Photosynthesis Research (2022) 152:97–105
https://doi.org/10.1007/s11120-022-00910-1

REVIEW

Photosystem 2 and the oxygen evolving complex: a brief overview

Charles F. Yocum[1]

Received: 24 November 2021 / Accepted: 28 February 2022 / Published online: 16 March 2022

Abstract
These special issues of photosynthesis research present papers documenting progress in revealing the many aspects of photosystem 2, a unique, one-of-a-kind complex system that can reduce a plastoquinone to a plastoquinol on every second flash of light and oxidize 2 H_2O to an O_2 on every fourth flash. This overview is a brief personal assessment of the progress observed by the author over a four-decade research career, including a discussion of some remaining unsolved issues. It will come as no surprise to readers that there are remaining questions given the complexity of PS2, and the efforts that have been needed so far to uncover its secrets. In fact, most readers will have their own lists of outstanding questions.

Keywords Photosystem 2 · Oxygen evolving complex · Polypeptides · Manganese · Calcium · Chloride

Introduction

Evolution of PS2 is currently believed to have begun in sea water about 3 billion years ago (Fournier et al. 2021). This has been sufficient time for evolution to produce and refine a redox enzyme that is a model of (almost) foolproof simplicity. If this were not so, life on earth would likely be a good deal more anaerobic than it is today. This overview encompasses about four decades dating from the appearance of active, highly resolved PS2 preparations from spinach; what we know now is overwhelming in comparison to the information that was available at the time when PS2 was named the "Inner Sanctum" of photosynthesis by Kok and Cheniae (1966). Advances achieved with thylakoid membranes, green algae and cyanobacteria should not be forgotten. The period 4 oscillations in oxygen yield and the S-state model (Joliot et al. 1969; Kok et al. 1969), and the demonstration of ATP synthesis in darkness, driven by an acid–base transition without electron transfer (Jagendorf and Uribe 1966), are among the important discoveries in research on molecular bioenergetics, two of the many significant discoveries made using thylakoid membranes or intact algae. Now PS2 and its OEC (O_2 evolving complex) are center stage, as documented by many reviews (for example Barber 2016; Cox et al. 2020; Shen 2015; Vinyard and Brudvig 2017). A comprehensive review by Junge (2019) adds an important historical component to an assessment of scientific progress. The author of this overview will comment on discoveries about PS2 made across a number of years, but no attempt will be made to match the depth of coverage afforded by Prof. Junge's more extensive review.

Probing the inner sanctum

The author's generation of PS2 researchers were confronted by a series of experimental results that were, in some instances, puzzling. For example, in the early 1980's, the simplicity with which pure, active PS2 preparations could be obtained (Berthold et al. 1981; Kuwabara and Murata 1982) went against the opinions of a number of senior investigators who believed this couldn't be accomplished. (A historical note: the BBY and K&M notations for these preparations *were not* an invention of the authors of these papers on PS2 isolation (see Dunahay et al. 1984)). Another very intriguing result at the same time was the discovery that the lumenal side of inside-out thylakoid vesicles, thought to be a hydrophobic domain, contained H_2O soluble polypeptides associated with PS2 activity (Åkerlund et al. 1982). This observation led to identification of the important extrinsic PS2 subunits now known as PsbO, P and Q in eukaryotes and PsbO, U, and V (a cytochrome, C550) in cyanobacteria

✉ Charles F. Yocum
cyocum@umich.edu

[1] Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

(reviewed by Bricker et al. 2012). Using purified PS2 from spinach, extraction of PsbP and Q inhibited O_2 evolution and created another puzzle. A general rule in biochemistry is that if extraction of a protein inhibits an activity, reconstitution of that protein should restore activity. No such luck in the case of PS2: repeated reconstitutions of these proteins were ineffective in restoring O_2 evolution, but addition of Ca^{2+}, a redox inert metal that would not have been a first choice at the time, was effective (Ghanotakis et al. 1984a). This was an odd result to say the least, and finding its cause led to the discovery that the PsbP and PsbQ extrinsic proteins form a barrier, along with PsbO, to prevent Ca^{2+} loss (Ghanotakis et al. 1984b) and also to protect the OEC from reductant induced Mn^{2+} formation and loss from the OEC (Ghanotakis et al. 1984c). Now it is well-established that PsbP has the key role in Ca^{2+} retention (Ifuku et al. 2005).

The early 1980's also brought the discovery, first in thylakoid membranes, that the S_2 state produced a multiline EPR signal (Dismukes and Siderer 1981), detected at liquid helium temperatures, which is now found in other S-states as well (Haddy 2007). Characterization of effects of Cl^- on reversible amine inhibition of steady state O_2 evolution activity revealed the presence two sites in the OEC where NH_3 inhibits O_2 evolution; inhibition at one of the sites is insensitive to interference by Cl^-, whereas at the second site both NH_3 and Tris (tris-(hydroxymethyl)-aminomethane) inhibitions are reversed by Cl^- (Sandusky and Yocum 1984). Another unexpected result was that a tyrosine radical ($Y_D^{\cdot}$) is the source of the EPR detectable Signal II species (Barry and Babcock 1987); site-directed mutagenesis identified two redox active residues as Y161(Y_Z) of PsbA (D1), and Y160 (Y_D) of PsbD (D2) (Debus et al. 1988; Vermaas et al. 1988). These discoveries joined many others in expanding research on PS2 in a number of areas. Electron paramagnetic resonance spectroscopy (EPR) was used to characterize a number of PS2 signals (Miller and Brudvig 1991). Optical absorbance changes from Mn oxidation state advancements were reported (Dekker et al.1984), and X-ray absorption spectroscopy became an important technique for probing Mn oxidation states and Mn–Mn, Mn-Ca^{2+} and Mn-ligand distances (Sauer et al. 2005). The identification of Ca^{2+} as a cofactor in the oxygen evolving reaction and a characterization of the Cl^- requirement for activity led to research to establish the stoichiometries of these ions in PS2. The results indicated that a single Ca^{2+} atom (Ádelroth et al. 1995) and a Cl^- atom with high and low affinity binding behavior (Lindberg et al. 1993, 1996) are present in active PS2 preparations. Large scale projects to provide DNA sequences of many species, but of cyanobacteria in particular, provided the background for application of site directed mutagenesis to identify other amino acids in addition to the redox-active tyrosines that are involved in PS2 reactions (Debus 2008). The techniques of molecular biology were also important factors in elucidating the steps in the mechanism for repair of reaction centers damaged by photoinhibition (Järvi et al. 2015) and for defining, in eukaryotes, which genes reside in the chloroplast, and which are found in the nucleus, such as genes for the extrinsic subunits and light harvesting antenna subunits, whose products are imported into chloroplasts (Theg 2018).

