3 RESULTS

Dataset of an already collected raw images was reprocessed with the new version of the XDS package [94, 131], which is optimized with a new scaling algorithm for mosaic detectors. This led to substantial improvement in data quality, and the resolution limit was extended to 2.9 Å (see Table 3). The amount of unique reflection data increased to 193,457 (compared to 155,340 for the previous 3.0 Å model [41]), and the amount of unique reflections used in the refinement increased from 129,965 [132] to 186,169, with $I/\sigma_I \geq 2.0$, allowing a more reliable refinement and calculation of maps with better quality. After numerous iterations of model building in Coot [122] and refinement with the CNS package [117] the hitherto most complete and realistic model of PSII has been obtained (R/R_{free} factors of 0.249/0.292, with r.m.s. deviations from ideal geometry of 0.010 Å for bond lengths and 1.453° for bond angles; see Table 3). Coordinates were deposited under 3BZ1 and 3BZ2 codes in the PDB bank.

Data collection	
Space group	$P2_12_12_1$
Unit cell dimensions	
a, *b*, *c* (Å)	127.7, 225.4, 306.1
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å)	30–2.9 (3.0–2.9)*
R_{sym}	0.104 (0.533)*
$I / \sigma I$	13.5 (2.22)*
Completeness (%)	94.2 (88.3)*
Redundancy	12.2
Refinement	
Resolution (Å)	30–2.9 (3.0–2.9)*
Reflections	186,169
R_{work} / R_{free}	24.9/ 29.2
No. atoms	50,234
Protein	41,052
Ligand/ion	9,182
Average B-factors	
Protein	73,83
Ligand/ion	79,99
r.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.453

Table 3. Dataset and refinement statistics. * indicates data in the highest resolution shell.

3.1 Protein subunits

Each monomer of PSII consists of 20 protein subunits (see Figs. 8, 14, 15 and Table 4), which can be divided into two major subclasses: intrinsic (embedded into the membrane) and extrinsic (attached to the membrane) protein subunits.

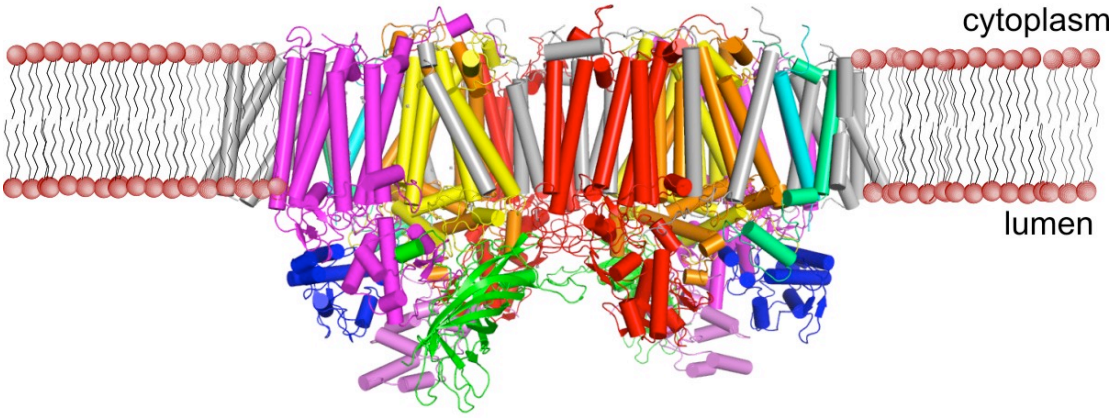


Figure 14. Protein subunits in the PSII homodimer. Colour coding is the same as in Figure 8, except for PsbO (green), PsbV (blue) and PsbU (light violet). Note the difference between intrinsic and extrinsic subunits.

