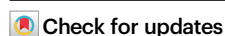


Cryo-EM structures of naturally occurring dimeric photosystem II complexes lacking the Mn_4CaO_5 cluster

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Robust oxygenic photosynthesis requires the efficient assembly and repair of the multi-subunit oxygen-evolving photosystem II (PSII) complex. Previous cryogenic electron microscopy (cryo-EM) structures of PSII assembly/disassembly intermediates have relied on the analysis of deletion mutants or removal of PSII subunits in vitro. Here we report the cryo-EM structures of naturally occurring dimeric PSII intermediates from the cyanobacterium *Thermosynechococcus vestitus* at a resolution of about 2.2 Å. These intermediates contain inactive dimers lacking the oxygen-evolving complex (OEC) and semi-active dimers with the OEC present in one of the two monomers. Our structural data provide a mechanism for how assembly and disassembly of the Mn_4CaO_5 cluster is coordinated with the binding and release of the extrinsic proteins: restructuring of the C-terminal tail of D1 subunit during assembly or disassembly of the Mn cluster triggers conformational changes in D2, CP47 and CP43 to drive the binding/release of the extrinsic proteins. A combination of structural and mass spectrometry data also suggests that the inactive PSII complexes may include damaged complexes containing oxidized D1-His332, a monodentate ligand to one of the Mn ions of the OEC.

Photosystem II (PSII) is the light-driven multi-subunit membrane complex found in cyanobacteria and chloroplasts that catalyzes the oxidation of water and reduction of plastoquinone during oxygenic photosynthetic electron transport. PSII is the only enzyme known in nature that is able to catalyze the water-splitting reaction, making it essential for producing oxygen and supporting aerobic life¹.

Assembly of PSII is a stepwise process involving multiple accessory factors, many conserved in cyanobacteria and chloroplasts, that promote and regulate biogenesis (Fig. 1)². During the early stage of PSII assembly in cyanobacteria, the Ycf48 accessory factor binds to the luminal side of the D1 and D2 reaction center complex (RC),

preventing premature binding of ions to the Mn_4CaO_5 cluster binding site^{3–6}. The PSII RC then binds the CP47 module (CP47_{mod}) to form the RC47 complex, followed by attachment of the CP43 module (CP43_{mod}) to create an inactive non-oxygen-evolving monomeric PSII complex^{7,8}. D1 is synthesized as a precursor protein (pD1) with a 16-residue C-terminal extension, which is cleaved by the CtpA protease in a two-step process before attachment of CP43^{9–12}. Subsequently, fully functional PSII dimers are formed by assembly of the Mn_4CaO_5 cluster in a light-driven process known as photoactivation¹³ and by attachment of extrinsic subunits to the luminal side of PSII to protect and stabilize the Mn_4CaO_5 cluster¹⁴. At what stage PSII complexes dimerize is still unclear.

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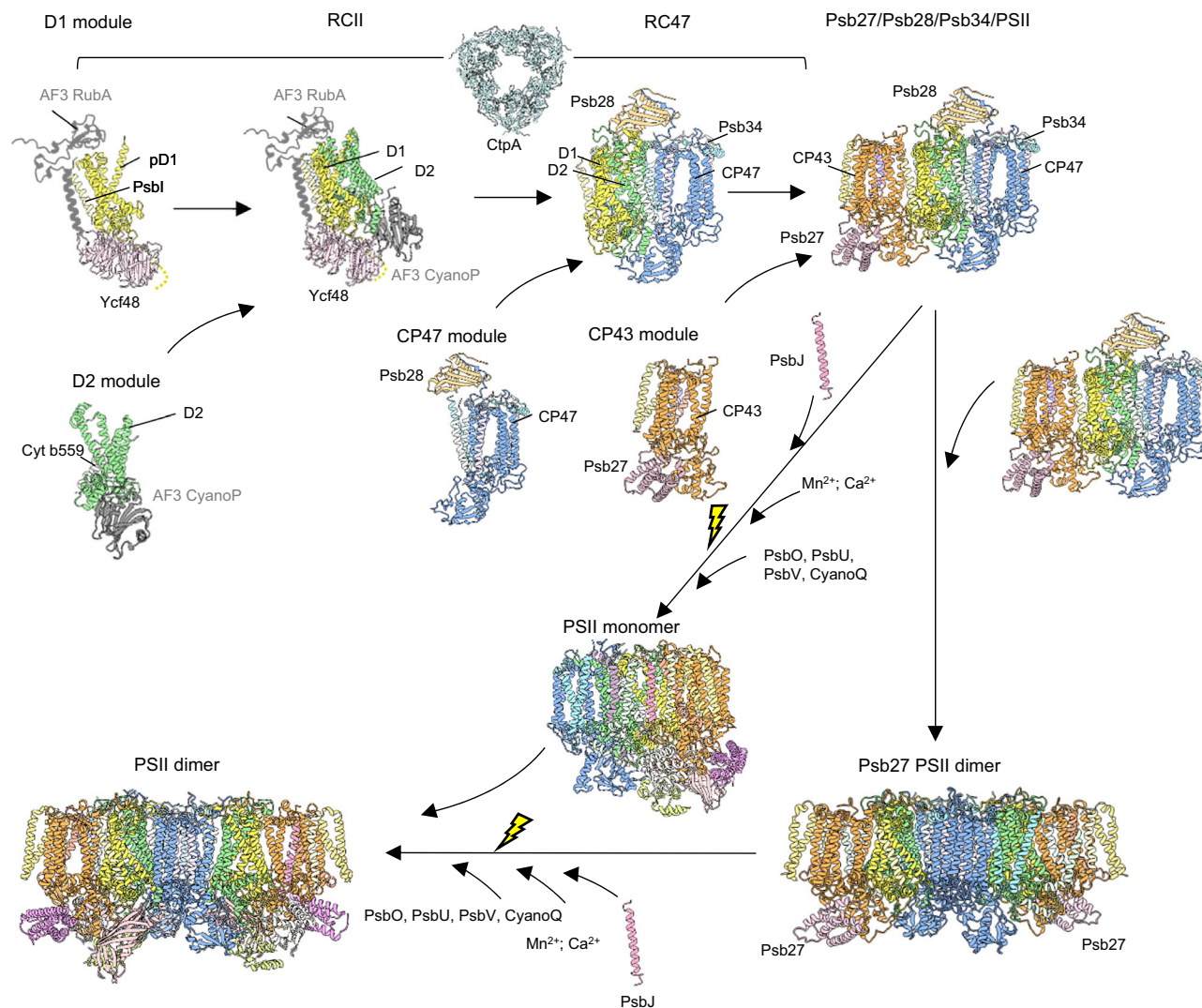


Fig. 1 | Model for assembly of PSII in cyanobacteria. PSII assembly begins with the formation of the D1 module, comprising precursor D1 (pD1), PsbI, accessory factor Ycf48, and the rubredoxin protein RubA. This module associates with the D2 module, which includes D2, cytochrome b-559 (Cyt b559), and accessory factor CyanoP, to form the reaction center assembly complex (RCII); the Ycf39/Hlips complex can bind to RCII to form RCII* (not shown). Subsequent addition of the CP47 module, including accessory factors Psb28 and Psb34, forms the RC47 complex. Addition of the CP43 module containing accessory factor Psb27 then produces a non-oxygen-evolving PSII monomer (represented here by Psb27/Psb28/Psb34/PSII). This PSII monomeric intermediate then binds PsbJ and undergoes photoactivation along with attachment of the extrinsic subunits (PsbO, PsbU, PsbV and CyanoQ) and detachment of accessory factors to form a fully functional

oxygen-evolving PSII monomer, which can further dimerize. Alternatively, PSII may first dimerize and then undergo photoactivation. C-terminal processing of precursor D1 (pD1) by the CtpA protease is largely complete by the time the CP43 module attaches. Psb27 is associated with both monomeric and dimeric complexes. Key assembly factors and main PSII subunits are labeled. Protein structures were obtained from PDB with the ID in brackets: CtpA (8SXH); D1 module, D2 module and RCII* (8ASL); CP47 module, CP43 module, RC47 and Psb27/Psb28/Psb34/PSII (7NHP); Psb27/PSII (7CZL); PSII monomer and PSII dimer (7RCV). The structures of assembly factors RubA and CyanoP, which have not yet been resolved in PSII structures, were predicted using AlphaFold3 (AF3) (<https://alphafoldserver.com>) and are shown in gray and labeled with the prefix “AF3”.

