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The heart of photosynthesis in glorious 3D

A. William Rutherford and Peter Faller

The input of solar energy into photosynthesis, and thence into the biosphere, occurs via chlorophyll-containing proteins known as reaction centres. There are two kinds of reaction centre in oxygenic photosynthesis: photosystem I (PSI) and photosystem II (PSII). The PSII reaction centre, alias the oxygen-evolving enzyme, the water-oxidizing complex or the water–plastoquinone photo-oxidoreductase, has now been crystallized and its structure solved to a resolution of 3.8 Å.

PSII reduces quinone at the start of the photosynthetic electron transfer chain, stripping the required electrons from H₂O and releasing oxygen and protons on one side (the inside) of the membrane (Fig. 1). The protons then contribute to the gradient that drives ATP synthesis whereas the oxygen diffuses into the environment. Using H₂O as the source of electrons allowed the photosynthetic species to colonize most of the planet while, at the same time, turning the biosphere aerobic and, thereby, allowing respiration and the consequent increases in the complexity of organisms. As many aspects of the structure and function of this important enzyme remain mysterious, the publication of an X-ray crystallographic model is an important event (Ref. 1) (Fig. 2).

This enzyme² is a complex membrane protein that weighs in at ~300 kDa and

comprises at least 17 subunits. It contains at least 13 redox-active cofactors not to mention at least 26 more chlorophylls (green pigments) with light-collection roles. All of the chemical action seems to occur in the central heterodimer (D1–D2); conceptually however, the complex can be divided into two parts: the photochemical part and the catalytic part.

The photochemical part³ contains the ultra-fast and ultra-efficient light-induced charge separation and stabilization steps that occur vectorially across the membrane when light is absorbed by chlorophyll. A good structural model already exists for this part. Based originally on spectroscopic comparisons⁴ with the simpler and better-characterized cousin of PSII, the bacterial reaction centre, this model was subsequently developed, tested and verified by amino-acid sequence analysis⁵, biochemistry⁶, site-directed mutagenesis², computer modelling (cited in Refs 1–3,6), more spectroscopy (for example, Refs 7–10) and, recently, electron crystallography with a resolution of 8 Å (Ref. 11).

The catalytic part of PSII is responsible for H₂O oxidation, a process involving a cluster of Mn ions close to a redox-active Tyr residue. In the absence of a structurally relevant biological analogue, spectroscopy-based modelling^{12,13} has relied instead on Mn-coordination

chemistry and the models have been relatively vague and ambiguous.

Although there is plenty of new information in there (see following text), the core of the new structure, on the face of it, looks like the purple bacterial reaction centre with a bunch of Mn ions stuck onto the base. This terse résumé of the PSII structure is equally applicable to the model that has dominated the field since the early 1980s (see Refs 2,4,5,7 for examples), thus, at this level, the new crystal structure provides no surprises. That said, the earlier PSII modellers would (or at least should) readily admit that their structures were based on measurements, interpretations and arguments that often suffered from varying degrees of ambiguity. One of the main contributions of the new structural model is in removing much of that ambiguity.

What is new?

In terms of the photochemical part, very little. Indeed, the new crystal structure does not resolve the amino-acid side chains, the orientation of the smaller cofactors or the position of the carotenoids. So, in some respects, the old model is in fact more detailed than that presented in the new crystal structure (for example, Refs 7–10). As for the proteins, the new picture for the core and peripheral heterodimers (the D1–D2 and 43–47-kDa

