Mechanism of photosynthetic water oxidation: combining biophysical studies of photosystem II with inorganic model chemistry

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Abstract

A mechanism for photosynthetic water oxidation is proposed based on a structural model of the oxygen-evolving complex (OEC) and its placement into the modeled structure of the D1/D2 core of photosystem II. The structural model of the OEC satisfies many of the geometrical constraints imposed by spectroscopic and biophysical results. The model includes the tetranuclear manganese cluster, calcium, chloride, tyrosine Z, H190, D170, H332 and H337 of the D1 polypeptide and is patterned after the reversible O2-binding diferric site in oxyhemerythrin. The mechanism for water oxidation readily follows from the structural model. Concerted proton-coupled electron transfer in the S2 → S3 and S3 → S4 transitions forms a terminal Mn(V)N=O moiety. Nucleophilic attack on this electron-deficient Mn(V)=O by a calcium-bound water molecule results in a Mn(III)-OOH species, similar to the ferric hydroperoxide in oxyhemerythrin. Dioxygen is released in a manner analogous to that in oxyhemerythrin, concomitant with reduction of manganese and protonation of a μ-oxo bridge. © 2001 Published by Elsevier Science B.V.

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1. Introduction

The mechanism of water oxidation by photosystem II (PSII) is yet to be fully understood despite the contributions of many researchers. Lately, a number of proposals have been put forth which consider data obtained from studies of inhibited states of the water oxidation cycle, theoretical investigations of the energetics involved, and bioinorganic modeling of the oxygen-evolving complex (OEC) [1–7]. In a recent paper, we proposed a model for the O-O bond-forming step of photosynthetic dioxygen evolution that involved the formation of a reactive electron-deficient manganese(V)-oxo species [3]. Our rationale for that mechanism was based in part upon an analysis of high-valent metal–oxo reactivity in inorganic model systems and the requirement for calcium, chloride, and key amino acids for optimal activity by PSII. In this review, we build upon these ideas by more stringently exploring the possible functions of the protein components in facilitating the formation of a terminal Mn(V)=O moiety and develop our ideas on the structure of the OEC in light of the available biophysical data.

PSII is the multi-subunit transmembrane complex located in the appressed regions of the chloroplast thylakoid membrane that couples the four-electron oxidation of water to the reduction of two molecules...
of plastoquinone. Because of the size and complexity of PSII, it has proven difficult to obtain a high-resolution crystal structure of the protein. However, an 8 Å structure of the D1/D2/CP47 complex obtained by electron crystallography [8,9] and theoretical models [10,11] based on sequence homology to the bacterial reaction center are available. The former method allows the helices of the protein subunits to be arranged in space while the molecular modeling results give the likely spatial arrangements of the individual amino acids.

The photochemistry of PSII is initiated by photo-induced charge separation from the reaction center chlorophylls (P680) to a pheophytin and subsequently to the membrane-bound quinone QA. To stabilize the charge-separated state, P680** is reduced by a redox-active tyrosine residue, Y161 of the D1 polypeptide (denoted YZ). The neutral radical YZ in turn oxidizes the tetranuclear manganese cluster (Mn4) through a cycle of five 'S-n-states' (for storage of oxidizing equivalents, n = 0–4) [12]. Fig. 1 depicts our proposed S-state cycle, which will be elaborated upon in this review. Protons are released into the thylakoid lumen during S-state advancement and O2 is released spontaneously during the S3 to S0 conversion.

The OEC is the site of water oxidation and consists of the Mn4 cluster, YZ, and Ca2+ and Cl− cofactors [13–16]. The exact arrangement of the Mn ions in the Mn4 cluster is unknown, but electron paramagnetic resonance (EPR) [17–19] and X-ray absorption spectroscopy (XAS) [16,20–24] data suggest that it is a tetramer of µ-oxo-bridged units with Mn–Mn separations of 2.7 Å and 3.3 Å. There is general agreement that the Mn4 cluster is oxidized in the S-state cycle. Based on the X-ray absorption near-edge structure (XANES) and EPR spectra of the S0-, S1-, and S2-states, the oxidation states of Mn in the S2-state are thought to be Mn(III)Mn(IV), [1,16,22], although Mn(III)3Mn(IV) cannot be ruled out [25,26].

Ca2+ and Cl− are required for water oxidation and advancement beyond the S2-state [27–31]. Extended X-ray absorption fine structure (EXAFS) data suggest that Ca2+ binds close to the Mn4 cluster [21,32–34]. It can be replaced by cations of similar size but only Sr2+ partially restores activity [30,35]. Depletion of either Ca2+ or Cl−, or treatment with acetate, inhibits the S-state cycle at an intermediate step where YZ is oxidized with the Mn4 cluster in the S2-state [1,27,29,31,36–41]. The resulting state, S2YZ, has a characteristic EPR spectrum that differs only slightly between calcium-depleted and acetate-treated samples. The S2YZ spectrum has been used to estimate the point-dipole distance between YZ and the Mn4 cluster as 7–10 Å [42–45], which is close enough for YZ to be hydrogen-bonded to a Mn-bound water molecule [3,46]. Acetate binds in competition with Cl− [37], and the proximity of acetate to YZ as measured by electron-nuclear double resonance (ENDOR) [47] places Cl− near both YZ and the Mn4 cluster.