Additional research on components and reactions involved in O_2 evolution was facilitated by a variety of biophysical techniques. X-ray absorption spectroscopy was applied to characterizations of Mn oxidation states in PS2, but the requirement for a variety of model Mn compounds to fit to the experimentally observed edge spectra made this a demanding research problem. The "high oxidation state" model for the S-states shown below has gained considerable experimental support (see, for example, Cheah et al. 2020).

S_O, [3Mn(III)/Mn(IV)]: S_1, [2Mn(III)/2Mn(IV)]: S_2, [Mn(III)/3Mn(IV)]: S_3, [4Mn(IV)]: and S_4 [?].

Measurements of H^+ release during S-state advancement revealed a stoichiometry of 1,0,1,2 (Junge et al. 2002) for the Mn oxidation state transitions.

Additional X-ray absorption experiments (EXAFS (Extended X-ray Absorption Fine Structure)) provided information on the positioning of the Mn atoms with respect to one another and to putative ligands, using comparisons of the experimental data from PS2 samples to data from Mn model compounds. Generally agreed distances were: 1.8Å (Mn–O); 2.7Å (Mn – Mn); 3.3–3.4 Å (Mn–Mn, Mn-Ca) (Sauer et al. 2005). Results of ENDOR (Electron Nuclear Double Resonance) spectroscopy on the S_2 state by Peloquin et al. (2000) produced a new proposal for the structure of the Mn cluster in PS2, a trimer of Mn atoms and a 4th atom, termed a "dangler", separated from the trimer. The combined results from biochemical and biophysical characterizations of PS2 at this point defined the stoichiometries of components of the OEC and provided important information on their possible arrangements, and Mn oxidation states, while data from site-directed mutagenesis experiments cited above, in cyanobacteria, identified amino acid residues of D1 and D2 that were involved in formation of a functional active site of water oxidation.

Viewing the inner sanctum

The first attempts to crystallize PS2, from spinach, were successful, but the crystals were not suitable for extended X-ray crystallographic analysis (Fotinou et al. 1993). A major breakthrough in PS2 research started in the new millennium with reports that PS2 isolated from thermophilic cyanobacteria (*Thermosynochococcus elongatus* and *T. vulcanus*) had been crystallized, and their structures were being determined (Shen and Kamiya 2000; Zouni et al. 2001). Viewed from a

distance, these structures fit existing topological data. The OEC was located towards the lumenal side of the structure, the plastoquinones on the opposite (stromal) side and the 6 Chl*a* and 2 Pheo*a*, bound to D1 and D2 were located as one would have predicted from the structure of the bacterial reaction center. A major advance in understanding reactions of the OEC was the report by Ferreira et al. (2004) of a more detailed structure of the metal cluster, one in which the model of Peloquin et al. (2000) was confirmed and expanded to 3 dimensions, with the "dangler" Mn atom appended to a distorted cube in which the Ca^{2+} atom replaced a Mn in the 4th corner of the cube near Y_Z. A significant problem has been overcome since then. Reduction of the Mn oxidation states by radiation exposure has been avoided with the "diffract and destroy" technique, using the XFEL (X-ray Free Electron Laser) technique (Suga et al. 2014), and at least 2 groups using the XFEL technique are in the process of elucidating the structures of the individual S-states (Young et al. 2016; Suga et al. 2017; Suga et al. 2019; Ibrahim et al. 2020).

The new millennium has also seen an increased contribution to research on PS2 structure and function from the application of computational methods (Vinyard and Brudvig 2017). New models for substrate H_2O binding have appeared (Wang et al. 2017), and with all of the data now available or emerging, it is reasonable to expect that key steps in the mechanism of H_2O oxidation are, and will be, based on data from crystals of PS2, and from experiments that utilize crystal structures for their design. The activities of thermophilic PS2 preparations in the crystalline and soluble forms (Ananyev et al. 2019; Ibrahim, et al. 2020), and structural correlations between metal–metal and metal–ligand distances from the crystals and from characterizations by X-ray absorption spectroscopy provide confidence that the current crystal structures present an accurate representation of the native structure and organization of the polypeptides of cyanobacterial PS2, and of the inorganic ion constituents of the OEC.

Remaining questions

If PS2 crystals are revealing a great deal more about one of the most important biological reactions on earth, should there be a concern over what, if anything, remains to be discovered? What follows offers some observations about the properties of PS2, clearly exposing the author's biases, that might profit from additional attention. The first of these is whether it will one day be possible to obtain high quality crystals from a eukaryotic source like spinach, where the majority of biochemical and biophysical characterizations have been carried out. The successful crystallization of eukaryotic PSI (Amunts and Nelson 2010) and the early results of Fotinou et al. (1993) suggest that eukaryotic PS2 crystals might one day be available. It would also be interesting to explore the origins of differences between thermophilic and mesophilic PS2 in cyanobacteria. The *T. volcanus* PS2 preparation requires much higher concentrations of NH_2OH (up to 50 mM) for Mn^{2+} release (Zhang et al. 2017) than does the mesophile *Synechocystis* sp PCC 6830 (1 mM; Anton P. Avramov et al. 2020). It seems unlikely that NH_2OH redox chemistry would be species dependent, so there may be significant differences in channels that lead to the OEC between thermophiles and mesophiles. What can be learned about structural properties of PS2 in these species might also account for the differences in access of a small reductant like NH_2OH to the OEC. CryoEM offers the prospect of an alternate technology that can be used to address this question, as it has in the cases of photoactivation of the OEC (Gisriel et al. 2020) and the structure of channels and other features of PS2 from plants, cyanobacteria, and green algae (Sakashita et al. 2017; Gisriel et al. 2022; Nathan Nelson Personal Communication).

There are a number of other properties of the OEC that might deserve additional experimental scrutiny. Starting with the S_0 state, there's a difficulty on account of its instability; S_0 is slowly oxidized to S_1 in a reaction catalyzed by $Y_D\cdot$ (see Rutherford et al. 2004). Therefore, obtaining highly resolved structural information on a homogeneous S_0 sample would be difficult. This is the OEC oxidation state where initial binding of substrate H_2O may occur, and any proposed mechanism for the oxidation-induced reduction of S_4 should account for the resulting S_0 structure. The core inorganic structure of S_0 does not appear to differ drastically from that of the other S-states (Ibrahim et al. 2020); Pantazis (2018) presents an in-depth analysis of possible arrangements of ions in S_0, and these observations might be a starting place to seek a better understanding of this S-state.

