# Crystal Structure of Psb27 from T. elongatus and its Association with Dimeric Photosystem II **Imperial College**

#### Franck Michoux<sup>1</sup>, Kenji Takasaka<sup>1,3</sup>, Marko Boehm<sup>1</sup>, James W. Murray<sup>2</sup> and Peter J. Nixon<sup>1</sup>

<sup>1</sup>Division of Biology and <sup>2</sup>Division of Molecular Biosciences, Imperial College London, South Kensington Campus, London SW7 2AZ, UK <sup>3</sup>Current address: Division of Bioscience, Faculty of Science, Okayama University, Okayama 700-8530, Japan Contact: franck.michoux@imperial.ac.uk

Several proteins have recently been identified as playing accessory roles in Photosystem II, either regulating activity or involved in the assembly and repair of the complex. This study focuses on Psb27, which is found in oxygenic phototrophs from cyanobacteria to higher plants.

Repair and assembly of Photosystem II



Accessory proteins (in red) involved in the repair, assembly and activity of cyanobacterial PSI

## Psb27 crystal structure

T. elongatus Psb27, lacking the signal peptide and the lipidbinding Cys<sub>22</sub> residue, was expressed in E. coli with an Nterminal His-tag, which was removed following purification. The crystal structure was solved at 1.7Å through molecular replacement using previously published NMR structure (pdb 2KMF). The structure is a four-helix bundle (H1-H4), similar to the recently published structures of Psb27 from Synechocystis 6803 obtained by NMR (Cormann et al., 2009; Mabbitt et al., 2009).



PSII accessory proteins include Psb27, Psb28, Psb29, CyanoP and CyanoQ. Psb27 is a lipoprotein (of approximately 11 kDa) which is thought to bind to the lumenal side of monomeric PSII core complexes, preventing binding of the PsbO, PsbU and PsbV extrinsic proteins (Nowaczyk et al., 2006). Psb27 has been assigned a role in regulating the assembly of the Mn<sub>4</sub>Ca cluster involved in water oxidation (Roose and Pakrasi, 2008). Recently, the crystal or NMR structures of several assembly factors have been reported. This study focuses on the crystal structure of Psb27 from Thermosynechococcus elongatus.

London



3D crystal structures of Ycf48 (BI e, pdb 2XBG), CyanoP (Orange, pdb 2XB3, Michoux et al., 2010) from T. elongatus and NMR structure of Psb28 from Synechocystis 6803 (Purple, pdb 2KVO).

#### Comparison with known Psb27 structures

The determination of the crystal structure of Psb27 from T. elongatus allowed a direct comparison with previously reported NMR structures of Psb27 from Synechocystis 6803 which shares 50% sequence identity. Psb27 from T. elongatus has an extra short helix (H\*) located between helices 2 and 3. The surface charge is similar to that calculated



## Identification of Psb27 in monomeric and dimeric PSII complexes

The presence of Psb27 within thylakoid membranes isolated from T. elongatus was assessed by immunoblotting 2D Blue Native (BN)/SDS PAGE gels (A, B) with specific antibodies raised against Psb27 and D1 (C, D). The results indicate that Psb27 is part of both monomeric and dimeric PSII, as well as larger complexes, in contrast to previous findings.



Dodecylmaltoside solubilised thylakoid proteins from *T. elongatus* were separated by BN-PAGE (A). The lanes were then cut, solubilised and run on a 18% (w/v) 2<sup>nd</sup> dimension SDS PAGE gel (B). Gels were stained using Coomassie (A), silver (B) or immunoblotted with specific antibodies raised against D1 (C) and Psb27(D).

## Conclusions

1- The 3D crystal structure of Psb27 from *T. elongatus* is more similar to the 2KMF NMR model from Synechocystis and contains a new helical region (H\*) not present in previous models.

2- The surface charge model of Psb27 from T. elongatus indicates that interaction between Psb27 and PSII may be localised to the H4 helix only instead of both H3 and H4 (Cormann et al., 2009).

3- Psb27 is found in both monomeric and dimeric PSII

#### References

Nowaczyk et al. (2006) The Plant Cell 18: 3121-3131. Roose and Pakrasi (2008) J. Biol. Chem. 283: 4044-4050. Michoux et al. (2010) Biochemistry, online publication August 10, 2010. Cormann et al. (2009) Biochemistry 48: 8768-8770. Mabbitt et al. (2009) Biochemistry 48: 8771-8773

menda et al. (2008) J. Biol. Chem. 282: 22390-22399

#### Acknowledgements

FM, MB and PJN were supported in this work by the BBSRC and EPSRC, as well as JWM by a BBSRC David Phillips Fellowship.





#### **3D structures of PSII assembly factors**