assigned small subunits PsbX, PsbY and ycf12 are coloured green, red and blue, respectively, and labelled. For better orientation the subunits D1 (yellow), D2 (orange), PsbE (light green) and PsbF (cyan) are also indicated.

Subunit ycf12 is located next to subunits PsbJ, PsbK and PsbZ (Figs. 15, 20, 22) with its N-terminus pointing towards the lumen. It consists of 46 residues and is folded into a single TMH, the N-terminus is probably very flexible, therefore it was not possible to model it. This subunit forms just few H-bonds (see Appendix Table 7.4); one of them is with the PsbK-Asp23 residue coordinating Ca^{2+}-ion. Subunit ycf12 is mainly bound by hydrophobic forces, which correlates with high values of hydropathicity for this subinit [166].

A recent study [167] showed that the deactivation of the corresponding gene leads to the normal phenotype, and a mutant lacking ycf12 possesses comparable oxygen activity as wild type, though this activity is lower under light stress.

Subunit PsbX is located next to PsbH and D2 (Figs. 15, 20, 22), its N-terminus being near the lumen. The full length of the mature form is 40 residues (methionine Met1 is cleaved); all residues were modelled except the C-terminal three residues. N- and C-termini form few H-bonds with subunits PsbH and D2, respectively (see Appendix Table 7.4), whereas the hydrophobic segment Gly22-Val29 shields $ChlZ_{D2}$ from the membrane phase.

PsbX is not essential for photo-autotrophic growth of cyanobacteria [168, 169] as well as plants [170] but leads to a reduced level of oxygen activity and stability of the PSII complex.

Subunit PsbY was assigned unambiguously in the electron density due to a characteristic break in the TMH that was predicted from secondary-structure elements [166]. It is located at the periphery of the complex, close to *cyt b-559*, and it comprises 41 residues, which were modelled as poly-Ala only, due to poor electron density in this particular area, most probably because PsbY is loosely attached to the complex. PsbY contacts the TMH of PsbE, with residues 14, 17 and 21 pointing toward the haem group of *cyt b-559* and partially shielding it from the membrane. The position of PsbY was recently confirmed independently by crystallographic studies on a ΔPsbY mutant [171]. The function of this subunit is still unclear.

Comparison of refinement statistics for low molecular weight subunits at 3.0 Å [41] and 2.9 Å is given in Appendix Table 7.2.

3.1.2 Membrane extrinsic protein subunits

In addition to membrane intrinsic protein subunits, three membrane extrinsic protein subunits are found at the lumenal side of cyanobacterial PSII. These subunits are 33kDa protein (PsbO), 12kDa protein (PsbU) and cytochrome c-550 (PsbV).

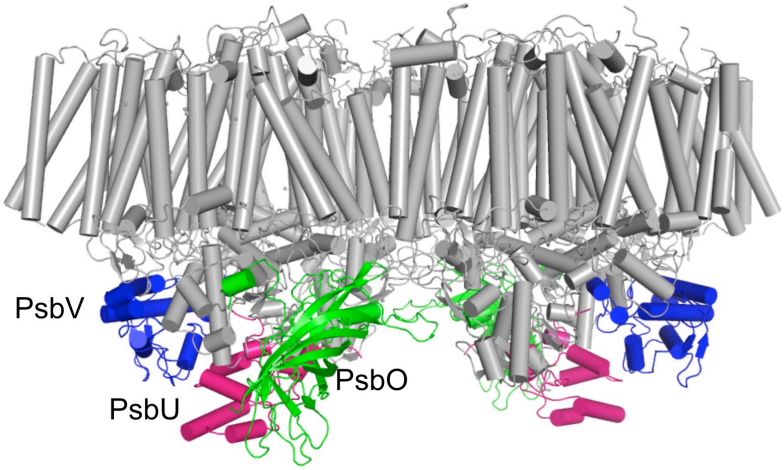

Figure 23. Extrinsic protein subunits of PSII. PsbO in green, PsbU in pink, PsbV in blue, other subunits grey.