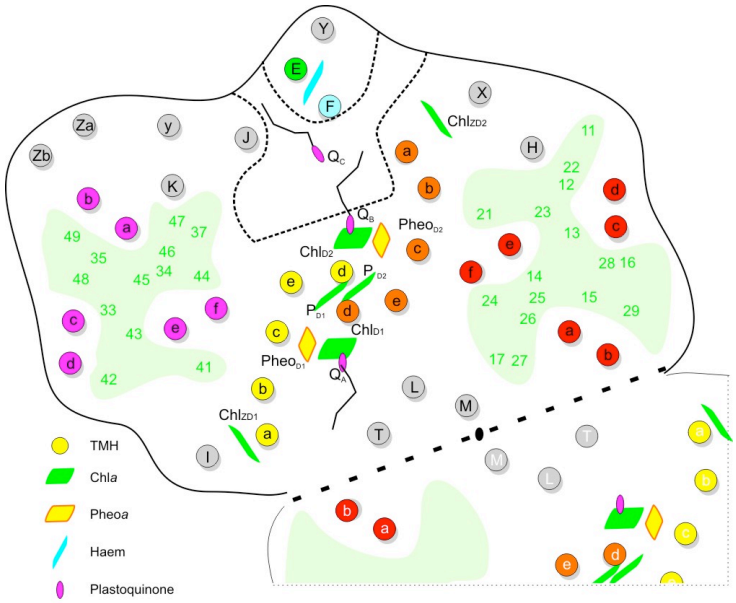


Figure 15. Schematic view of TMHs in PSII and cofactors of the ETC. Shown is one monomer and part of the other momoner of the homodimeric complex, and positions of Chl in CP43 and CP47 are indicated by green numbers. TMHs of the main subunits are highlighted in yellow (D1), orange (D2), red (CP47), magenta (CP43), green and cyan (cytochrome *b- 559* α- and β-subunit); low molecular mass subunits in grey. The 5 to 6 TMHs of D1, D2, CP43 and CP47 are labelled a-e/f and the TMHs of the small subunits are labelled by capital letters with the exception of ycf12, labelled y. The cofactors of the ETC are shown in labelled in black. A black dashed line indicates monomer-monomer interface and a black ellipse indicates C_2 symmetry axis relating two monomers.

Chain	Subunit name	Number of amino acids	Location within the thylakoid membrane
A	D1, reaction centre subunit	344	intrinsic
B	CP47, antenna subunit	510	intrinsic
C	CP43, antenna subunit	461	intrinsic
D	D2, reaction centre subunit	352	intrinsic
E	*Cytochrome b-559*, α-subunit	84	intrinsic
F	*Cytochrome b-559*, β-subunit	45	intrinsic
H	psbH	66	intrinsic
I	PsbI	38	intrinsic
J	PsbJ	40	intrinsic
K	PsbK	37	intrinsic
L	PsbL	37	intrinsic
M	PsbM	36	intrinsic
O	Manganese-stabilizing protein	246	extrinsic
T	PsbT	32	intrinsic
U	12 kDa extrinsic protein	104	extrinsic
V	*Cytochrome c-550*	137	extrinsic
y	ycf12	46	intrinsic
X	PsbX	41	intrinsic
Y	PsbY	41	intrinsic
Z	PsbZ	62	intrinsic

Table 4. Protein subunits of PSII.

3.1.1 Membrane intrinsic protein subunits

3.1.1.1 Reaction Center

The Reaction Center (RC) is formed by protein subunits D1 (PsbA) and D2 (PsbD) (Fig. 15) in analogy with Reaction Center from Purple Bacteria (PBRC) that is formed by L and M polypeptides [133, 134].

There is about 30% of sequence similarity between D1 of PSII and subunit L of PBRC and 31% between D2 of PSII and subunit M of PBRC. Moreover, the RC of PSI also shows a similar folding (D1 corresponds to the N-terminal part of a subunit PsaA and D2 to the N-terminal part of a subunit PsaB), which might indicate gene duplication from a common ancestor (see Fig. 2). Each polypeptide of RC is folded into five TMHs **a** to **e** (Figs. 15,