The S_1 state has received considerable attention and is certain not to be ignored in the future. The unique properties of S_2, including the appearance of the EPR multiline signal, has caused researchers to sit up and take notice. Early on, Frasch and Cheniae (1980), using thylakoid membranes, showed that irreversible inhibition of O_2 evolution by Tris buffer under their conditions was maximum in S_2, and Velthuis (1975) had shown that NH_3 blocked electron transfer beyond S_2. Addition of NH_3 along with Tris blocked irreversible Tris inhibition of PS2 activity (Frasch and Cheniae 1980). Setting aside other results on amine binding behavior of the OEC for the time being, what new property of S_2, not observed in S_1, is responsible for increased Tris sensitivity? A subtle change in structure (a channel?) that allows a large amine to gain access to a site of inhibition, the presence of another Mn(IV), or another factor, not discovered, that increases inhibition kinetics under the experimental conditions (0.8 M Tris in the reaction mixture)?

The mechanism of Ca^{2+} extraction from the OEC is also an interesting question related to S_2. Miyao and Murata (1986) showed that Ca^{2+} depletion of the OEC is accelerated by illumination in concert with release of extrinsic polypeptides. Subsequently, Boussac and Rutherford (1988) identified S_2 and S_3 as susceptible states for Ca^{2+} loss from PS2 samples exposed to high salt (1.2 M NaCl) to remove PsbP and PsbQ. The result of Ca^{2+} removal under illumination creates a modified multiline EPR signal and an unusually stable S_2 state that decays upon addition of Ca^{2+} (Boussac et al. 1989). Illuminated Ca^{2+}-depleted samples also produce an EPR signal that was later shown to originate from Y_Z (Gilchrist et al. 1995). These results show that loss of Ca^{2+} from S_2 interferes with electron transfer from the Mn cluster to Y_Z and raise a number of questions. What is the mechanism of the accelerated release of Ca^{2+} from its site in the distorted Mn_3Ca cube? Is it possible that $Ca^{2+}$is displaced by another monovalent cation from the solvent during extraction? Potassium and Cs^+ are effective competitors for the Ca^{2+} site in PS2, but Na^+ is not (Ono et al. 2001). High concentrations of NaCl (1.2 M), used for polypeptide extraction, would be difficult to test for competition with Ca^{2+} binding. Is it possible that Ca^{2+} release in S_2 and S_3 is due to electrostatic repulsion arising from formations of additional positive charges (Mn(IV) in the Mn_3Ca cube? The last, and most complex issue is the question of why Ca^{2+} release blocks electron transfer from S_2 to Y_Z; the answer could be informative in achieving a complete understanding of the mechanism of O_2 evolution.

It's also curious that the S_2 state, after Ca^{2+} extraction, exhibits a robust stability (Boussac et al. 1989). Positive charges on the metals in dark-stable S_1 state and in a putative Ca^{2+}-depleted, monovalent cation substituted S_2 state might, in theory, be the same (+16) in both states. A major structural reorganization of the metal cluster after Ca^{2+} removal seems an unlikely cause of stability, given the rapid recovery of activity upon Ca^{2+} reconstitution in depleted samples during assays of steady state activity (Homann 1988b). Finally, Ca^{2+} extraction and replacement of the vacant site with Sr^{2+} slows the steady state rate of O_2 evolution, produces changes to the S_2 multiline signal, and slows the decay of Y_Z•on the $S_3 \rightarrow S_O$ transition (Boussac et al. 2004). Does the crystal structure of Sr^{2+} substituted PS2 (Koua et al. 2013) provide any clues to this observation, which may be linked to the difference in Lewis acidities of Ca^{2+} and Sr^{2+} (Vrettos et al. 2001)? Finally, the role of Ca^{2+} in formation of the Mn_4Ca cluster is still being examined; Anton P. Avramov et al. (2020) have characterized the Ca^{2+}- Mn competition in photoactivation and present a model for how the stability of high affinity Mn-binding sites depend on the presence of Ca^{2+}.

The S_2 state is probably as good a place as any to consider the Cl^- questions(s) and amine inhibition of the OEC. Chloride is not required for formation of S_2 in terms of Mn oxidation, but it is required to observe the S_2 multiline signal (Ono et al. 1986). Chloride is required for advancement beyond S_2 to S_3, and for the $S_3 \rightarrow S_0$ transition (Wincencjusz et al. 1997). The majority of biochemical data on Cl^- function has been generated using thylakoids and isolated PS2 from spinach and other plants, while structures and the effects of site-directed mutants on Cl^- function come from cyanobacteria. Chloride is an essential cofactor for the O_2 evolving reaction in eukaryotic PS2; its K_M, from reconstitution experiments, is 0.9 mM in thylakoids (Kelly and Izawa 1978), and lower in PS2 preparations depending on assay conditions, such as the presence or absence of extrinsic polypeptides (Homann 1988a) or the assay pH (Baranov and Haddy 2017). Linear double reciprocal plots are characteristic of a single site of activation of O_2 evolution by Cl^-. Unfortunately, buffer and sucrose solutions contain residual Cl^- contaminations, which are likely to be utilized by PS 2 (see, for example, Kelley and Izawa (1978) and Ishida et al. (2008)). In cyanobacterial and plant PS2, evidence for Cl^- involvement in the O_2 evolving reaction also comes from detection of a slowing of the $S_3 \rightarrow S_0$ decay by biosynthetic replacement of Cl^- with Br^- in *T. elongatus* (Ishida et al. 2008) or with Br^-, I^-, or NO_3^{2-} in spinach PS2 by incubation reconstitution (Wincencjusz et al. 1999). In PS2 crystals, Cl^- is detected at two sites, Cl1 near a narrow channel leading to the dangler Mn4 in the OEC, and Cl2, in a wider channel (Kawakami et al. 2009; Ibrahim et al. 2020). The distances from the metal cluster (6–7 Å) place Cl^- in the outer shell around the cluster. In the case of Cl1, the PS2 structure has been used to guide directed mutagenesis experiments using *Synechocystis* 6803. The mutant D2K312A negatively affects O_2 evolution activity, appears to abolish a large fraction of Cl^- dependent activity, but leaves behind activity at low rates and a multiline signal that is weaker than the wildtype signal (Pokhrel et al. 2013). Is this signal a result of retention of low levels of Cl^-? Otherwise, the hypothesis that Cl1is a structural component in PS2, organizing a hydrogen bonding water network leading away from the Mn/Ca cluster of the OEC (Kawakami et al. 2009; Rivalta et al. 2011)) is consistent with the results obtained with D2K312A.