Light not only drives photosynthesis and photoactivation but also damages the photosynthetic complexes, leading to chronic photoinhibition¹⁵. Acceptor- and donor-side mechanisms of visible-light photodamage are still under debate^{15,16}. The acceptor-side mechanism suggests that photodamage results from an over-reduced acceptor-side of PSII and blockage of forward electron transfer, which leads to the production of reactive oxygen species, especially singlet oxygen^{15,17,18}. In contrast, donor-side mechanisms propose that excitation of Mn^{3+}/Mn^{4+} ions by visible light or the production of reactive oxygen species (ROS) by the Mn_4CaO_5 cluster leads to photodamage^{16,19–21}.

Recently, cryo-EM has provided structural insights into various cyanobacterial PSII assembly intermediates, including the PSII RC⁶ and

RC47 monomers^{22,23} and a Psb27/PSII dimer²⁴. These structures were obtained from mutants with deletions of specific PSII subunits that block assembly of PSII to enhance the accumulation of low-abundance PSII intermediates^{6,23,24}. Additionally, structures of PSII lacking the Mn_4CaO_5 cluster were obtained by treating oxygen-evolving PSII with NH_2OH , EDTA and $NaCl$ ^{25,26}. The PSII structure missing both the Mn_4CaO_5 cluster and extrinsic subunits is termed apo-PSII²⁶ (see Supplementary Table 1 for further details of these structures).

Previous work by Nowaczyk and colleagues revealed that purification of His-tagged PSII from WT *Thermosynechococcus vestitus* BP-1 (*T. vestitus*) yielded four fractions when separated by anion-exchange chromatography²⁷: a Psb27/PSII monomer, an oxygen-evolving PSII monomer, a fully active oxygen-evolving PSII dimer and, the fourth, a

less active oxygen-evolving PSII dimer fraction, here termed PSII_{peak4}, which has not been fully characterized.

Here we use cryo-EM to determine three structures of the dimeric complexes present in PSII_{peak4} at a resolution of around 2.2 Å. This pool of PSII dimers consists of active PSII dimers with extrinsic subunits (PsbO, PsbU and PsbV) bound to both monomers, semi-active PSII dimers with extrinsic subunits attached to only one monomer and inactive dimers that lack extrinsic subunits. Although lacking extrinsic subunits, the inactive PSII monomers in the semi-active and inactive dimers contain all the transmembrane subunits, including PsbJ with partial occupancy and all the key cofactors apart from the Mn₄CaO₅ cluster and one of the two chloride anions, termed Cl-2. Compared with fully functional PSII, the inactive PSII monomers also show structural differences in D1, D2, CP43, and CP47 around the donor side close to the binding site of the Mn₄CaO₅ cluster. These changes allow Mn²⁺ and Ca²⁺ ions access to and from the binding site of the Mn₄CaO₅ cluster. In addition, the structure of the D1-His332 residue in the inactive monomer, which is a ligand to the Mn cluster, suggests oxidation of the imidazole side chain. This oxidation could potentially reflect an early stage of photodamage to PSII on the donor-side of PSII.

Results

Purification and properties of PSII_{peak4} fraction

PSII_{peak4} was purified from *T. vestitus* using His-tagged CP47, as described in ref. 28. Consistent with the findings of Nowaczyk and colleagues, PSII_{peak4} is a less active PSII dimer fraction²⁷ (Supplementary Figs. 1, 2). SDS-PAGE with Coomassie brilliant blue staining revealed reduced amounts of the three extrinsic subunits in PSII_{peak4} compared to fully functional oxygen-evolving PSII (Supplementary Figs. 1, 2), in line with the lower oxygen evolution activity of PSII_{peak4}. It is unlikely that the extrinsic subunits were removed by salt during anion-exchange chromatography, as PSII retains its activity up to 90 mM MgSO₄²⁹, whereas PSII_{peak4} was eluted at a MgSO₄ concentration of less than 60 mM (Supplementary Fig. 1b), which is insufficient to detach extrinsic subunits. Negative staining electron microscopy confirmed that PSII_{peak4} contained dimeric PSII complexes similar in size to fully functional PSII (Supplementary Fig. 3).

Overall structures of PSII intermediates in the PSII_{peak4} mixture

The composition and structures of the PSII complexes in PSII_{peak4} were determined by cryo-EM single-particle analysis. The dataset was collected with the grid tilted 30 degrees to mitigate the effects of preferential orientation of the sample. Extensive 2D and 3D classification resulted in 1.06 million good particles, which were separated into three classes: active PSII dimer (34.5% of total particles), inactive PSII dimer (39.6% of total particles), and semi-active PSII dimer (25.8% of total particles) (Fig. 2 and Supplementary Figs. 4, 5). The presence of inactive complexes explains the reduced oxygen-evolution activity in the PSII_{peak4} mixture (Supplementary Fig. 2c). The distinction between these classes is based on the presence or absence of the extrinsic subunits (PsbO, PsbU, and PsbV) and the Mn₄CaO₅ cluster on the luminal side of PSII. Active dimers contain both sets of extrinsic subunits and the Mn₄CaO₅ cluster; inactive dimers lack both sets of extrinsic subunits and the Mn₄CaO₅ cluster, and semi-active dimers have one monomer that contains extrinsic subunits and the Mn₄CaO₅ cluster while the other monomer lacks them. The overall resolution for all three classes is about 2.2 Å (active dimer: 2.22 Å; inactive dimer: 2.15 Å; semi-active: 2.24 Å). The data processing pipeline is outlined in Supplementary Fig. 5, and the refinement statistics are shown in Supplementary Table 2. Examples of the density fit are shown in Supplementary Figs. 6–9, and statistics of modeling are shown in Supplementary Tables 3–5.

PSII monomers containing the oxygen-evolving complex (PSII(+ OEC)) in the active and semi-active dimers contain the D1, D2, CP43

and CP47 subunits, 12 low-molecular-mass transmembrane subunits PsbH, PsbX, Cyt b559 α&β, PsbI, PsbK, Psb30, PsbZ, PsbL, PsbT, PsbM and PsbJ, and three extrinsic subunits, PsbO, PsbU and PsbV (Fig. 2a–c). The active monomer shows high similarity to the high-resolution crystal structure of *Thermosynechococcus vulcanus* (*T. vulcanus*) PSII³⁰ with a root mean square deviation (rmsd, calculated using the backbone Cα atoms from all chains) of 0.28 Å. PSII monomers lacking the oxygen-evolving complex (PSII(-OEC)) in the inactive dimer and semi-active dimer contain all 16 transmembrane subunits and are highly similar to the transmembrane portion of fully functional PSII, although the single helix PsbJ subunit, involved in the late stage of PSII assembly⁸, had a lower EM density due to either reduced occupancy or higher flexibility (Fig. 2c). Although trace amounts of Psb27 were detected in PSII_{peak4} as a faint band in Coomassie-stained gels (Supplementary Fig. 1c) and by immunoblotting (Supplementary Fig. 10), no corresponding density was observed in the cryo-EM maps. On the luminal side, PSII(-OEC) lacks not only the extrinsic subunits and the Mn₄CaO₅ cluster, but also the Cl-2 ion and density for the D1 C-terminal tail from D1-N335 to D1-A344, as well as the luminal region of CP43 from CP43-G313 to CP43-L325 (residue numbers throughout are according to *T. vestitus*, unless stated otherwise) (Fig. 2d, Supplementary Tables 3, 5).

The PSII(-OEC) monomer in the semi-active dimer closely resembles those in the inactive dimer, with no discernible structural differences. The PSII(+ OEC) monomer in the semi-active dimer is highly similar to that in the active dimer, showing no distinct differences. Therefore, subsequent structural descriptions and comparisons are based on the PSII(-OEC) and PSII(+ OEC) complexes in the semi-active dimer PSII, unless stated otherwise.

Cofactors in the PSII(-OEC) complex

In the PSII(+ OEC) complex, the arrangement of key cofactors is highly similar to that of fully functional oxygen-evolving PSII determined previously (Supplementary Fig. 11). In the PSII(-OEC) complex, apart from the Mn₄CaO₅ cluster and Cl-2, the other key cofactors and residues in the D1/D2 heterodimer are all present at their expected positions, including P_{D1}, P_{D2}, Chl_{D1}, Chl_{D2}, Pheo_{D1}, Pheo_{D2}, Q_A, Q_B, the non-heme iron, bicarbonate, D1-β-carotene, D2-β-carotene, ChlZ_{D1}, ChlZ_{D2}, Cl-1 (Fig. 2d) as are the chlorophylls and β-carotenes in CP43 and CP47 (Supplementary Fig. 12). It is known that the Mn₄CaO₅ cluster is not stable without the extrinsic subunits³¹ and that Cl-2, proposed to be involved in a proton-exit pathway, binds to D1-N338, D1-F339 and CP43-E342 (E354 in *T. vulcanus*) close to the interface of D1 and the extrinsic subunits^{30,32,33}. Therefore, the absence of Cl-2 is likely associated with the lack of a folded D1 C-terminal tail (Fig. 3c).