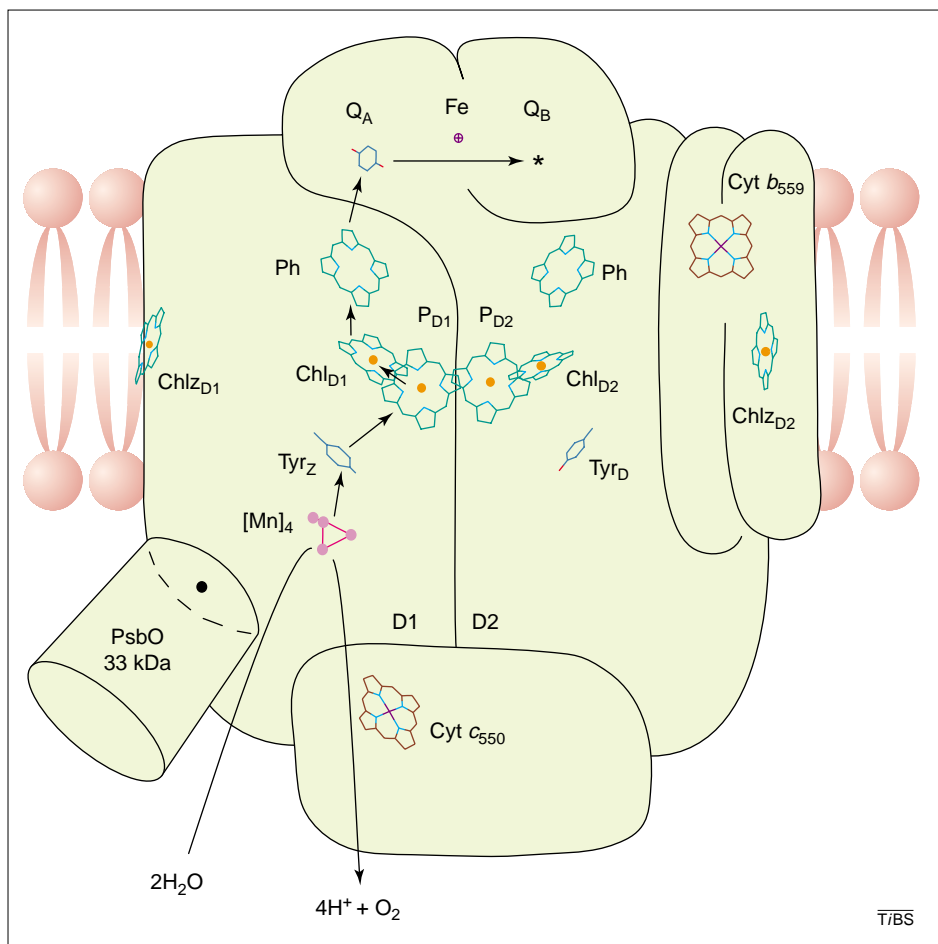


Fig. 1. Structural model of the oxygen evolving enzyme (PSII) focusing on the electron transfer cofactors and based on the crystal structure¹. The arrows indicate the pathway of electron transfer occurring upon the absorption of light. Please note that these arrows were not resolved by crystallography (or at least they were not reported in Ref. 1), they have been added gratuitously by the current authors to emphasize a functional view. Only 6 of the 17 polypeptides are shown and these are drawn in cartoon style. These are the central heterodimer D1–D2, the 33-kDa extrinsic polypeptide (PsbO), the cytochrome c_{550} and the two subunits making up cytochrome b_{559} . For simplicity, the other polypeptides have been left out of this drawing. The major absentees are the membrane-spanning parts of the large 47- and 43-kDa polypeptides (CP47 and CP43 in Fig. 1), each having six transmembrane α helices and containing 14 and 12 chlorophylls, respectively. These large proteins are located symmetrically in front and behind the D1–D2 core as drawn in this view and contribute much of the protein protruding from the membrane (i.e. on the lower side of this drawing). The crystal structure defines the positions of 12 more transmembrane α helices, some of which are tentatively assigned to some of the other ten minor subunits expected to be present (see Fig. 2 and Ref. 1). Most of the protein outside of the membrane is poorly defined; thus, the inside and outside boundaries of the D1–D2 heterodimer are unclear. The cytochrome c_{550} is well-defined, as is about half of the 33-kDa protein which makes up the barrel-like structure. The black sphere at the inside terminus of the barrel is an adventitious Cd^{2+} ion (see text). Abbreviations and explanations: Chl, chlorophyll [subscripts distinguish between chlorophylls mainly on the basis of their parent proteins (N.B. P also represents chlorophyll molecules, see below)]; Cyt, cytochrome; Fe, a non-heme iron ion; $[Mn]_4$, the manganese cluster; P, the specific chlorophylls that bear the cation detectable by spectroscopy; Ph, pheophytin; Q_A and Q_B , plastoquinone molecules (note, the crystal structure lacks Q_B); Tyr_Z and Tyr_D, redox-active Tyr residues (only Tyr_Z is kinetically competent).

proteins, respectively) had already been proposed on the basis of comparisons with other reaction centres and work with lower-resolution crystallography¹⁴. The 'hot stuff' from the new structure was at the level of the Mn, the extrinsic proteins and the cytochrome b_{559} , and in that order.

The Mn cluster: structure

The Mn cluster that constitutes the active site and the interface between the one-electron photochemistry and the

four-electron water–oxygen chemistry, has been the focus of arguments for years concerning how many Mn ions there are and how closely they are grouped. Four Mn ions have consistently cropped up in biochemical and biophysical studies². Tetramers, two separate dimers and monomer–trimer structures have all been proposed but the mainstream have been fairly solidly in the tetramer camp. The next level of debate has focused on the geometry of the tetramer and, in recent

years, linked-dimers and 3+1 structures (i.e. three tightly associated Mn ions linked to a fourth, less tightly associated Mn ion) have both found favour over, for example, cubanes and adamantanes^{12,13}.