The use of inhibitors is often one sign that the biochemist using them is unable to vary the substrate concentration, as in the case of PS2 with 55.4 M H_2O. Inhibition of O_2 evolution activity by primary amines involves Cl^- (Sandusky and Yocum 1984, 1986). The Cl^- insensitive site binds NH_3, and is a Mn atom (Britt et al. 1989) that has been identified by Oyala et al. (2015) as Mn4 in the OEC, near Cl1. Ammonia binding displaces an H_2O from the dangler (Marchiori et al 2018). Manganese binding and displacement of a possible substrate H_2O would account for NH_3 inhibition of O_2 evolution and the identity of S_2 as the NH_3-sensitive state

(Velthuis 1975). The problem with respect to Cl^- is that all amines, from NH_3 and CH_3NH_2 through Tris to t-butylamine, also inhibit steady state activity (Ghanotakis et al 1983); the inhibition constant (I_{50}) is proportional to the amine pKa, suggesting a metal (Lewis acid) as the binding site of the free bases of these amines (Angelici, 1973). Characterization of this inhibition also showed that all amines compete with Cl^- for the second binding site, and again, a linear relationship between inhibition constants and amine pKa values was obtained (Sandusky and Yocum 1986).

What is the identity of the site where all amines tested compete with Cl^- and inhibit OEC activity under illumination in the steady state? Is the assumption valid that this site is a Lewis acid? There doesn't appear to be any EPR evidence for direct binding of larger amines to the Mn cluster, and the steady-state inhibition experiments would indicate that Cl^- and amines must exchange rapidly with the second binding site. There is another issue. At room temperature NH_3 binding to illuminated thylakoid samples produced the Y_Z EPR signal (Yocum and Babcock 1981), whose amplitude increased with increasing microwave power up to 200 mW. This was interpreted to arise from a spin–spin interaction between the organic radical and Mn in the OEC. Additional experiments (Ghanotakis et al. 1983) showed that larger amines that do not irreversibly inhibit activity also elicited the Y_Z signal under room temperature illumination, but the spin–spin interaction was altered; signal saturation occurred at ~ 25 mW for both CH_3NH_2 and 2-ethyl-2 amino propanediol. Effects of Cl^- on amine binding to the OEC were unknown at the time these experiments were conducted and additional experiments were not pursued.

The data on amine-Cl^- interactions in eukaryotic PS2 are difficult to rationalize with the crystal structures. Ammonia binding to the dangler Mn4 has been discussed above. The Cl2 site is distant from the metal cluster and Y_Z, but the environment around Y_Z includes space that might accommodate both NH_3 and larger amines to affect spin–spin interactions between the Mn cluster and the tyrosine radical. This would imply that at least under turnover conditions, the Lewis acid for which amines and Cl^- compete could be Ca^{2+}, which is already proposed to be involved in substrate H_2O binding to the OEC (Vrettos et al. 2001); the substrate analog CH_3OH is reported to bind to Ca^{2+} in S_2 as well (Oyala et al. 2014). In the case of Cl^- and amines, these species would have to be involved as inner shell ligands, but not to Mn. This is certain to be an unpopular suggestion because none of the available crystal structures support Cl^- binding to Ca^{2+}. However, EPR and ESEEM (Electron Spin Echo Envelope Modulation) results with acetate, which also competes with Cl^-, are interpreted to show binding of the former ligand near Y_Z (Szalai et al. 1998; Klemens et al. 2002). If, as these data might suggest, Cl^- binds to Ca^{2+}, what is its role in the mechanism of H_2O oxidation? Structural, as part of a substrate H_2O binding site on Ca^{2+}? Displacement of a non-substrate H_2O? Catalytic, by affecting the Lewis acidity of Ca^{2+}? The rate of O_2 evolution activity is sensitive to the Lewis base present: $Cl^- > Br^- > I^- \sim NO_3^{2-}$ (Wincencjusz et al. 1999).

Determination of the mechanism of the $S_3 \rightarrow S_0$ reaction will reveal how PS2 oxidizes $2H_2O$ to O_2. A question here is the identity of the electron donors to $S_3Y_Z^{\bullet}$; Cox et al (2020) present a thorough discussion of this issue. In any case the reaction involves an oxidation-induced reduction of both Y_Z and 3 Mn(IV). In the meantime, Pantazis's review (2018) provides an in-depth analysis of various remaining issues regarding H_2O oxidation, and the crystal structures identify the plentiful supply of potential substrates.

A final OEC issue concerns one of the many site-directed mutations in the mesophilic cyanobacterium *Synechocystis* 6803. These mutants have proven crucial in identification of essential residues involved in Mn and/or Ca^{2+} ligation, for example, and have been confirmed by crystal structures. One residue, D1E181 is clearly the donor of oxo ligands, one each to Mn and to Ca^{2+} in the OEC. An unusual result was the discovery that substitution of a positively charged lysine (E189K) produced an active OEC. This result has been discussed in detail by Kim and Debus (2020), who present evidence from FTIR spectroscopy supporting normal functioning of H_2O molecules in the mutant OEC. An alternate, less likely explanation would be that another residue near E189 has substituted for E189K. This has been observed in a H_2O-soluble metalloenzyme with highly conserved Fe-ligating cys residues. A conserved cysteine residue (C20A) was mutagenized in a 4Fe/4S cluster in Ferredoxin I from *Azotobacter vinelandii* (Martin et al. 1991). The mutation retained its 4Fe/4S cluster and X-ray crystallography revealed that a nearby Cys residue (C24) had replaced the ligand function of the mutagenized residue. This seems to be a rare occurrence in site-directed mutagenesis experiments and is not likely to explain E189K's properties.