Redox-active tyrosines Y_Z (D1-Tyr161) and Y_D (D2-Tyr160), along with the related H-bonding histidine residues D1-H190 and D2-H189, are also located at the expected positions (Fig. 2d). The distance between Y_Z and His190 is crucial for rapid reduction of P_{D1}⁺³⁴. In the PSII(-OEC) complexes in the semi-active and inactive dimers, the Y_Z-H190 distances are all around 2.7 Å, close to the value of 2.5–2.6 Å seen in the PSII(+ OEC) complexes in the semi-active and active dimers, but slightly longer than the 2.4 Å distance reported for fully functional PSII³⁰. Additionally, water molecules, which contribute to shortening the distance between Y_Z and D1-H190³⁴, are observed in similar positions in the PSII(-OEC) and PSII(+ OEC) complexes (Supplementary Fig. 13). D1-N298, which is H-bonded to D1-H190 and is necessary for efficient oxidation of Y_Z³⁴, is also located at the expected position (Fig. 2d, Supplementary Fig. 13).

In general, the PSII(-OEC) complex displays well-resolved cofactor density on the acceptor side, including Q_B (Supplementary Fig. 9), and closely resembles the structure of fully functional PSII in this region (Supplementary Fig. 14), despite lacking cofactors on the donor side (Fig. 2d).

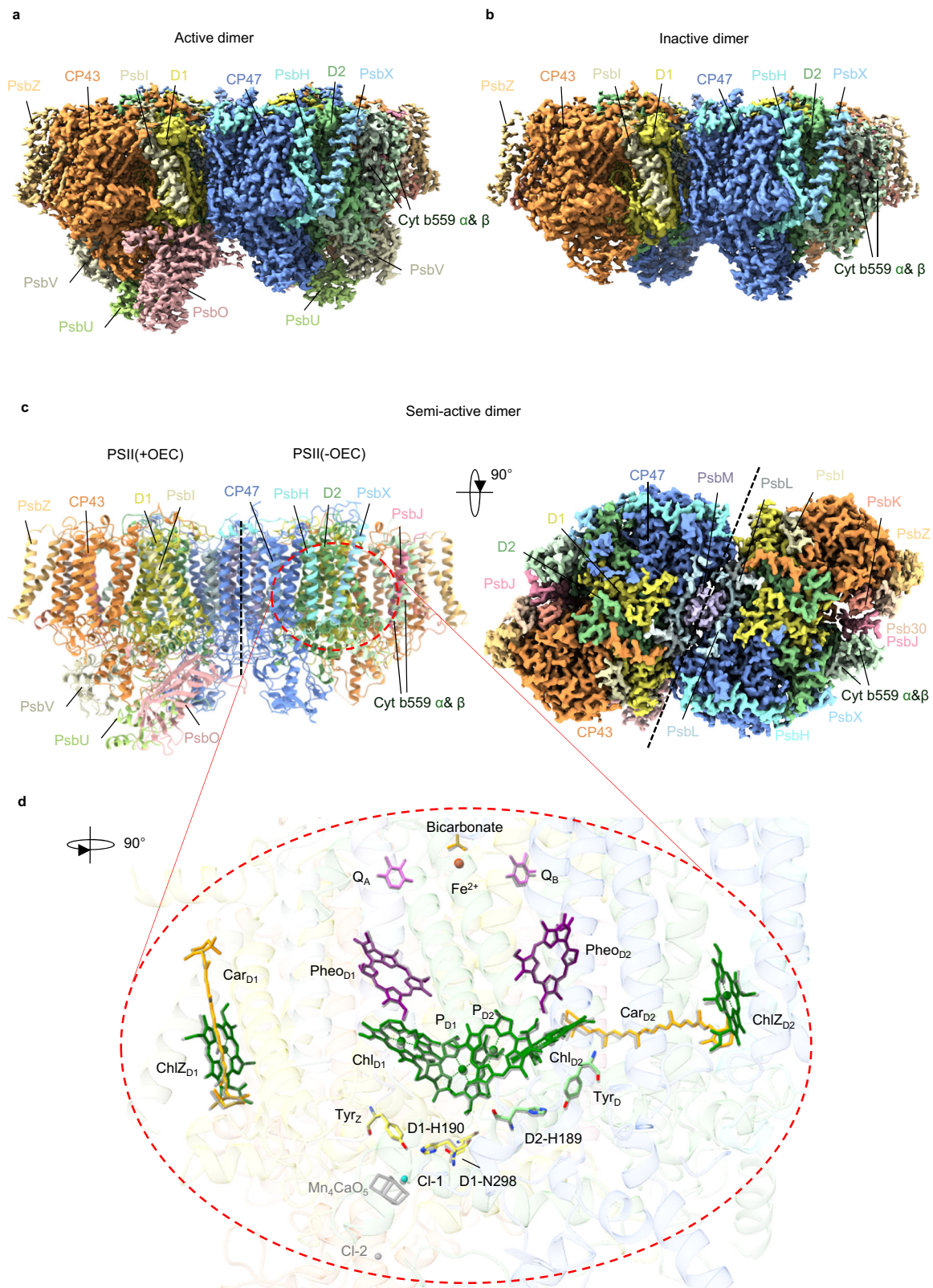


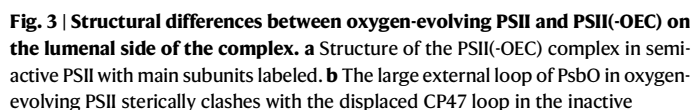
Fig. 2 | The cryo-EM maps and models of the three PSII_{peak4} complexes. a Active dimer map. **b** Inactive dimer map. **c** The structure and map of the semi-active dimer. **d** Comparison of cofactors in the OEC-depleted PSII (PSII(-OEC)) and OEC-

containing PSII (PSII(+OEC)). The cofactors of fully functional PSII are shown in gray and the PSII(-OEC) cofactors are shown in color.

PSII dimer is maintained in the absence of extrinsic subunits

Previous studies of PSII from the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 have suggested that PsbO stabilizes the dimeric state of PSII^{26,35–38}. The lack of PsbO in our inactive PSII dimer and

Psb27/PSII dimer²⁴ would suggest that PsbO is not obligatory for maintaining the PSII dimer in the thermophiles and that attachment of extrinsic subunits to the two monomers in a dimer may occur independently.



monomer. The red dashed circle in **(b,c)** indicates the displacement of CP47 and the clashes with the expected binding site of PsbO. The PsbO loop from N- to C-terminus is indicated by arrows. **c** Overlay of the fully functional PSII (PDB ID: 3WU2, gray) and PSII(-OEC) (colored).

We found here that a β -sheet in CP47 (CP47-H343 to CP47-R347), previously reported to be disordered upon removal of extrinsic proteins²⁶, remains ordered in PSII(+OEC) in the semi-active PSII dimer and identical to that of oxygen-evolving PSII, despite the absence of PsbO in the adjacent PSII(-OEC) complex (Supplementary Fig. 16). The disordered β -sheet in this region is therefore likely caused by a loss of low-molecular-mass subunits, PsbJ, PsbY and PsbZ in apo-PSII²⁶.

Compared with the fully functional PSII complex, the PSII(-OEC) complex exhibits not only the absence of extrinsic subunits but

In the PSII-(OEC) complex, the luminal region of CP43 from T293 to P388 shifts away from the Mn_4CaO_5 cluster, especially the CP43 helix that interacts with the D1 C-terminal tail (Figs. 3c, 4c). However, CP43-E342 (E354 in *T. vulcanus*), which acts as a ligand to the Mn_4CaO_5 cluster, is present at the expected position (Fig. 3c). The density of the

side chain of CP43-E342 cannot be resolved clearly, suggesting different rotamers.

Previous work has indicated that the CP43 soluble domain is flexible and involved in assembly of the Mn cluster^{14,40}. An atomic force microscopy (AFM) study of *T. vulcanus* has suggested that assembly of the Mn_4CaO_5 cluster induces changes in the positions of CP43-E342 (E354 in *T. vulcanus*) and CP43-R345 (R357 in *T. vulcanus*), subsequently triggering conformational changes in the luminal region of CP43¹⁴. While our data do support a shift in this region of CP43, the CP43-E342 and CP43-R345 residues are found at the same positions as in oxygen-evolving PSII, and the overall structural changes are of a significantly lower magnitude compared to that proposed in the AFM study (Fig. 3c).