The crystal structure¹ shows a glob of electron density forming a very asymmetrical triangle, the bulbous corners of which are likely positions for three Mn ions. The crystallographers have proposed that a fourth Mn might fit more or less in the middle of the cluster (Fig. 2b). If this is correct, then the whole structure seems to have the geometry of a bent paint-scraper: not very romantic perhaps, but functional (Fig. 2b).

Many questions spring to mind. Where is the famous Ca^{2+} ion: that enigmatic cofactor required for H_2O oxidation (at least in plant PSII)²? (No sign yet.) How sure is the fourth Mn? (Better than 50:50?) And dare we consider the heretical trimer? (Only in private.) Does the '4-Mn paint-scraper' model agree with the spectroscopy? This geometry can perhaps be thought of either as a variation of two interacting dimers or as a 3+1 cluster: not too far removed from the most recent models based on electron paramagnetic resonance and X-ray absorption spectroscopy^{12,13}. At this moment, several labs could be reassessing their spectroscopic data in the light of this new geometry, asking themselves whether it is feasible given the restrictions imposed by their data. If not, something will have to give and precedence shows that it is not always the spectroscopy.

For the structure of the Mn cluster, the new crystal structure eliminates less-favoured options, such as models in which the cluster comprises two separate dimers, and establishes that the Mn ions form a tightly grouped cluster¹. However, the precise geometry of the cluster remains unclear: there are still niggling doubts over its nuclearity and there is no information about its coordination. The chemistry groups that specialize in the synthesis of biologically inspired models, and who need the cluster's structure as a target before they can start the race to develop true biomimetic catalysts of water oxidation, will have to remain under starter's orders for a while longer.

The Mn-cluster: localization

The crystal structure shows that the Mn cluster is asymmetrically positioned close to the predicted site of the redox active

Tyr (Tyr_Z) and, indeed, a somewhat poorly resolved electron density at this site is attributed to Tyr_Z itself. This result puts an end to various flirtations with the aesthetically pleasing idea of the Mn being on the enzyme's symmetry axis, and also to the associated idea that it could be further away from the Tyr (Ref. 4). Spectroscopic studies had shown that the Mn cluster was close enough to the Tyr_Z to interact via electronic exchange¹³ but the deduced distance estimations (~5–10 Å) are vague and unreliable¹⁵. The 7 Å distance reported in the new crystal structure appears to be the distance between the Tyr and the nearest Mn ion of the cluster¹. If this distance holds up to refinement, then it has important repercussions on mechanistic thinking.

The concept that is currently de rigueur in mechanistic circles focuses on the Tyr being not merely an oxidant for the Mn ion but also the direct oxidant for H₂O, extracting not only electrons but also protons, possibly via an H-atom transfer process^{13,16}. Now, true H-atom transfer is predicted to be feasible only at very short distances so, for it to occur, the present structure would imply that the substrate H₂O would have to be positioned between the Mn and the Tyr, and even then this distance might be too far. Proton-coupled electron transfer, a process which, compared to H-atom transfer, can occur over greater distances, seems the feasible option^{13,16}. An accurate and reliable Tyr–Mn distance measurement could allow elimination of the H-atom transfer option. In any case, one thing is for certain: from now on the structure will be a less flexible parameter in mechanistic models.

Extrinsic polypeptides and cytochromes

An unexpected finding concerns the main extrinsic protein², the 33-kDa protein (also known as PsbO), that stabilizes the Mn cluster and protects it from reductive attack (i.e. acts as insulation) but is not absolutely required for activity. The part of the protein that was resolved contains what looks like a tunnel, 14 Å wide and 35 Å long, stretching from the outside towards the active site. Tantalizingly, the resolution peters out 30–40 Å away from the Mn cluster. So, what could be the function of such a structure? To get the H₂O in or the O₂ and/or protons out? Or is it merely a structural quirk of the insulation? More tantalizing still, consultation of the Protein Data Bank