Moving away from the OEC to the protein structure of PS2, the effects of removing the extrinsic proteins on PS2 activity raise some questions. In the case of the PsbP and Q subunits, extraction is remediated by additions of Ca^{2+} and Cl^-. The main problem here, and it may be a minor one, is that the activity recovered by this approach does not completely restore the original activity; elevated concentrations of inorganic ions do not compensate completely for activity loss caused by polypeptide extraction (Miyao and Murata 1985). This could be due to a failure to overcome the release of these cofactors in the light (such as expulsion of Ca^{2+}), or to some subtle structural changes induced by the treatments used to remove the polypeptides. In the case of PsbO, it was once known as Manganese Stabilizing Protein (Burnap and Sherman 1991) because its removal caused a slow loss of Mn(II) and activity. Miyao and Murata (1984)

showed that this denaturation event could be attenuated by incubation of extracted samples with Cl^-, and it has further been shown that the residual activity after PsbO extraction requires high Cl^- concentrations (Miyao and Murata 1985). Is this due to structural changes to channels, caused by PsbO extraction? The effect appears to be specific to Cl^-. It has also been shown that PsbO is tightly bound to PS2 (Leuschner and Bricker (1996)) and extraction of this subunit from PS2 requires exposure to urea/NaCl or 1 M $CaCl_2$, neither of which can be classified as gentle treatments; extraction procedures might contribute to the properties of PsbO-depleted PS2. The majority of information on the interactions between extrinsic subunits and PS2 come from experiments with eukaryotic PS2. It is difficult to predict what differences might emerge from research on the comparative properties of the extrinsic proteins found in cyanobacteria (Gisriel, et al. 2022). Lastly, a very interesting observation was reported by Ettinger and Theg (1991), who showed that thylakoid membranes from pea and spinach contained unassembled PsbO, P and Q subunits. One cause of this is proposed to be that these proteins are waiting to come to the rescue of photodamaged PS2 reaction centers. Could overexpression of these subunits in vivo provide additional stability to PS2 when plants are under stressful conditions that might destabilize binding of the extrinsic subunits?

Coming finally to the other function of PS2, and to thylakoid membranes as well, it is still unclear how electrons get from Q_BH_2 to the cytochrome b_6f complex, which reduces plastocyanin, the mobile carrier that reduces $P700^+$ (Höhner et al. 2020). This question has been raised by Bill Cramer in annual conversations (now by Zoom, unfortunately) with the author, who has no answers. What is the nature of interactions between the b_6f complex and the reducing side of PS2 that promote rapid transfers of reducing equivalents to the cytochrome complex? Special channels? Diffusion? This is certainly not the only issue left to explore, and the current cohort of PS2 investigators, including the authors contributing to these special issues, with new ideas and techniques can provide substantial additions to any list of needed experiments, along with new answers. That this is the case is a positive sign of the health of photosynthesis research overall. The challenge now is to get back together in person at meetings and international congresses. In the meantime, the author apologizes for losing track of all of the important questions that he's overlooked in this overview.

Acknowledgements Thanks are due to many people who aided the author's research efforts with advice and critiques: my collaborators, Jerry Babcock, Bob Sharp, Jim Penner-Hahn, and Hans van Gorkom, the B's (Bridgette Barry, Terry Bricker, David Britt, Gary Brudvig, and Rob Burnap), the rest of the alphabet (George Cheniae, Bill Cramer, Rick Debus, Nathan Nelson, and Bill Rutherford) and last, but certainly not least, my graduate students and post-docs, all of whose names appear on my publications. At the start of my career, the National Institutes of Health returned my first research proposal, unreviewed, with a note saying that biochemical research on O2 production by photosynthesis was not relevant to human health. I am deeply grateful to the National Science Foundation and the United States Department of Agriculture for their more enlightened outlook on my requests for funding.

References

Ádelroth P, Lindberg K, Andréasson L-E (1995) Studies of Ca^{2+} binding in spinach photosystem II using $^{45}Ca^{2+}$. Biochem 34:9021–9027. https://doi.org/10.1021/bi00028a010

Åkerlund H-E, Jansson C, Andersson B (1982) Reconstitution of photosynthetic water splitting in inside-out thylakoid vesicles and identification of a participating polypeptide. Biochim Biophys Acta 681:1–10. https://doi.org/10.1016/0005-2728(82)90271-7

Avramov AP, Hwang HG, Burnap RL (2020) The role of Ca^{2+} and protein scaffolding in the formation of nature's water oxidizing complex. Proc Natl Acad Sci USA 117:28036–28045. https://doi.org/10.1073/pnas.2011315117

Amunts A, Toporik H, Borovikova A, Nelson N (2010) Structure determination and improved model of plant photosystem I. J Biol Chem 285:3478–3486. https://doi.org/10.1074/jbc.M109.072645

Ananyev G, Roy-Chowdhury S, Gates C, Fromm P, Dismukes GC (2019) The catalytic cycle of water oxidation in crystallized photosystem II complexes: performance and requirements for formation of intermediates. ACS Catal 9:1396–1407. https://doi.org/10.1021/acscatal.8b04513

Angelici RJ (1973) Stability of coordination compounds. In: Eichorn K (ed) Inorganic Biochemistry, vol 1. Elsevier, Amsterdam, pp 63–101

Baranov S, Haddy A (2017) An enzyme kinetics study of the pH dependence of chloride activation of oxygen evolution in photosystem II. Photosynth Res 131:317–332. https://doi.org/10.1007/s11120-016-0325-z

Barber J (2016) Photosystem II: the water splitting enzyme of photosynthesis and the origin of oxygen in our atmosphere. Quart Rev Biophys 49:1–21. https://doi.org/10.1017/S0033583516000093

Barry BA, Babcock GT (1987) Tyrosine radicals are involved in the photosynthetic oxygen evolving system. Proc Natl Acad Sci USA 84:7099–7103. https://doi.org/10.1073/pnas.84.20.7099

Berthold DA, Babcock GT, Yocum CF (1981) A highly resolved oxygen-evolving photosystem II preparation from spinach thylakoid membranes: EPR and electron transport properties. FEBS Lett 134:231–234. https://doi.org/10.1016/0014-5793(81)80608-4

Boussac A, Rutherford AW (1988) Ca^{2+} binding to the oxygen evolving enzyme varies with the redox state of the Mn cluster. FEBS Lett 236:432–436. https://doi.org/10.1016/0014-5793(88)80071-1

Boussac A, Zimmermann J-L, Rutherford AW (1989) EPR signals from modified charge accumulation states of the oxygen-evolving enzyme in calcium-deficient photosystem II. Biochem 8:8984–8989. https://doi.org/10.1021/bi00449a005

Boussac A, Rappaport F, Carrier P et al (2004) Biosynthetic Ca^{2+}/Sr^{2+} exchange in the photosystem II oxygen-evolving enzyme of *Thermosynechococcus elongatus*. J Biol Chem 22:22809–22819. https://doi.org/10.1074/jbc.M401677200

Bricker TM, Roose JL, Fagerlund RD, Frankel LK, Eaton-Rye J (2012) The extrinsic proteins of Photosystem II. Biochim Biophys Acta 1817:121–142. https://doi.org/10.1016/j.bbabio.2011.07.006

Britt RD, Zimmermann J-L, Sauer K, Klein MP (1989) Ammonia binds to the catalytic manganese of the oxygen-evolving complex of photosystem II. Evidence by electron spin-echo envelope modulation spectroscopy. J Am Chem Soc 111:3522–3532. https://doi.org/10.1021/ja00192a006