Similar shifts in CP43 from T293 to P388 have been reported in other PSII intermediates, including apo-PSII, the Psb27/PSII dimer and Psb28/Psb27/PSII complex^{22,24,26}. The shift in this area does not cause steric clashes with the expected positions of PsbO, PsbU, or PsbV (Supplementary Fig. 18). Instead, the displacement of the CP43 luminal domain shifts it away from the Mn_4CaO_5 cluster, resulting in the disengagement of CP43 from the binding interface for the extrinsic subunits.

Additionally, the luminal loop CP43-G313 to CP43-L325 in the PSII(-OEC) complex exhibits poor density and cannot be modeled, suggesting high flexibility in this region (Supplementary Fig. 19). Furthermore, the CP43-G326 to Y328 region shows an alternative orientation to that observed in oxygen-evolving PSII so that CP43-K327 points away from the D1 C-terminal tail (Figs. 3c, 4c). Other PSII intermediates lacking the extrinsic subunits also lack density in this area (Supplementary Fig. 20)^{22–24,26}.

In agreement with Gisriel and colleagues²⁶, the flexibility of CP43 is likely due to the absence of extrinsic subunits. However, we notice two key differences between CP43 in the PSII(-OEC) complex and Gisriel's apo-PSII structure²⁶. First, CP43-E342 (E354 in *T. vulcanus*), which shifts 3 Å away from its expected Mn-coordinating position in apo-PSII, remains unchanged in PSII(-OEC). Second, while the region from CP43-G389 to F404 is difficult to model in apo-PSII²⁶, most of this region exhibits clear density in the PSII(-OEC) complex. Possibly the loss of low-molecular-mass subunits, PsbJ, PsbY, and PsbZ, in apo-PSII might increase the flexibility of the soluble domain of CP43.

Flexible D1 C-terminal tail

Although the Mn_4CaO_5 cluster-coordinating residues D1-D170 and D1-E189 remain at their expected positions in the inactive PSII complexes, D1-E333 and D1-R334 show ambiguous side-chain density and possibly show a different conformation to that in fully active PSII (Fig. 3c). The backbone of D1-E333 undergoes a ~4 Å shift, with its side chain possibly orientated toward the Mn_4CaO_5 cluster, in an opposite orientation to that modeled in apo-PSII²⁶ (Supplementary Fig. 21). Density corresponding to the D1 C-terminal tail from N335 to A344 could not be observed in PSII(-OEC) likely due to the high flexibility in this region (Fig. 4a) as mass spectrometry data confirmed that the D1 C-terminus was not truncated.

Previous structures of PSII intermediates have also identified D1-D170 and D1-E189 at the Mn coordinating positions^{6,22–24,26}. Notably, D1-D170 and D1-E189 are already positioned to bind the Mn cluster in the early reaction center assembly intermediate (RCII)⁶ with D1-D170, coordinating Mn_4 , previously identified as a high-affinity Mn^{2+} binding site⁴¹. Additionally, a Psb27/Psb28/PSII assembly complex²² contained an unknown metal ion density close to D1-D170. These observations further support the hypothesis that the primary high-affinity Mn^{2+} binding site is close to Mn_4 and Ca^{2+} ^{26,42}.

Unexpectedly, the side chain of D1-His332 in PSII(-OEC) was found to have an extra density in addition to the imidazole ring (Fig. 5a and Supplementary Fig. 22a,b). Mass spectrometry data confirmed the presence of singly oxidized D1-H332 in PSII_{Peak4} (Fig. 5b,

Supplementary Table 6a), so this density may arise from the oxidation of the imidazole ring to form hydroxylated histidine, similar to that previously reported for D2-H336⁴³. To fit this density, D1-H332 needs to adopt a flipped rotamer from its Mn-coordinating conformation. The extra density could also reflect a mixture of rotamers, as D1-H332 lacks coordinating interactions in the PSII(-OEC) complex, although the fitting of two rotamers did not account for the extra density as well as an oxidized imidazole side chain. The 2.2 Å resolution of the map would be insufficient to resolve protonation as an explanation for the extra density, and the density is too close to the imidazole ring to be due to a ligated metal ion⁴⁴. In RC47²³, Psb27/Psb28/PSII²² and apo-PSII²⁶, D1-H332 was found to be close to Cl-I, but in our structure, this histidine is approximately 4 Å away from Cl-I and so unable to coordinate it.

One possibility to explain the possible oxidation of D1-H332 is that D1-H332 undergoes photodamage due to oxidation by ROS generated during water oxidation and that the inactive complexes represent damaged complexes rather than assembly complexes. D1-H332 is close to the site of water oxidation as it ligates Mn_1 of the cluster, which is thought to bind the O_x water involved in the formation of O_2 through an oxo-oxyl coupling mechanism³³, possibly involving a peroxide intermediate⁴⁵. Although 2-oxo-histidine appears to be more commonly observed in biology under ROS-mediated conditions^{46,47} than hydroxylated histidine (Supplementary Fig. 22d), the specific oxidation product likely depends on the local protein environment and structure⁴⁸. Early photoinduced oxidation of D1-H332 has also been observed in experiments using isolated cyanobacterial PSII complexes⁴⁹ and plant thylakoid membranes⁵⁰.

In addition, D1-H332 oxidation is not unique and occurs alongside oxidation of other residues, including M328, M331, and E333 (Supplementary Table 6a). Single and double methionine oxidations were detected by mass spectrometry in the D1 C-terminal tail (M328 and M331) (Fig. 5b). However, apart from a slight extra density at D1-M331 in active dimers, no clear additional density was observed on the side chains of D1-M328 or M331 in either PSII(+OEC) or PSII(-OEC) (Supplementary Fig. 23). The poor density for D1-E333 prevents distinguishing potential oxidation at this site. M328, M331, H332, and E333 have been previously reported as photodamage sites⁵⁰. In our cryo-EM maps, significant extra density was observed only on the side chain of His332. The overall oxidation level of residues from A324 to R334 in PSII_{Peak4} is higher than in oxygen-evolving active PSII_{Peak3} (Supplementary Table 6b), suggesting that PSII_{Peak4} likely contains damaged PSII complexes. Single oxidation of histidine is only observed under special conditions^{51,52}, such as exposure to singlet oxygen⁵³, a signature ROS generated during photodamage⁴⁵, or during metal-ion-catalyzed oxidation (MCO)⁴⁸. Therefore, the observed oxidation of D1-H332 is more likely a consequence of photodamage.

D1 C-terminus unfolding allows flipping of D2 C-terminus and displacement of CP47

In oxygen-evolving PSII, the D1 C-terminal tail not only provides ligands (H332, E333, D342, A344) to the Mn_4CaO_5 cluster (Fig. 4a), but also interacts with the C-terminal tail of D2 (Fig. 4b), the CP43 L312-Y328 loop (Fig. 4c), and a helix from T293 to G313 of CP43. The backbone oxygens of D1-R334 and D1-H337 H-bond to the backbone nitrogen and side chain of D2-N350 (Fig. 4b), respectively. The side chain of D1-N338 H-bonds to the backbone of CP43-K327. In addition, D1-F339 exhibits a hydrophobic interaction with CP43-F305 (F317 in *T. vulcanus*), and CP43-Q301 (Q313 in *T. vulcanus*) also H-bonds to D1-L341 (Fig. 4c).

In the absence of a structured D1 C-terminal tail, the C-terminal tail of D2 flips to the opposite orientation, interacting with CP47-R384 within a small loop of CP47 (Fig. 4b). This interaction induces a minor shift in the loop of CP47 and results in clashes with PsbO-G163 to D169 in the large external loop of PsbO (Fig. 3b), a crucial region for the attachment of PsbO⁵⁴. Interestingly, mutation of CP47-R384 and R385