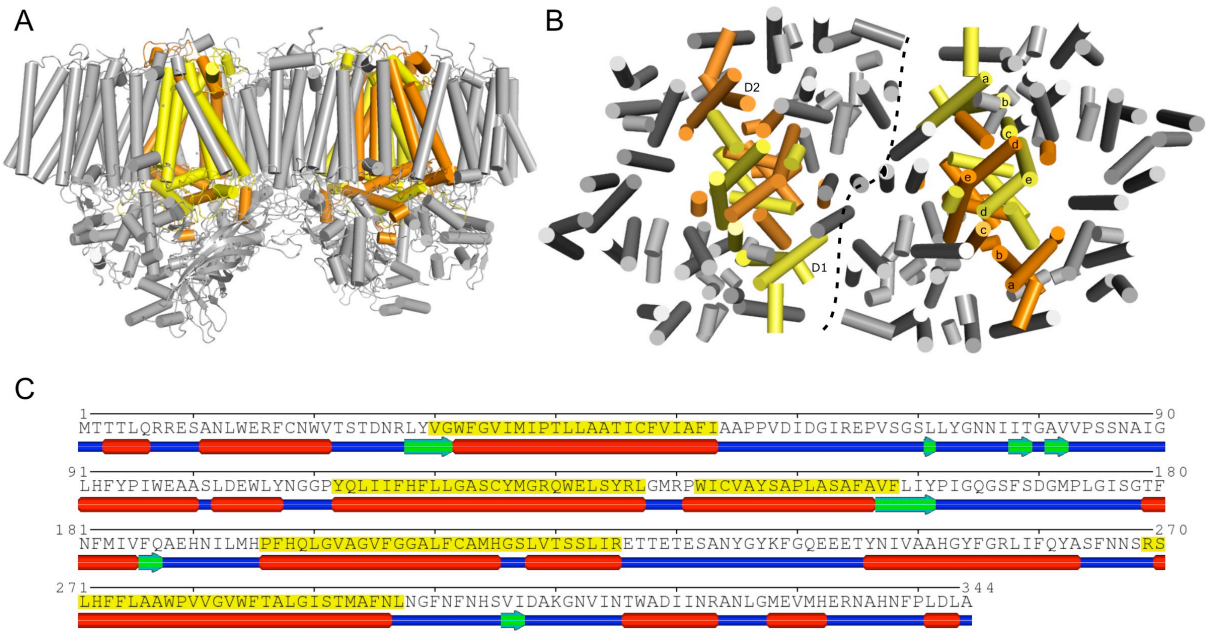
16B,C,D;) and both subunits are related in a handshake motif with a local pseudo two-fold axis (pseudo C_2 (Fe^{2+}) axis) passing the non-haem iron. Both subunits have their N-termini located at the cytoplasm and C-termini at the lumen, respectively. The overall fold of D1 and D2 is very similar (rmsd 1.5 Å), even with similar positions for amino acids, which coordinate numerous cofactors, leading to two symmetrical branches in the ETC (Fig. 17).

D1 and D2 harbour all cofactors of the ETC, namely four Chl *a* molecules (P_{D1}, P_{D2}, Chl_{D1}, Chl_{D2}), two Pheo *a* molecules ($Pheo_{D1}$ and $Pheo_{D2}$) and two plastoquinone molecules (Q_A and Q_B, see chapter 3.2.2 for details about a third plastoquinone Q_C). Moreover, D1 provides most of the ligating amino acids for the unique Mn_4Ca cluster (see section 3.2.3), located close to the electron donor site of RC (Fig. 17).

Another noticeable feature of subunit D1 is its high turnover rate (~30 minutes under high light conditions) [44, 45, 135] associated with light-induced photodamage. See section 3.2.1.3 for discussion of lipids role in D1-turnover.

The TMH **a** of D2 forms part of the channel for plastoquinone exchange (see section 3.2.2.4).

Both subunits D1 and D2 were fully modelled, except for the flexible N-terminus of D1 (nine N-terminal residues; the full length of the mature form is 344 residues) and the C-terminus of D2 (last 19 residues; the full length of the mature form is 352 residues). The electron density allowed modelling of missing side chains and better refinement in comparison with the previous 3.0 Å resolution structure [41] (see Appendix Table 7.1 for details).



