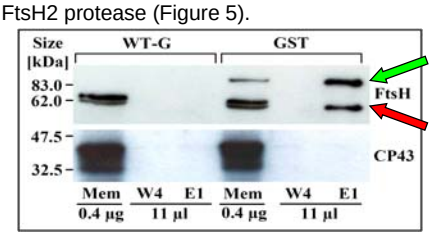

Figure 4: Specific co-purification of an FtsH homologue (FtsH3; Slr1604) with FtsH2-GST. Immunoblotting with primary antibodies against *E.coli* FtsH on fractions from wild-type (WT-G) and FtsH2-GST (GST) *Synechocystis* sp. PCC 6803 strains. Crude membranes (Mem), final GST column wash 4 (W4), elution 1 (E1). Green arrow = FtsH2-GST; red arrow = co-purifying FtsH3 (Slr 1604) found by N-term. sequencing (not shown).

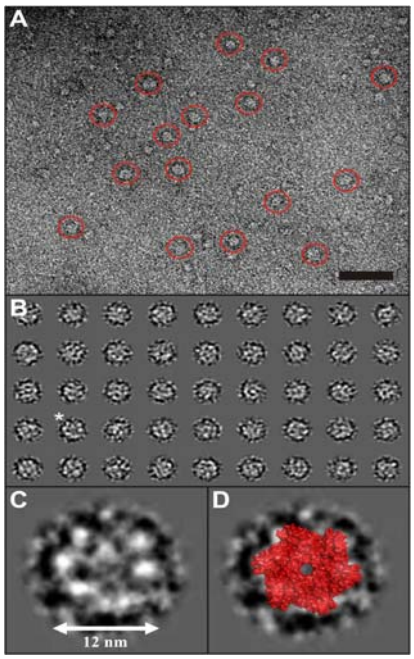

Figure 5: TEM and single particle analysis of purified FtsH2-GST complexes. (A) A micrograph region with E1 (Figure 4) FtsH2-GST particles (circled, red). **(B)** 2D averages of the purified FtsH2-GST complex ~12 nm diameter. **(C)** Magnified FtsH2-GST average; see * in (B). **(D)** This average with an overlay of the NSF-D2 AAA domain (PDB: 1D2N, Lenzen et al., 1998). Bar = 50 nm.

Conclusions

- **FtsH2 resides in the thylakoids of *Synechocystis* sp. PCC 6803.**
- **An FtsH homologue copurifies with the FtsH2-GST and found to be FtsH3 (Slr1604) by N-terminal sequencing.**
- **FtsH2 forms a ring-like hetero-hexameric protein complex with an approximate diameter of 12 nm.**

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