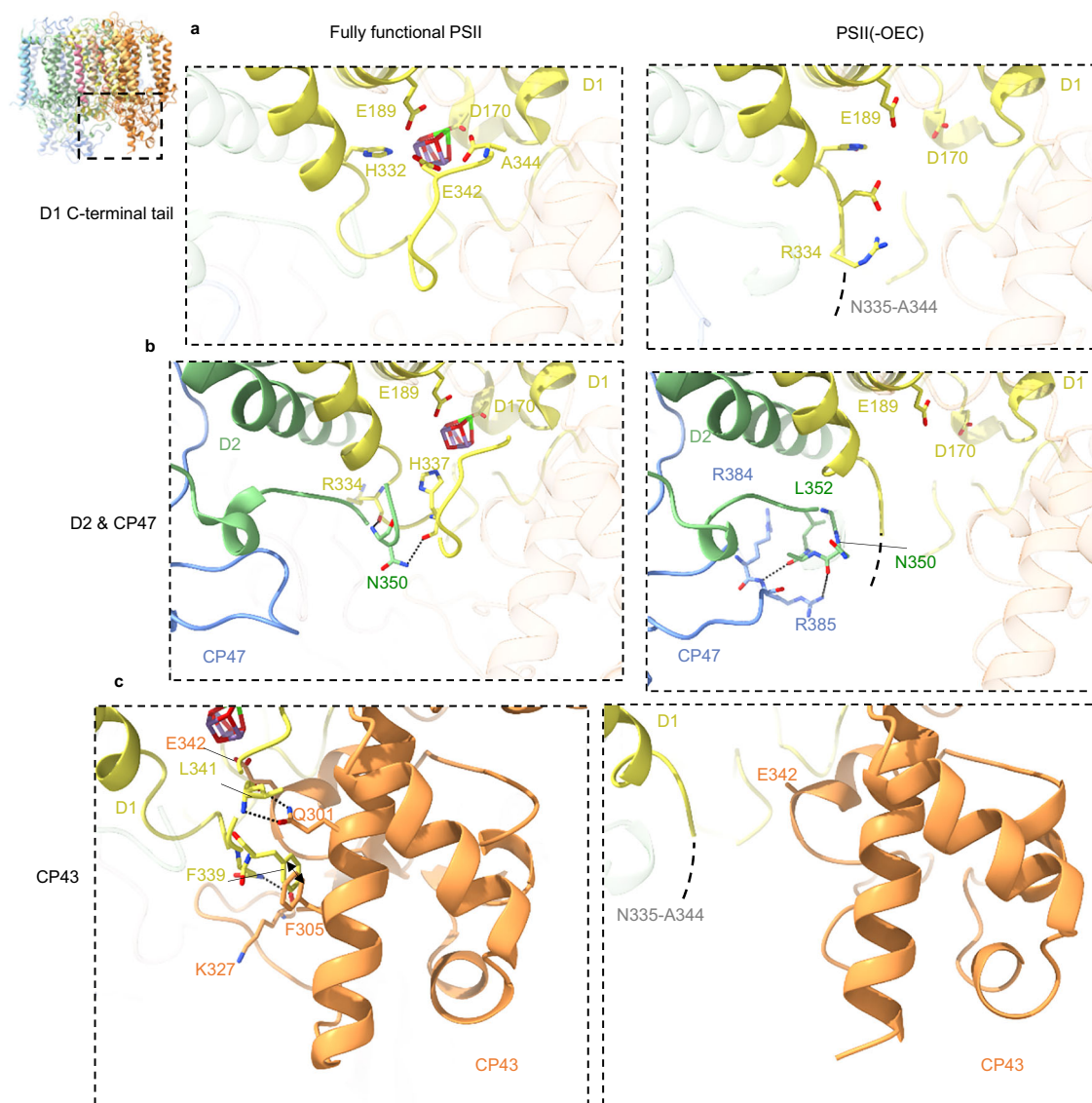


Fig. 4 | Structural changes in the D1, D2, CP47 and CP43 subunits in PSII(-OEC). Structural features from fully functional PSII (PDB ID: 3WU2, left panels) and PSII(-OEC) (right panels) **a** D1 C-terminal tail. Left panel, view of the D1 C-terminal tail and the Mn_4CaO_5 cluster. Extrinsic subunits are hidden for clarity. Right panel, loss of density for D1 residues beginning at D1-R334 in PSII(-OEC). Residues N335–A344 were not resolved in the density map and are represented by a dashed line and labeled in gray. **b** D2 C-terminal tail and the CP47 loop (CP47-P382 to CP47-E387). Left panel, interactions between the D1 C-terminal tail and the D2 C-terminus.

Extrinsic subunits are hidden for clarity. Right panel, interactions between the D2 C-terminus and CP47. In the fully functional state, D2-L352 hydrogen bonds with CP47-R384 (3.0 Å), and the CP47-R385 side chain—though showing ambiguous density—is positioned near the D2-N350 backbone oxygen (4.6 Å). **c** luminal side of CP43. Left panel, interactions between the D1 C-terminal tail and the CP43 luminal helix (CP43-E296 to CP43-L312) and loop (L312–Y328). Black dashed lines indicate hydrogen bonds (~ 3.0 Å), and double arrow denotes hydrophobic contacts (~ 4.2 Å). Right panel, luminal CP43 conformation in PSII(-OEC).

decreases the binding of extrinsic subunits⁵⁵, suggesting that this interaction between the C-terminal tail of D2 and CP47 is important for assembly and possibly disassembly of PSII.

Discussion

We have determined the structures, at a resolution of about 2.2 Å, of active, semi-active and inactive dimeric PSII complexes of *T. vestitus* that co-purify in a single peak by anion-exchange chromatography²⁷, here termed PSII_{Peak4}. PSII_{Peak4} represents about 5% of total PSII (Supplementary Fig. 1) and contains the three different types of complex at similar levels, which explains the reduced oxygen-evolving activity displayed by this fraction.

The OEC-containing dimeric PSII shows no significant protein or cofactor differences compared with previous structures of dimeric

oxygen-evolving PSII³⁰. The reason why the OEC-containing PSII dimers elute in Peak 4 rather than Peak 3 remains unclear, but may be due to subtle protein variations (e.g., in the unresolved regions) or differences in associated lipid or detergent molecules. It is also conceivable that active PSII dimers observed in Peak4 are coming with the trailing edge of Peak 3, which is substantially larger than Peak 4 (Supplementary Fig. 1).

The semi-active and inactive PSII complexes may in principle represent either assembly intermediates or photodamaged disassembly complexes or a mixture of both. Mass spectrometry data together with the structural data of PSII(-OEC) raise the interesting possibility that D1-His332, a ligand to the Mn cluster, may be oxidized, although more work is needed to confirm this assignment given the resolution of the structures. However, from a physiological