Subunit PsbO is located next to PsbU and to lumenal loops of D1, D2, CP43 and CP47 from the other monomer which provide conserved residues for binding of PsbO to the PSII complex. It is 272 residues long, but the first 26 residues serve as signal peptide and are cleaved in the mature form. All residues were modelled, except three N-terminal residues. PsbO is folded in the form of an extended cylindrical β-barrel (Fig. 24) of 15 Å in diameter and 35 Å in length.

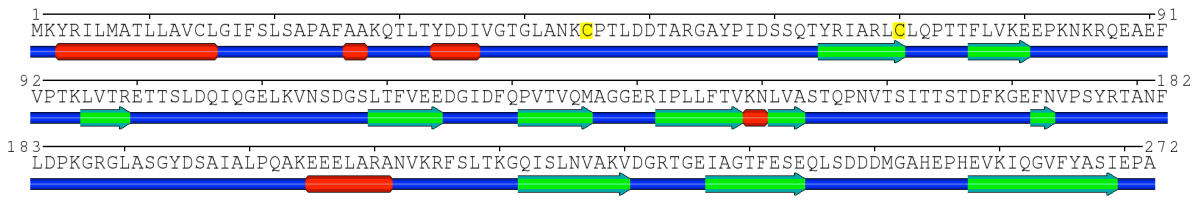

Figure 24. Secondary structure of subunit PsbO. Cysteine residues involved in disulphide bridge formation are highlighted in yellow, α-helices in red, β-strands in green, coil in blue.

PsbO is tightly associated with CP43 and its C-terminal part interacts with CP47 (see Appendix Table 7.5). Several strong H-bonds with D1, D2 and PsbU are also found (see Appendix Table 7.5) which is in good agreement with biochemical data [52, 172-179].

PsbO was shown to be essential for efficient oxygen evolution; removal of this subunit leads to the destabilization and disintegration of the Mn_4Ca cluster [180]. This corresponds well with the position of PsbO in PSII, which provides shielding of the Mn_4Ca cluster from bulk solvent. Therefore this subunit is also referred to as manganese-stabilizing protein (MSP). Since the activity of PSII might be retained under non-physiological Ca^{2+} and Cl^- concentrations [181], it is assumed that PsbO might also regulate the concentration of these ions [182]. PsbO might indirectly [183] stabilize the Mn_4Ca cluster as it is closely associated with loops of D1 that provide the majority of ligating residues for the cluster. Among the long speculated functions of PsbO is the transport of small molecules to / from Mn_4Ca via β-barrel hollow (Fig. 25), but a combination of theoretical calculations with experimental tracing of channels (see section 3.3) excluded this possibility.

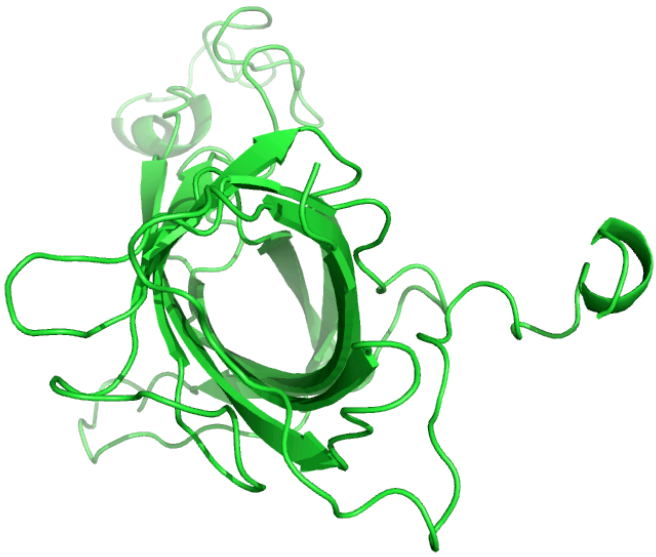

Figure 25. PsbO hollow. View from the lumen.