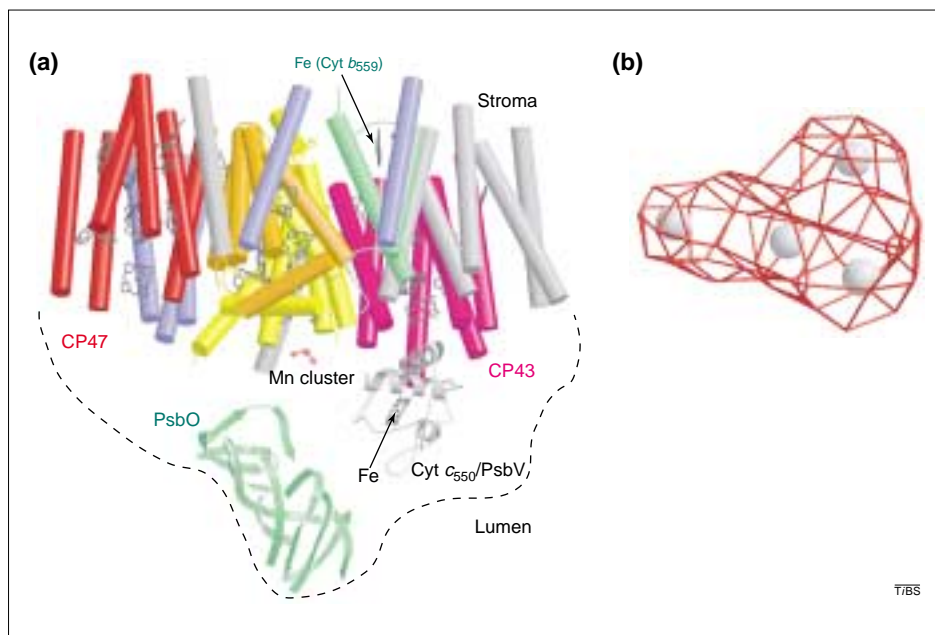


Fig. 2. (a) Structural model of PSII from X-ray crystallography (reproduced, with permission, from Ref. 1). The helices are shown as coloured cylinders. The yellow and orange helices are from the central core heterodimer, D1 and D2 (see Fig. 1). The red and magenta helices are from the 47- and 43-kDa proteins that comprise the peripheral heterodimer that bears chlorophylls with light-collection roles. The green helices are from cytochrome *b*₅₅₉, whereas the blue and grey helices are minor polypeptides, the grey being unassigned. The green ribbon structure labelled PsbO is also termed the 33-kDa protein elsewhere in the text. (b) The electron density ascribed to the Mn cluster showing the possible position of the Mn ions.

(1FE1) shows a Cd²⁺ ion hovering mysteriously over the internal mouth of the tunnel. Cd²⁺ was used as a heavy atom derivative to solve the structure, so its presence is clearly an artefact. However, as Cd²⁺ binds well in Ca²⁺ sites, we might ask whether this represents the elusive Ca²⁺ site? The answer is that this seems unlikely. The important Ca²⁺ is not thought to be associated with the 33-kDa protein and it would be surprising (and disappointing for many of the researchers working on the enzyme mechanism) if the Ca²⁺ were so far away (>28 Å) from the active site. Significantly perhaps, Cd²⁺ binds strongly to the mouth of the proton channel in the bacterial reaction centre, thereby inhibiting its function¹⁷. In PSII, the Cd²⁺ could be an indication that the 33-kDa subunit does indeed represent a channel. Experimentation (e.g. computer modelling, site-directed mutagenesis, effects of heavy metals) will doubtless focus on potential channel roles.

As for hemes, there are two visible in the structure, those of cytochromes *b*₅₅₉ and *c*₅₅₀. The latter is not found in plants and is not required for enzyme function¹⁸. The crystal structure places it too far from the other cofactors for it to have an electron transfer role. Some kind of protective role, possibly as a catalase or peroxidase, is

worth considering. Cytochrome *b*₅₅₉ is present in PSII from all sources and, although its role is unknown, it is often suggested to be involved in a 'cyclic' electron transfer pathway around the periphery of the reaction centre, playing a protective role under some circumstances¹⁹. A lot of the speculation can now be more or less ruled out as there is only one *b*₅₅₉ heme (and not two as has been commonly argued in the past) and this is rather distant from the other cofactors. However, there is a wild card for the *b*₅₅₉ speculator: spectroscopic studies indicated that a redox-active β-carotene, a 25 Å-long cofactor, probably winds through the reaction centre allowing electron transfer contact between the *b*₅₅₉ heme, the peripheral oxidizable chlorophyll(s) (i.e. Chl_Z) and the central photo-oxidizable chlorophylls²⁰. Because the crystal structure contains no sign of β-carotene, and as two of these molecules are likely to be present (one on each side?), scope for cyclic electron transfer pathways still exists.

Impacts and perspectives

The present structure represents an important milestone in research in this area. It resolves doubts, raises confidence in existing spectroscopic studies, verifies models, eliminates some of the more

fanciful ideas and provides some totally new insights that then raise new questions. Research in this area is moving into a new era in which the focus will change from understanding the large-scale structural features to conducting known-structure based studies of the enzyme mechanism. For this transition to be completed, a structure with details of the Mn ligands and geometry, and of the special environment of the redox active Tyr_Z, is needed and, given the present breakthrough, the chances are that this will not be too long in coming.

The groups from Berlin that provided the PSII crystal structure deserve congratulations, especially because they also solved the monumental structure of PSI. That is an impressive two out of two, in a sense 'the full set'. It seems that for H.T. Witt, the man who initiated the project and who has worked on photosynthesis since the 1950s, the PSI structure was just practice for the altogether trickier and more grail-like structure of PSII. So, for now, we will digest and appreciate the current structure but bring on the next; many of us are on the edges of our seats.

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