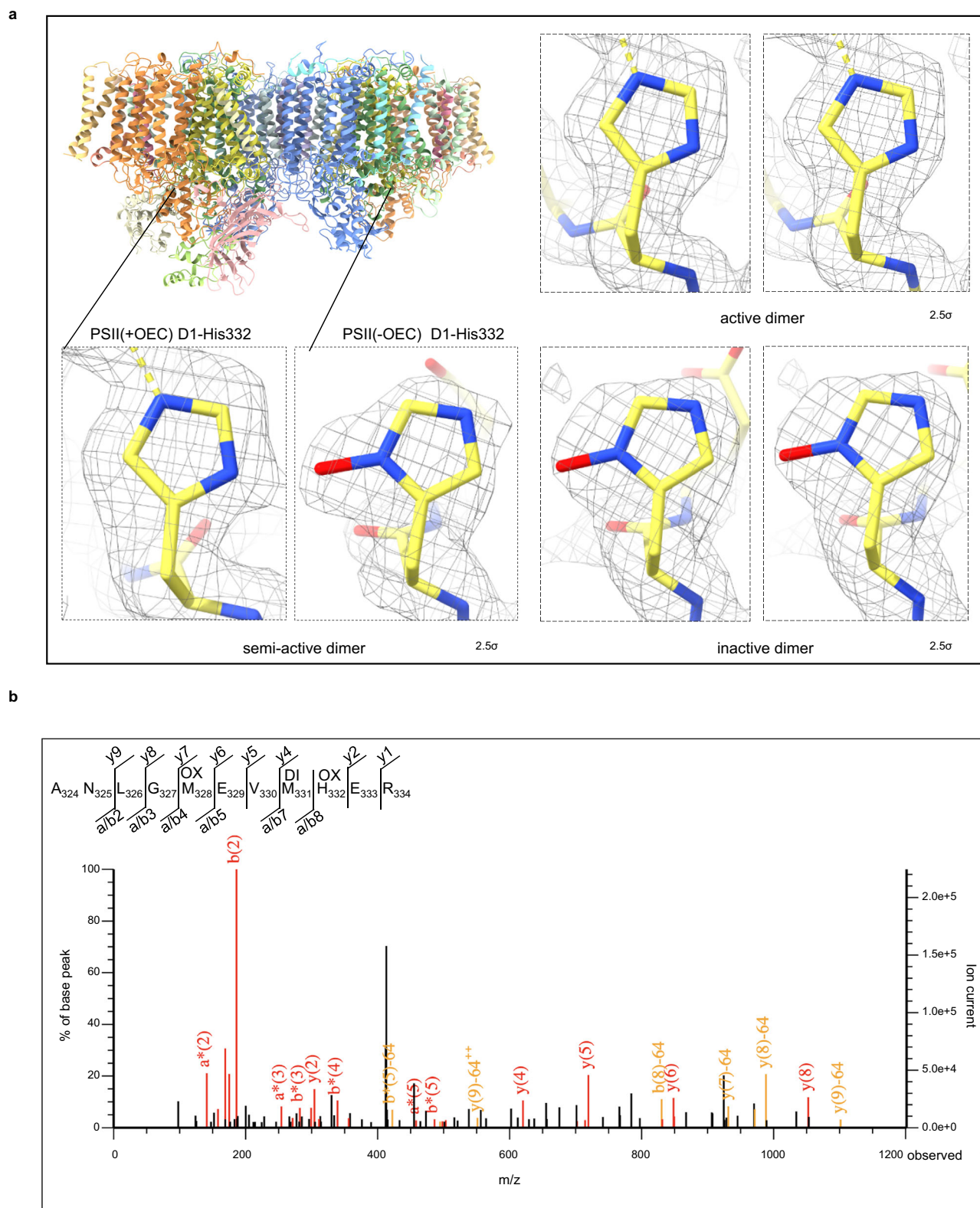


Fig. 5 | Oxidation of D1-H332 in PSII(-OEC). **a** Cryo-EM density of D1-His332 in the inactive, semi-active and active dimer. Densities are shown at the contour level of 2.5σ. **b** The figure shows a tandem mass spectrum of the quadruply-oxidized D1 peptide (A324–R334). Fragmentation via collisionally activated dissociation (CAD) confirms the presence of a single oxidation on M328 (OX), dioxidation on M331 (DI), and a single oxidation on H332 (OX), with ion assignments provided by Mascot

software. Mass spectrometry verifies oxidation of the C-terminal tail of D1. The peptide exhibits a mass increase of 64 Da relative to the non-oxidized form, corresponding to four oxidation events in total. Fragment ions y2 and y4 indicate three oxygens on M331 and H332, while ions a/b8 confirm two oxygens on M331 and one on H332.

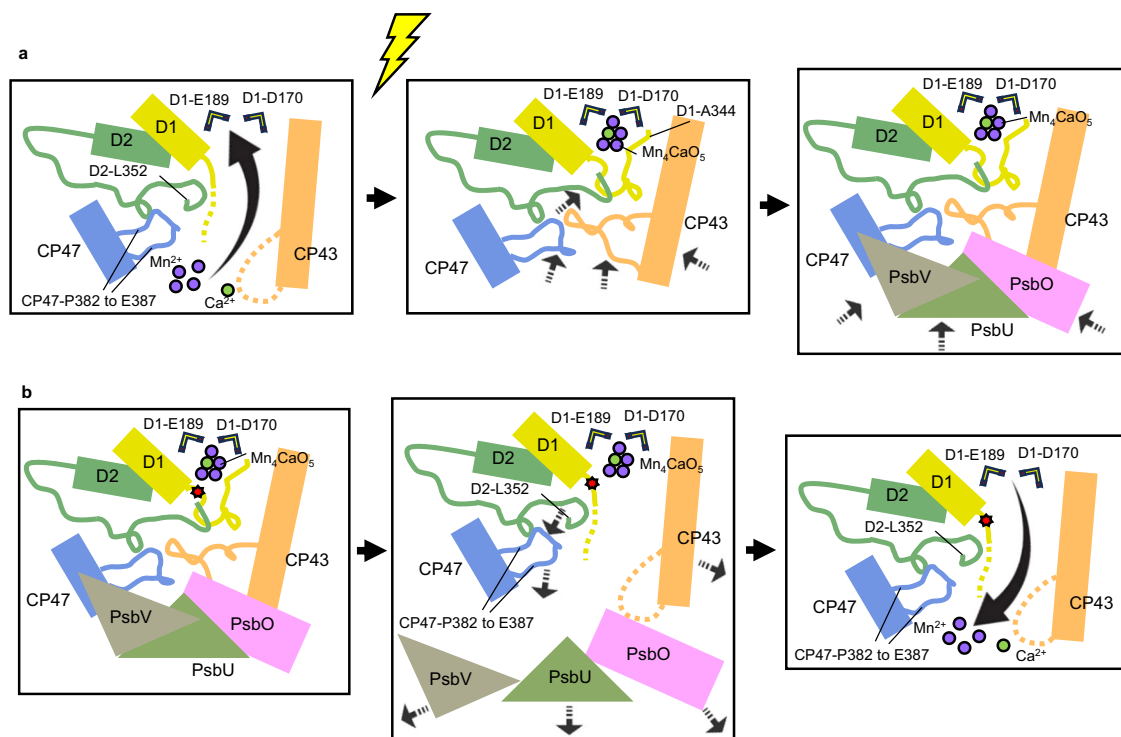


Fig. 6 | Model for PSII assembly/disassembly. Cartoon (a) represents the assembly pathway, while (b) represents the disassembly pathway. **a** Mn^{2+} and Ca^{2+} ions are initially delivered to the binding site of the Mn_4CaO_5 cluster. Photoactivation (photons are indicated by a “flash” cartoon) then drives the assembly of the Mn_4CaO_5 cluster and the folding back of the D1 C-terminal tail. The folding of D1 induces the flipping of the D2 C-terminal tail, structural rearrangements in the CP47 loop, and movement of the CP43 loop toward the Mn_4CaO_5 cluster. Finally, the extrinsic subunits attach to the luminal side of the PSII, completing the formation of

fully functional PSII. The attachment of extrinsic subunits may occur in a sequential manner with PsbV binding to the luminal side early in the process, while PsbU is likely incorporated at a later stage. **b** Upon irreversible oxidation of D1 residues (indicated by a red star), unfolding of the D1 C-terminal tail leads to destabilization of the luminal regions of D1, D2, CP43, and CP47 and detachment of the extrinsic subunits from the luminal side. As structural rearrangements proceed, the Mn cluster is more accessible from the lumen, allowing the release of Mn^{2+} (reduced from $\text{Mn}^{3+}/\text{Mn}^{4+}$) and Ca^{2+} ions from PSII.

perspective, prompt detachment of the extrinsic proteins triggered by photodamage to D1 C-terminal residues, would allow access of reductant to the Mn cluster to promote disassembly⁵⁶, thereby preventing the formation of further reactive oxygen species and further damage to PSII. A semi-active PSII dimer has also been observed after affinity purification of twin-strep tagged PsbO followed by anion-exchange chromatography. Whether it is an assembly or disassembly complex (or a mixture) remains unclear⁵⁷.

A recent structure of a Psb32/PsbV/Psb27/PSII assembly intermediate has revealed the binding of the Psb27 and Psb32 assembly factors, along with the PsbV extrinsic subunit, to the luminal side of PSII⁵⁸. In this complex, the transmembrane helix of Psb32 occupies the position of PsbY and assists in binding of PsbV, suggesting that extrinsic subunits are not incorporated spontaneously, but rather in a sequential and regulated manner with the assistance of Psb32.

In contrast, the PSII(-OEC) structure shows clear density for PsbY but lacks all extrinsic subunits. Either the PSII(-OEC) complexes are derived from photodamaged PSII or they represent a late-stage assembly intermediate in a parallel pathway to that involving Psb32. Deletion mutants of cyanobacteria and plants lacking Psb32 exhibit phenotypic deficiencies only under stress conditions^{59,60}, implying that Psb32 is not absolutely essential for PSII assembly and that alternative PSII assembly pathways exist.

A striking feature of the structures so far determined for PSII assembly intermediates lacking the OEC and PSII disassembly complexes that have had the extrinsic proteins and OEC removed is their high degree of structural similarity^{22–24,26}. Consequently, we suggest that assembly/disassembly of PSII follow similar trajectories but

proceed in opposite directions. Based on the structural differences between the inactive and active PSII complexes, we propose a mechanism (Fig. 6) for the assembly/disassembly of oxygen-evolving PSII.

In the case of assembly, we propose that photoactivation, which drives the assembly of the Mn cluster, is coupled to the remodeling of the C-terminal tail of D1 within the complex. The folded D1 triggers the movement of the luminal domain of CP43 and releases the CP47 loop from the D2 C-terminal tail to allow attachment of the PsbO extrinsic subunit (Fig. 6a). The current two-quantum photoactivation model, based largely on biochemical data, fits well with our mechanism¹³. After the first flash oxidizing a Mn^{2+} ion bound to the high-affinity Mn-binding site, PSII undergoes a slow ‘dark rearrangement’ that possibly corresponds to the movement of D1, D2, CP43 and CP47 in the luminal region. A second photon then oxidizes a second Mn^{2+} ion to generate the first stable intermediate in the assembly of the Mn cluster.