Burnap RL, Sherman LA (1991) Deletion mutagenesis in *Synechocystis* sp. PCC6803 indicates that the Mn-stabilizing protein of photosystem II is not essential for O_2 evolution. Biochem 30:440–446. https://doi.org/10.1021/bi00216a020

Cheaha MH, Zhang M, Shevela D, Mamedov F, Zouni A, Messinger J (2020) Assessment of the manganese cluster's oxidation state via photoactivation of photosystem II microcrystals. Proc Natl Acad Sci USA 117:141–145. https://doi.org/10.1073/pnas.1915879117

Clemens KL, Force DA, Britt RD (2002) Acetate binding at the photosystem II oxygen evolving complex: an S_2-state multiline signal ESEEM study. J Am Chem Soc 124:10921–10933. https://doi.org/10.1021/ja012036c

Cox N, Pantazis DA, Lubitz W (2020) Current understanding of the mechanism of water oxidation in photosystem II and its relation to XFEL. Ann Rev Biochem 89:795–820. https://doi.org/10.1146/annurev-biochem-011520-104801

Debus RJ (2008) Protein ligation of the photosynthetic oxygen-evolving center. Coord Chem Rev 252:244–258. https://doi.org/10.1016/j.ccr.2007.09.022

Debus RJ, Barry BA, Babcock GT, McIntosh L (1988) Site-directed mutagenesis identifies a tyrosine radical involved in the photosynthetic oxygen-evolving system. Proc Natl Acad Sci USA 85:427–430. https://doi.org/10.1073/pnas.85.2.427

Dekker JP, Van Gorkom HJ, Wensink J, Ouwehand L (1984) Absorption difference spectra of the successive redox states of the oxygen evolving apparatus of photosynthesis. Biochim Biophys Acta 767:1–9. https://doi.org/10.1016/0005-2728(84)90073-2

Dismukes GC, Siderer Y (1981) Intermediates of a polynuclear manganese center involved in photosynthetic oxidation of water. Proc Natl Acad Sci USA 78:274–278. https://doi.org/10.1073/pnas.78.1.274

Dunahay TG, Staehelin LA, Seibert M, Ogilvie PD, Berg SP (1984) Structural, biochemical, and biophysical characterization of four oxygen-evolving photosystem II preparations from spinach. Biochim Biophys Acta 764:179–193. https://doi.org/10.1016/0005-2728(84)90027-6

Ettinger WF, Theg SM (1991) Physiologically active chloroplasts contain pools of unassembled extrinsic proteins of the photosynthetic oxygen evolving enzyme complex in the thylakoid lumen. J Cell Biol 115:321–328. https://doi.org/10.1083/jcb.115.2.321

Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303:1831–1838. https://doi.org/10.1126/science.1093087

Fotinou C, Kokkinidis M, Fritzsch G et al (1993) Characterization of a photosystem II core and its three-dimensional crystals. Photosynth Res 37:41–48. https://doi.org/10.1007/BF02185437

Fournier GP, Moore KR, Rangel LT, Payette JG, Momper L, Bosak T (2021) The Archean origin of oxygenic photosynthesis and extant cyanobacterial lineages. Proc R Soc B 288:1–10. https://doi.org/10.1098/rspb.2021.0675

Frasch WD, Cheniae GM (1980) Flash inactivation of oxygen evolution. Identification of S_2 as the target of inactivation by tris. Plant Physiol 65:735–745. https://doi.org/10.1104/pp.65.4.735

Ghanotakis DF, O'Malley PJ, Babcock GT, Yocum CF (1983) Structure and inhibition of components of the oxidizing side of photosystem II. In: Inoue Y et al (eds) The oxygen evolving system of photosynthesis. Academic Press, Tokyo, pp 87–98

Ghanotakis DF, Babcock GT, Yocum CF (1984a) Calcium reconstitutes high rates of oxygen evolution in polypeptide-depleted photosystem II preparations. FEBS Lett 167:127–130. https://doi.org/10.1016/0014-5793(84)80846-7

Ghanotakis DF, Topper JN, Babcock GT, Yocum CF (1984b) Water-soluble 17and 23 kDa polypeptides restore oxygen evolution activity by creating a high- affinity binding sites for Ca^{2+} on the oxidizing side of photosystem II. FEBS Lett 170:169–173. https://doi.org/10.1016/0014-5793(84)81393-9

Ghanotakis DF, Topper JN, Yocum CF (1984c) Structural organization of the oxidizing side of photosystem II: Exogenous reductants reduce and destroy the Mn-complex of PSII membranes depleted of the 17 and 23 kDa polypeptides. Biochim Biophys Acta 767:524–531. https://doi.org/10.1016/0005-2728(84)90051-3

Gilchrist ML, Ball JA, Randall DW, Britt RD (1995) Proximity of the manganese cluster of photosystem II to the redox-active tyrosine Y_Z. Proc Natl Acad Sci USA 92:9545–9549. https://doi.org/10.1073/pnas.92.21.9545

Gisriel CJ, Zhou K, Hao-Li Huang H-L, Debus RJ, Xiong Y, Brudvig GW (2020) Cryo-EM structure of monomeric photosystem II from *Synechocystis* sp. PCC 6803 lacking the water-oxidation complex. Joule 4:2131–2148. https://doi.org/10.1016/j.joule.2020.07.016

Gisriel CJ, Wang J, Liub J, Flesher JA, Reissa KM et al (2022) High-resolution cryo-electron microscopy structure of photosystem II from the mesophilic cyanobacterium, Synechocystis sp PCC 6803. Proc Natl Acad Sci USA 119:e2116765118. https://doi.org/10.1073/pnas.2116765118

Haddy A (2007) EPR spectroscopy of the manganese cluster of photosystem II. Photosynth Res 92:357–368. https://doi.org/10.1007/s11120-007-9194-9

Höhner R, Pribil M, Herbstová M et al (2020) Plastocyanin is the long-range electron carrier between photosystem II and photosystem I in plants. Proc Natl Acad Sci 117:15354–15362. https://doi.org/10.1073/pnas.2005832117

Homann PH (1988a) Chloride relations of photosystem II membrane preparations depleted of, and resupplied with, their 17 and 23 kDa extrinsic polypeptides. Photosyn Res 15:205–220. https://doi.org/10.1007/BF00047353

Homann PH (1988b) The chloride and calcium requirement of photosynthetic water oxidation: Effects of pH. Biochim Biophys Acta 934:1–13. https://doi.org/10.1016/0005-2728(88)90113-2