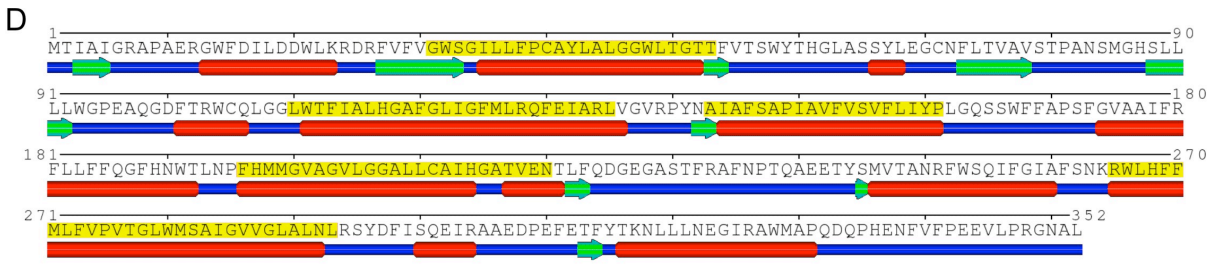


Figure 16. Reaction Centre subunits: A) side view of the PSII homodimer, all proteins subunits in grey, except D1 is in yellow, D2 is in orange; B) top view on the PSII homodimer from the cytoplasmic side, colours as in panel A, transmembrane α-helices of D1 and D2 are labelled from a to e; C) secondary structure and sequence details of D1, TMHs are highlighted in yellow, α-helices in red, β-strands in green, coil in blue; D) the same for D2.

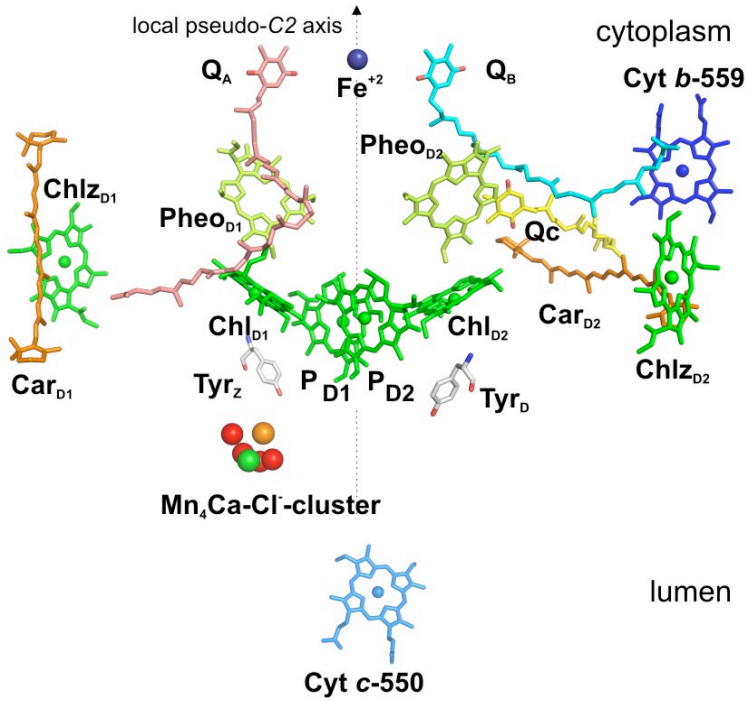


Figure 17. Electron transfer chain with auxiliary cofactors, all named and colour-coded as follows: chlorophylls P_{D1}, P_{D2}, Chl_{D1}, Chl_{D2}, $ChlZ_{D1}$, $ChlZ_{D2}$ in green, phytyl tails are omitted for clarity; Haems of *cyt b-559* and *cyt c-550* in blue and light blue, respectively; carotenoids Car_{D1} and Car_{D2} in orange; pheophytines $Pheo_{D1}$ and $Pheo_{D2}$ in lemon-yellow; plastoquinones Q_A, Q_B and Q_C in salmon red, cyan and yellow, respectively; cations are shown as spheres – manganese in red, calcium in orange, chloride in green and iron in deep blue. Active tyrosine Z and its symmetry mate Tyr D are shown in grey, oxygens in red, nitrogens in blue.

3.1.1.2 Antenna subunits

The other two largest protein subunits found in PSII are antenna subunits CP47 (PsbB) and CP43 (PsbC) (Figs. 15, 18), both harbouring all chlorophyll molecules, except those which belong to the RC. CP47 is located close to the monomer-monomer interface and