Our structure of the PSII(-OEC) complex revealed the presence of a putative channel for the delivery of metal ions for Mn cluster assembly (Supplementary Fig. 17). Gisriel et al. suggested that D1-E333 in the C-terminal tail could deliver Mn^{2+} to the Mn cluster binding site²⁶. However, this remains an open question, as E333 adopts a different conformation in apo-PSII compared to our PSII(-OEC) structure. (Supplementary Fig. 21). It is feasible that one of the water channels, the O1 channel, which has a minimum radius exceeding 0.9–1.1 Å^{61,62}, slightly larger than the ionic radius of Mn^{2+} (0.8 Å), may also serve as a Mn delivery channel, assuming Mn is dehydrated in the channel. However, mutations of O1 channel bottleneck residues do not appear to affect the assembly or structure of the Mn_4CaO_5 cluster⁶³.

In the case of disassembly, oxidation of PSII residues close to the Mn cluster leads to destabilization of the D1 C-terminal tail, triggering the detachment of extrinsic subunits from the luminal side and opening of the luminal channel. Subsequently, Mn^{2+} and Ca^{2+} ions are released into the lumen, resulting in disassembly of the PSII complex (Fig. 6b). Damaged subunits, such as D1, are then degraded and replaced by newly synthesized copies. Notably, the PSII structure in which the OEC was removed after crystallization shows an empty Mn_4CaO_5 site while retaining a folded D1 C-terminal tail and all three extrinsic subunits²⁵. This suggests that Mn and Ca ions may exit PSII via alternative pathways rather than direct luminal release, although no fully enclosed Mn-binding site has been observed in in vivo OEC-depleted PSII intermediates.

Inactive PSII complexes show Q_B density (Supplementary Fig. 9), while lacking cofactors on the donor side (Fig. 2d). This observation is similar to the recently reported Psb32/Psb27/PsbV/PSII assembly intermediate⁵⁸. In addition, the acceptor side of inactive PSII resembles that of the structure of fully functional PSII (Supplementary Fig. 14).

The flexible C-terminal tail of D1 is a feature of all PSII intermediates^{23,24,26}, apart from the Psb27/Psb28/PSII complex which shows an unknown metal bound to the luminal side of PSII²² (Supplementary Fig. 21). For all structures of PSII intermediates, the reorientated D2 C-terminal tail is present (Supplementary Fig. 21), but density for the loop from CP43-G313 to L325 is always missing, even though in some cases this region was still built in the model (Supplementary Fig. 20)^{22–24}.

The structure of the soluble domain of CP43 in PSII(-OEC) is highly similar to that of the *T. vulcanus* Psb27/PSII dimer²⁴ and *T. vestitus* Psb28/Psb27/PSII monomer²² (Supplementary Fig. 24). When PSII(-OEC) is superimposed on the two Psb27-containing structures^{22,24}, only minor clashes are observed between Psb27 and CP43 in PSII(-OEC) (Supplementary Fig. 24). Additionally, several CP43 residues previously reported to conflict with and disrupt interactions with PsbO were found in similar positions in PSII(-OEC)²⁴ (Supplementary Fig. 25). These observations suggest that the existence of Psb27 is unlikely to induce structural changes in CP43. Instead, Psb27 likely acts as a ‘door wedge’, blocking the necessary movement of CP43 required for Mn_4CaO_5 cluster assembly. If the soluble domain of CP43 from the Psb27/PSII structure is moved to its position in the fully functional PSII, Psb27 sterically clashes with PsbO (Supplementary Fig. 26)²⁴, indicating that with Psb27 bound, the soluble domain of CP43 cannot shift toward the Mn_4CaO_5 cluster and properly accommodate PsbO. As a result, the proper ‘dark rearrangement’ during photoactivation may be impeded.

Methods

Purification and characterization of Peak 4 PSII

His-tagged CP47 *T. vestitus* cells were grown under $\sim 60 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity, and thylakoid membranes were solubilized using 1% (w/v) DDM for 10 min at 4 °C at a chlorophyll concentration of 1 mg/ml. The solubilized PSII was purified by nickel affinity chromatography followed by anion exchange chromatography according to Nowaczyk et al.^{27,28} with the following modifications: PSII complexes eluted from nickel affinity column were loaded directly on the Bio-Rad UNO Q-12 column using a AKTA purifier 10 system; PSII were then eluted with 5–200 mM MgSO_4 gradient²⁸. The purification methods were derived from Nowaczyk et al.²⁷ and described in Michoux et al.²⁸.

SDS-PAGE and CN-PAGE Electrophoresis

Protein samples containing 3 μL of chlorophyll were mixed with 2 $\times$ SDS loading buffer (125 mM Tris/HCl, pH 6.8; 4% (w/v) SDS; 20% (v/v) glycerol; 0.1% (w/v) bromophenol blue; and 10% (v/v) 2-mercaptoethanol) and loaded onto a 12% Tris–Tricine gel containing 6 M urea⁶⁴. Electrophoresis was performed at 5 mA for 7 h

at 4 °C. Gels were then stained with InstantBlue™ (Expedeon, UK) for 2 h.

Clear Native PAGE (CN-PAGE) was carried out using NativePAGE™ 3–12% Bis-Tris pre-cast gels (Life Technologies, UK), as previously described⁶⁵. Protein samples were mixed with native loading buffer to a final composition of 50 mM Bis-Tris/HCl (pH 6.5), 7% (v/v) glycerol, and 0.01% (w/v) Ponceau S. Electrophoresis was performed at 8 mA and 4 °C using an anode buffer (50 mM Bis-Tris/HCl, pH 7.0) and a cathode buffer (50 mM Tricine, 15 mM Bis-Tris/HCl, pH 7.0, 0.05% (w/v) sodium deoxycholate, and 0.03% (w/v) β -DDM).

The Psb27 antibody was raised against the *Thermosynechococcus vestitus* BP-1 protein expressed in *E. coli*²⁸ and immunoblotting was performed as described previously⁶⁶.

Measurement of oxygen evolution activity

Oxygen evolution activity was assessed using an OxyI-Oxylab device (Hansatech Instruments, UK). Cells were adjusted to a chlorophyll a (Chl a) concentration of 1 $\mu\text{g}/\text{ml}$. The activity was measured with 1 mM potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and 0.1 mM 2,6-dichlorobenzoquinone (DCBQ) at a light intensity of 2000 $\mu\text{mol photons m}^{-2}\text{ s}^{-1}$. For PSII protein samples, 1 μg of Chl a was mixed with 1 ml of a buffer solution containing 50 mM MES-NaOH (pH 6.5), 500 mM sucrose, 30 mM CaCl_2 , 10 mM MgCl_2 , 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, and 0.1 mM DCBQ. The oxygen evolution activity was then measured under the same light conditions of 2000 $\mu\text{mol photons m}^{-2}\text{ s}^{-1}$.

Mass spectrometry

PSII_{peak4} and PSII_{peak3} protein samples were analyzed by bottom-up mass spectrometry ($n=1$ for each sample). This mass spectrometry workflow and data analysis pipeline are based on extensively validated protocols routinely applied to a wide range of biological samples and species. Protein samples were heated (95 °C, 5 min.) with lysis buffer (100 μL , 12 mM sodium lauroyl sarcosine, 0.5% sodium deoxycholate, 50 mM triethylammonium bicarbonate (TEAB)). The samples were treated with tris(2-carboxyethyl) phosphine (10 μL , 55 mM in 50 mM TEAB, 30 min, 37 °C) followed by treatment with chloroacetamide (10 μL , 120 mM in 50 mM TEAB, 30 min., 25 °C in the dark). They were then diluted 5-fold with aqueous 50 mM TEAB, and incubated overnight with Sequencing Grade Modified Trypsin (1 μg in 10 μL of 50 mM TEAB; Promega, Madison, WI), following which an equal volume of ethyl acetate/trifluoroacetic acid (TFA, 100/1, v/v) was added. After vigorous mixing (5 min) and centrifugation (13,000 $\times$ g, 5 min), the supernatants were discarded, and the lower phases were dried in a centrifugal vacuum concentrator.

The samples were then dissolved in acetonitrile/water/TFA (solvent A, 100 μL , 2/98/0.1, v/v/v) and loaded onto a small portion of a C18-silica disk (3 M, Maplewood, MN) placed in a 200 μL pipette tip. Prior to sample loading, the C18 disk was prepared by sequential treatment with methanol (20 μL), acetonitrile/water/TFA (solvent B, 20 μL , 80/20/0.1, v/v/v), and finally with solvent A (20 μL). After loading the sample, the disc was washed with solvent A (20 μL , eluent discarded) and eluted with solvent B (40 μL)⁶⁷. The collected eluent was dried in a centrifugal vacuum concentrator. The eluants were reconstituted in water/acetonitrile/FA (solvent E, 10 μL , 98/2/0.1, v/v/v), and aliquots (5 μL) were injected onto a reverse phase nanobore HPLC column (AcuTech Scientific, C18, 1.8 μm particle size, 360 μm $\times$ 20 cm, 150 μm ID), equilibrated in solvent E and eluted (500 nL/min.) with an increasing concentration of solvent F (acetonitrile/water/FA, 98/2/0.1, v/v/v: min./% F; 0/0, 5/3, 18/7, 74/12, 144/24, 153/27, 162/40, 164/80, 174/80, 176/0, 180/0) using an Eksigent NanoLC-2D system (Sciex (Framingham, MA)).