Ibrahim M et al (2020) Untangling the sequence of events during the S2 → S3 transition in photosystem II and implications for the water oxidation mechanism. Proc Natl Acad Sci USA 117:12674–12675. https://doi.org/10.1073/pnas.2000529117

Ifuku K, Yamamoto Y, Ono T-A, Usgugara SM, Sati F (2005) PsbP protein, but not PsbQ protein, is essential for the regulation and stabilization of photosystem II in higher plants. Plant Physiol 139:1175–1184. https://doi.org/10.1104/pp.105.068643

Ishida N, Sugiura M, Rappaport F, Lai T-L, Rutherford AW, Boussac A (2008) Biosynthetic exchange of bromide for chloride and strontium for calcium in the photosystem II oxygen-evolving enzymes. J Biol Chem 283:13330–13340. https://doi.org/10.1074/jbc.M710583200

Jagendorf AT, Uribe E (1966) ATP formation caused by acid-base transition of spinach chloroplasts. Proc Natl Acad Sci USA 55:170–177. https://doi.org/10.1073/pnas.55.1.170

Järvi S, Suorsa M, Aro EM (2015) Photosystem II repair in plant chloroplasts—regulation, assisting proteins and shared components with photosystem II biogenesis. Biochim Biophys Acta 1847:900–909. https://doi.org/10.1016/j.bbabio.2015.01.006

Joliot P, Barbieri G, Chabaud R (1969) Un nouveau modele des centres photochimiques du systeme II. Photochem Photobiol 10:309–329. https://doi.org/10.1111/j.1751-1097.1969.tb05696.x

Junge W (2019) Oxygenic photosynthesis: history, status and perspective. Quart Rev Biophys 52:1–17. https://doi.org/10.1017/S0033583518000112

Junge W, Haumann M, Ahlbrink R, Mulkidjanian A, Clausen J (2002) Electrostatics and proton transfer in photosynthetic water oxidation. Philos Trans R Soc Lond B Biol Sci 29:1407–1420. https://doi.org/10.1098/rstb.2002.1137

Kawakami K, Umena Y et al (2009) Location of chloride and its possible functions in oxygen-evolving photosystem II revealed by X-ray crystallography. Proc Natl Acad Sci USA 106:8567–8572. https://doi.org/10.1073/pnas.0812797106

Kelley PM, Izawa S (1978) The role of chloride ion in photosystem II. I. Effects of chloride ion on photosystem II electron transport and on hydroxylamine inhibition. Biochim Biophys Acta 502:198–210. https://doi.org/10.1016/0005-2728(78)90042-7

Kim CJ, Debus RJ (2020) Roles of D1-Glu189 and D1-Glu329 in O_2 formation by the water-splitting Mn_4Ca cluster in photosystem II. Biochem 59:3902–3917. https://doi.org/10.1021/acs.biochem.0c00541

Kok B, Cheniae G (1966) Kinetics and intermediates of the oxygen evolution step in photosynthesis. In: Sanadi DR (ed) Current Topics in Bioenergetics, vol 1. Academic Press, New York, pp 1–47

Kok B, Forbush B, McGloin M (1969) Cooperation of charges in photosynthetic O_2 evolution-I. A linear four step mechanism. Photochem Photobiol 11:457–475. https://doi.org/10.1111/j.1751-1097.1970.tb06017.x

Koua FH, Umena Y, Kawakami K, Shen JR (2013) Structure of Sr-substituted photosystem II at 2.1Å resolution and its implications in the mechanism of water oxidation. Proc Natl Acad Sci USA 110:1889–1894. https://doi.org/10.1073/pnas.1219922110

Kuwabara T, Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. Plant Cell Physiol 23:533–539. https://doi.org/10.1093/oxfordjournals.pcp.a076378

Leuschner C, Bricker TM (1996) Interaction of the 33 kDa extrinsic protein with photosystem II: rebinding of the 33 kDa extrinsic protein to photosystem II membranes which contain four, two, or zero manganese per Photosystem II reaction center. Biochem 35:4551–4557. https://doi.org/10.1021/bi9522615

Lindberg K, Andréasson L-E (1996) A one-site, two-state model for the binding of anions in photosystem II. Biochem 35:14259–14267. https://doi.org/10.1021/bi961244s

Lindberg K, Vanngard T, Andréasson L-E (1993) Studies of the slowly exchanging chloride in photosystem II of higher plants. Photosynth Res 38:401–408. https://doi.org/10.1007/BF00046767

Marchiori D, Oyala P, Debus R, Stich T, Britt RD (2018) Structural effects of ammonia binding to the Mn_4CaO_5 cluster of photosystem II. J Phys Chem B 122:11588–11599. https://doi.org/10.1021/acs.jpcb.7b11101

Martin AE, Burgess BK, Stouts CD et al (1991) Site-directed mutagenesis of *Azotobacter vinelandii* ferredoxin I: [Fe-S] cluster-driven protein rearrangement. Proc Natl Acad Sci USA 87:598–602. https://doi.org/10.1073/pnas.87.2.598

Miller AF, Brudvig GW (1991) A guide to electron paramagnetic resonance spectroscopy of photosystem II membranes. Biochim Biophys Acta 1056:1–18. https://doi.org/10.1016/s0005-2728(05)80067-2

Miyao M, Murata N (1984) Role of the 33-kDa polypeptide in preserving Mn in the photosynthetic oxygen-evolution system and its replacement by chloride ions. FEBS Lett 170:350–354. https://doi.org/10.1016/0014-5793(84)81342-3

Miyao M, Murata N (1985) The Cl^- effect on photosynthetic oxygen evolution: interaction of Cl^- with 18-kDa, 24-kDa and 33-kDa proteins. FEBS Lett 180:303–308. https://doi.org/10.1016/0014-5793(85)81091-7

Miyao M, Murata N (1986) Light-dependent inactivation of photosynthetic oxygen evolution during NaCl treatment of photosystem II particles: The role of the 24-kDa protein. Photosynth Res 10:489–496. https://doi.org/10.1007/BF00118315

Ono T-A, Zimmermann J-L, Inoue Y, Rutherford AW (1986) EPR evidence for a modified S-state transition in chloride-depleted photosystem II. Biochim Biophys Acta 851:193–201. https://doi.org/10.1016/0005-2728(86)90125-8

Ono T-A, Rompel A, Mino H, Chiba N (2001) Ca^{2+} function in photosynthetic oxygen evolution studied by alkali metal cations substitutions. Biophys J 81:1831–1840. https://doi.org/10.1016/s0006-3495(01)75835-3