Subunit PsbV (*cyt c-550*) is located opposite to PsbO but has no contact with this subunit and is in contact with subunits D1, D2, CP43 as well as with α-subunit of *cyt b-559*, PsbJ and PsbU. The full length of psbV is 175 residues, of which 35 residues belong to the signal peptide that is cleaved in the mature form. All the remaining residues were fully modelled. PsbV contains bihistidine-ligated haem group [184] (Fig. 26) with unusually low potential of -240mV (unbound form) or -80mV (bound to PSII) [184, 185].

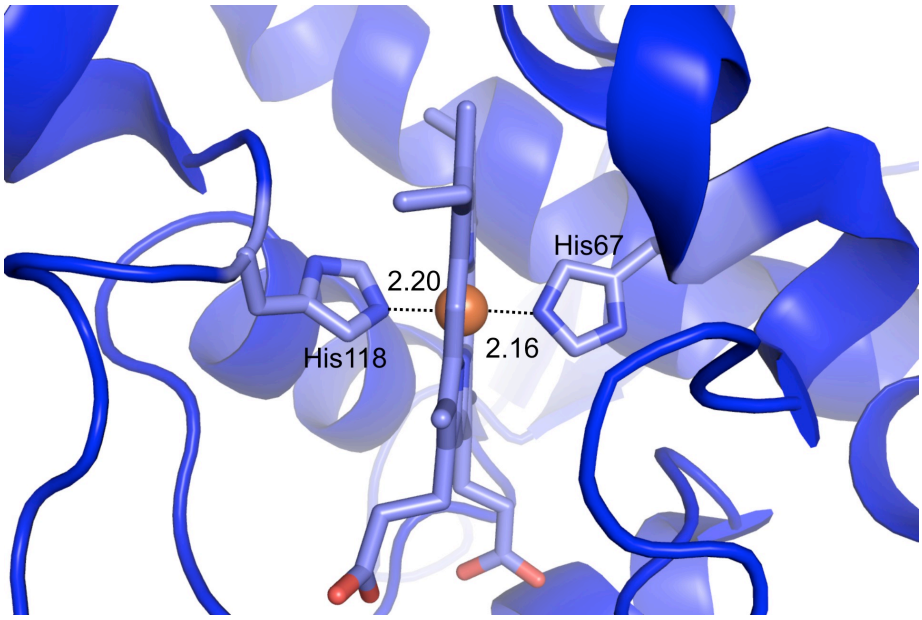

Figure 26. Haem group of PsbV. Ligating histidines are shown as sticks and labelled, distances are given in Å.

Several factors are considered [186] to explain this potential: (i) solvent exposure of the haem group [185], which generally lowers the potential of cytochromes [187]; (ii) presence of ionisable groups nearby [185, 188]; (iii) bihistidine ligation [189].

Up to date, there is no evidence for participation of this haem in ET, and taking into account the fact that there is no equivalent cytochrome in plant PSII, it is hard to assign any functional role to the haem group in PsbV. Nevertheless, several hypotheses emerged regarding the role of *cyt c-550* in PSII – anaerobic removal of electrons [190] or PsbV acts as an electron acceptor from ferredoxin during NADPH oxidation. In addition, mutagenesis studies showed that PsbV might regulate ion environment [50, 51, 191] and the absence of PsbV leads to decreased amount of RCs capable of S-state transitions [192], diminished oxygen evolution and reduced thermostability [193].

Subunit PsbU is located in-between subunits PsbO and PsbV and additionally forms contact with CP43, CP47, D1 and D2. The mature form is 104 residues in total (the cleaved signal peptide is 30 residues long), all were modelled except the N-terminal seven residues.

PsbU is believed to enhance the stability of PSII, optimize the ionic environment [51, 194], protect PSII from dark inactivation [51, 195] and increase thermostability [196, 197].

Lack of PsbU leads to decreased photosynthetic efficiency [194], lowered oxygen evolution [198], impaired electron flow [194, 199] and rapid photodegradation of D1 [194].

A comparison of refinement statistics for extrinsic subunits at 3.0 Å [41] and 2.9 Å resolution is given in Appendix Table 7.2.