The effluent from the column was directed to a nanospray ionization source connected to a high-resolution orbitrap mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific) acquiring mass spectra in a data-dependent mode alternating between a full scan (m/z

350–1700, automated gain control (AGC) target 3×10^6 , 50 ms maximum injection time, FWHM resolution 70,000 at m/z 200) and up to 10 MS/MS scans (quadrupole isolation of charge states ≥ 2 , isolation width 1.2 Th) with previously optimized fragmentation conditions (normalized collision energy of 32, dynamic exclusion of 30 s, AGC target 1×10^5 , 100 ms maximum injection time, FWHM resolution 35,000 at m/z 200).

Raw data files were converted from .raw to.mgf format using MSConvert version 3.0 and analyzed with Mascot Server 2.8.2 and Mascot Daemon 2.8.0. Spectra were searched against the UniThermoelelongatus 20221202 database together with a contaminants database (20160129). Trypsin was specified as the protease with up to two missed cleavages allowed. Carbamidomethylation of cysteine was set as a fixed modification, while oxidation (A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y) and dioxidation of methionine were included as variable modifications. Precursor and fragment ion mass tolerances were set to 10 ppm and 50 ppm, respectively. The minimum peptide length was set to eight amino acids, and peptide identifications with Mascot scores greater than 34 were considered. Statistical significance was assessed at $p < 0.05$.

Negative staining with electron microscopy

PSII samples were diluted to a protein concentration of 0.01–0.04 $\mu\text{g}/\mu\text{L}$ (estimated from chlorophyll concentration). Samples were applied to 200-mesh copper grids, washed three times with pure water, and stained with 2.5% uranyl acetate for 1 min. Images were collected using an FEI Tecnai T12 Spirit transmission electron microscope.

Grid preparation

The sample, concentrated (using Amicon 100 kDa MWCO (Millipore) concentrators) to 0.3 mg/ml in 20 mM MES pH 6.5, 2.5 mM MgCl_2 , 2.5 mM CaCl_2 , 7 mM MgSO_4 , 0.03% DDM, 0.05% CHAPS, was frozen on Quantifoil 0.6/1, 300 mesh copper grids manually coated with a continuous layer of carbon approximately 1 nm thick⁶⁸. The grids were glow-discharged for 5 s at 25 mA prior to freezing. The sample was prepared using a Vitrobot Mark IV, set to 4 °C, 100% humidity, 2 s blot time, and 25 blot force. 3 μL of sample was applied to each grid. The sample was manipulated under green light until frozen.

Data collection

The micrographs were collected on a 300 kV Titan Krios G3i, equipped with a Gatan K3 BioQuantum direct detector. Preliminary data collection indicated that, although showing good particle spread, the sample adopts preferential orientation on continuous carbon. Therefore, the high-resolution dataset was collected with the grid tilted 30 degrees, to mitigate the effects of preferential orientation. As shown in Supplementary Fig. 6, about 9173 movies were collected in 72 h, using the software Serial-EM for 48 h and EPU for 24 hours. The grid was aligned so that rows of holes would sit along the tilt axis: this allowed us to collect nine holes with beam-image shift while keeping the beam tilt below 10 mrad⁶⁹. The data collection details are summarized in Supplementary Table 1.

Data processing

The processing outline is described in Supplementary Fig. 6. After optics group assignment, the raw micrographs were aligned using their own implementation of motion correction from Relion 3.1⁷⁰. CTFIND⁷¹ was used for initial CTF estimation, then Relion 3.1 was used for particle picking, 2D and 3D classification, polishing, and CTF/3D refinements, except for per-particle defoci, which were estimated using GCTF⁷² upon particle picking, to immediately assign the correct defoci to each particle. This step was important, since CTFIND only estimates per-micrograph defocus, not considering the defocus change resulting from the stage tilt. Initial picking was performed with LoG in Relion, resulting in 5.9 Mln particles, then subjected to 2D

classification. 3.4 M good particles were selected, pooled, globally refined, then filtered based on LogLikelihoodContribution (up to 91300), Autopick Figure of Merit (FoM) (max 0.2), CTF FOM (min 0.1), NormCorrection (0.57–0.61) to remove particles featuring strong artefacts in the micelle area. The resulting 2.65 M particles were polished and CTF-refined, then separated by 3D classification without alignment (20 Å lowpass filter of reference map, T64 and 10 classes), to resolve the compositional heterogeneity (presence vs absence of the Psb O-U-V subunits). Pre-alignment of particles via global refinement and subsequent classification without alignment resulted in better separation than 3D classification with alignment. This strategy allowed us to isolate two good classes, without artefactual streaks: a class of 636,664 particles, featuring a mixture of active (Psb O-U-V present in both PSII monomers) and semi-active (Psb O-U-V present only in one PSII monomer) particles, and one class of 420178 inactive (Psb O-U-V absent) particles. The two classes were then further classified without alignment (20 Å lowpass filter of reference map, T64 and three classes) to fully separate the particles, and this led to the three final classes: active PSII (363811 particles, 2.4 Å), semi-active PSII (272,215 particles, 2.2 Å), and inactive PSII (417537 particles, 2.5 Å). Since the active and inactive maps were perfect dimers, the maps were also postprocessed with C2 symmetry to further improve resolution, yielding 2.2 Å maps. Post-processing and local resolution estimation were performed in Relion 3.1.

Model fitting

The crystal structure of *T. vestitus* monomeric PSII (PDB ID: 3KZI)⁷³ and the cryo-EM structure of *T. vestitus* monomeric PSII lacking PsbJ and extrinsic subunits (PDB ID: 7NHO)²² were placed into the cryo-EM density of PSII(+OEC) and PSII(-OEC) within the structures of PSII dimers using phenix.dock_in_map^{74,75}. The model of PsbJ from monomeric PSII⁷³ was used as the initial model for PsbJ in the PSII(-OEC). Subsequently, the structures of PSII(+OEC) and PSII(-OEC) were corrected and adjusted in COOT to match cryo-EM density⁷⁶. Next, the refinement was carried out using phenix.real_space_refine with cofactor restraints generated by phenix.elbow^{74,75}. PSII(+OEC) and PSII(-OEC) were then merged to create three PSII dimer structures, which were further refined with COOT and phenix.real_space_refine. Residues in the structures were carefully inspected in COOT, and iterative rounds of manual refinement were performed using COOT and phenix.real_space_refine until no significant errors were present in COOT and MolProbity (<http://molprobity.biochem.duke.edu/>)⁷⁷ validation reports. Density fitting and refinement statistics are shown in Supplementary Figs 7–10 and Supplementary Tables 2–4.

Structural analysis

Figures were prepared using ChimeraX. Root-mean-square deviations (rmsd) between two structures were calculated in ChimeraX based on the C α atoms of the backbone, representing the structural differences across all residue pairs. The channel reaching the Mn cluster was calculated by MoleOnline (<https://mole.upol.cz/>). Electrostatic solvent accessible surface potentials were calculated using the PyMOL APBS electrostatics Plugin (<https://apbs.readthedocs.io/en/latest/>) with default template settings.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The cryo-EM maps are deposited in the Electron Microscopy Data Bank under accession number EMD- 51100 (inactive dimer), EMD- 51102 (active dimer) and EMD-51101 (semi-active dimer). The models are deposited in the Protein Data Bank under accession numbers 9G6F (inactive dimer), 9G6H (active dimer) and 9G6G (semi-active dimer).

Mass spectrometry data was uploaded to MassIVE with accession code [MSV000101057](#). Source data are provided with this paper.

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Author contributions

P.J.N. and L.A.S. conceived the study. Z.Z., I.V., J.P.W., P.J.N. and L.A.S. designed the experiments. W.B. and K.M. performed sample purification and performed biochemistry experiments shown in Supplementary Fig. 1 and Supplementary Fig. 10. Z.Z. performed the rest of the biochemistry experiments. I.V. performed structural biology experiments and determined cryo-EM maps. Z.Z. built and refined structural models. J.P.W. performed mass spectrometry experiments. Z.Z., I.V. and J.P.W. analyzed the results. Z.Z., I.V., J.P.W., P.J.N. and L.A.S. wrote the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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