Oyala PH, Stich TA, Stull JA, Yu F, Pecoraro VL, Britt RD (2014) Pulse electron paramagnetic resonance studies of the interaction of methanol with the S_2 state of the Mn_4O_5Ca cluster of photosystem II. Biochem 53:7914–7928. https://doi.org/10.1021/bi501323h

Oyala PH, Stich TA, Debus RJ, Britt RD (2015) Ammonia binds to the dangler manganese of the photosystem II oxygen-evolving complex. J Am Chem Soc 137:8829–8837. https://doi.org/10.1021/jacs.5b04768

Pantazis DA (2018) Missing pieces in the puzzle of biological water oxidation. ACS Catal 8:9477–9507. https://doi.org/10.1021/acscatal.8b01928

Peloquin JM, Campbell KA et al (2000) ^{55}Mn ENDOR of the S_2-state multiline EPR signal of photosystem II: implications on the structure of the tetranuclear Mn cluster. J Am Chem Soc 122(10):926–10942. https://doi.org/10.1021/ja002104f

Pokhrel R, Service RJ, Debus RJ, Brudvig, GW (2013) Mutation of lysine 317 in the D2 subunit of photosystem II alters chloride binding and proton transport. Biochem 52:4758–4773. https://doi.org/10.1021/bi301700u

Rivalta I, Amin M, Luber S, Vassiliev S et al (2011) Structural-functional role of chloride in photosystem II. Biochem 50:6312–6315. https://doi.org/10.1021/bi200685w

Rutherford AW, Boussac A, Faller P (2004) The stable tyrosyl radical in photosystem II: why D? Biochim Biophys Acta 1655:222–230. https://doi.org/10.1016/j.bbabio.2003.10.016

Sakashita N, Watanabe HC, Ikeda T, Ishikita H (2017) Structurally conserved channels in cyanobacterial and plant photosystem II. Photosynth Res 133:75–85. https://doi.org/10.1007/s11120-017-0347-1

Sandusky PO, Yocum CF (1984) The chloride requirement for photosynthetic oxygen evolution: Analysis of the effects of chloride and other anions on amine inhibition of the oxygen-evolving complex. Biochim Biophys Acta 766:603–611. https://doi.org/10.1016/0005-2728(84)90121-X

Sandusky PO, Yocum CF (1986) The chloride requirement for photosynthetic oxygen evolution: Factors affecting nucleophilic displacement of chloride from the oxygen evolving complex. Biochim Biophys Acta 849:85–93. https://doi.org/10.1016/0005272 8(86)90099-X

Sauer K, Yano J, Yachandra VK (2005) X-ray spectroscopy of the Mn_4Ca cluster in the water oxidation complex of photosystem II. Photosyn Res 85:73–86. https://doi.org/10.1007/s11120-005-0638-9

Shen J-R (2015) The structure of photosystem II and the mechanism of water oxidation in photosynthesis. Ann Rev Plant Biol 66:23–48. https://doi.org/10.1146/annurev-arplant-050312-120129

Shen J-R, Kamiya N (2000) Crystallization and the crystal properties of the oxygen-evolving photosystem II from *Synechococcus vulcanus*. Biochem 39:14739–14744. https://doi.org/10.1021/bi001402m

Suga M, Akita F, Hirata K et al (2014) Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. Nature 517:99–103. https://doi.org/10.1038/nature13991

Suga M et al (2017) Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. Nature 543:131–135. https://doi.org/10.1038/nature21400

Suga M et al (2019) An oxyl/oxo mechanism for oxygen-oxygen coupling in PSII revealed by an x-ray free-electron laser. Science 366:334–338. https://doi.org/10.1126/science.aax6998

Szalai VA, Kuhne H, Lakshmi KV, Brudvig GW (1998) Competitive binding of acetate and chloride in photosystem II. Biochem 37:13594–13603. https://doi.org/10.1021/bi990341t

Theg SM (2018) Chloroplast transport and import. Photosynth Res 138:261–262. https://doi.org/10.1007/s11120-018-0593-x

Velthuis BR (1975) Binding of the inhibitor NH3 to the oxygen-evolving apparatus of spinach chloroplasts. Biochim Biophys Acta 396:392–401. https://doi.org/10.1016/0005-2728(75)90145-0

Vermaas WFJ, Rutherford AW, Hansson Ö (1988) Site-directed mutagenesis in photosystem II of the cyanobacterium Synechocystis sp. PCC 6803: Donor D is a tyrosine residue in the D2 protein. Proc Natl Acad Sci USA 85:8477–8481. https://doi.org/10.1073/pnas.85.22.8477

Vinyard DJ, Brudvig GW (2017) Progress toward a molecular mechanism of water oxidation in photosystem II. Ann Rev Phys Chem 68:101–116. https://doi.org/10.1146/annurevphyschem-052516-044820

Vrettos JS, Stone DA, Brudvig GW (2001) Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: evidence for direct involvement of Ca^{2+} in O_2 formation. Biochem 40:7937–7945. https://doi.org/10.1021/bi010679z

Wang J et al (2017) Crystallographic data support the carousel mechanism of water supply to the oxygen-evolving complex of photosystem II. ACS Energy Lett 2:2299–2306. https://doi.org/10.1021/acsenergylett.7b00750

Wincencjusz H, van Gorkom HJ, Yocum CF (1997) The photosynthetic oxygen evolving complex requires chloride for its redox state S2→S3 and S3→S0 transitions but not for S0→S1 or S1→S2 transitions. Biochem 36:3663–3670. https://doi.org/10.1021/bi9626719

Wincencjusz H, Yocum CF, van Gorkom HJ (1999) Activating anions that replace Cl^- in the O_2 evolving complex of photosystem II slow the kinetics of the terminal step in water oxidation and destabilize the S_2 and S_3 states. Biochem 38:3719–3725. https://doi.org/10.1021/Bi982295N

Yocum CF, Babcock GT (1981) Amine-induced inhibition of photosynthetic oxygen evolution: A correlation between the microwave power saturation properties of signal IIf and photosystem II-associated manganese. FEBS Lett 130:99–102. https://doi.org/10.1016/0014-5793(81)80674-6

Young ID, Ibrahim M, Chatterjee R et al (2016) Structure of photosystem II and substrate binding at room temperature. Nature 540:453–474. https://doi.org/10.1038/nature20161

Zhang M, Bommer M, Chatterjee R et al (2017) Structural insights into the light-driven auto-assembly process of the water oxidizing Mn_4CaO_5-cluster in photosystem II. Elife 6:1–20. https://doi.org/10.7554/eLife.26933

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W, Orth P (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. Nature 409:739–743. https://doi.org/10.1038/35055589