Based on both computational [48] and experimental [49] estimates of the O–H bond strengths of water and hydroxide bound to high-valent Mn, and the close proximity of YZ to the Mn4 cluster, several mechanisms for water oxidation propose that YZ abstracts H-atoms from Mn-bound water to form a Mn=O moiety [1–5,50]. Removal of Ca2+ or Cl− may disrupt the hydrogen-bonding network required for this process, thus inhibiting S-state advancement. Furthermore, a number of amino acids are also required for optimal activity, including H190 [51–54], H332 [55,56], H337 [55], E189 [51], and D170 [51,57] of the D1 polypeptide. (Synechocystis numbering). These are thought to be either ligands to Mn or serve as part of the hydrogen-bonding network.

In this minireview, we focus first on the involvement of proton-coupled electron transfer (PCET) during S-state advancement and the effects of pH, H/D substitution, and the protein environment surrounding the OEC. Next, we propose a structure for the manganese complex and its local environment based on modeling studies and analogy to other protein systems. We show that our model would promote O=O bond formation through reaction of an electrophilic Mn(V)=O moiety with a water bound to Ca2+. This mechanism is considered in light of the available biophysical data on the OEC and in analogy with the release of O2 from the diferric center in oxyhemerythrin.
depend on the temperature and the pH of the medium, and the kinetics of these processes exhibit deuterium isotope effects. YZ is protonated [58] but, in both Mn-depleted and oxygen-evolving PSII, oxidized YZ is a neutral radical (Y\textsuperscript{O}Z) [59] and so must be deprotonated upon oxidation. Consequently, there must be a proton acceptor from YZ during its oxidation and also a proton donor to Y\textsuperscript{Z} during its re-reduction. In untreated PSII, the oxidation and reduction of YZ proceed rapidly. However, in Mn-depleted PSII, these processes are slowed. These observations led to the ideas that, in the presence of the Mn\textsubscript{4} cluster: (1) the OEC has a well-ordered hydrogen-bonding network that facilitates proton transfer.

Fig. 1. Proposed S-state cycle. The di-manganese unit which does not undergo redox changes during S-state advancement [Mn\textsuperscript{IV}O\textsubscript{X}] is omitted beyond S\textsubscript{0} for clarity. Solid arrows indicate light-driven steps and dashed arrows denote spontaneous steps. Steps that involve H-atom abstraction by Y\textsuperscript{O}Z are emphasized by including the reduction of Y\textsubscript{Z}O\textsuperscript{*} in the figure. The role of a protein residue acting as a base, B, and Ca-OH\textsubscript{2} in the O-O bond-forming step is shown in the S\textsubscript{4}-S\textsubscript{4}' step, but the proposed structure of Ca-OH\textsubscript{2} and Cl would be present in all of the earlier S-states as well.
between \( Y_Z \) and its proton acceptor and donor and (2) that S-state advancement is proton-coupled. The latter idea was key in the formulation of the H-atom abstraction model for water oxidation [1–5,50]. In this section, we consider the involvement of PCET during the S-state cycle. We focus in particular on the effects of changing the pH, H/D substitution, temperature dependencies and the effects of the protein environment on both the oxidation and the reduction of \( Y_Z \).

In manganese-depleted PSII, the rates for both the oxidation and reduction of \( Y_Z \) are pH-dependent [60–62]. It has been observed that the rate of oxidation of \( Y_Z \) increases at higher pH, becoming extremely rapid near pH 9 with measured \( \mathrm{p}K_a \) values of \( \sim 8 \) or 10 and \( \sim 5–7 \) [53,54]. The high \( \mathrm{p}K_a \) has been assigned as that of \( Y_Z \) while the lower value is assigned to its proton acceptor, which is most likely D1-H190. The somewhat low \( \mathrm{p}K_a \) of 8 for tyrosine (compared to a value of 10 for tyrosine in solution) is explained by the participation of \( Y_Z \) in two hydrogen bonds, one with the proton acceptor (H190) and another with a protonated acidic group, such as a carboxylic acid [54]. On the other hand, the value of 10 was measured in a mutant strain lacking H190 and so reflects the \( \mathrm{p}K_a \) of \( Y_Z \) lacking one of its putative H-bonding partners [53]. Therefore, at a pH > 9, \( Y_Z \) is already largely deprotonated and so its oxidation is no longer proton-limited and proceeds rapidly. In an intermediate pH regime, the oxidation of \( Y_Z \) is proton-coupled. At a pH below the \( \mathrm{p}K_a \) of the accepting base, both \( Y_Z \) and the base are protonated and \( Y_Z \) oxidation is retarded.

The involvement of a base with a \( \mathrm{p}K_a \) of \( \sim 6 \) forming a hydrogen-bonded pair with \( Y_Z \) is supported by the observation that the reduction of \( Y_Z^* \) is also modulated by a protonatable group with a \( \mathrm{p}K_a \) of 6 [62]. In oxygen-evolving PSII, the rate of \( O_2 \) evolution exhibits a maximum at pH 6.5 and decreases significantly below pH 5 and above pH 8 [60]. Just as in Mn-depleted PSII, it was concluded that a base with a \( \mathrm{p}K_a \) \( \sim 6 \) accepts a proton from \( Y_Z \), and at low pH the protonation of this group retards \( Y_Z \) oxidation. The identification of this base as D1-H190 is strongly supported by site-directed mutagenesis studies [52,53]. It was found that in D1-H190 mutants, \( Y_Z \) oxidation only occurs at high pH values, when \( Y_Z \) is already deprotonated [53,54]. Furthermore, the rates of P680** reduction, \( Y_Z \) oxidation and \( Y_Z^* \) reduction in the mutants were all significantly enhanced by the addition of small organic bases, such as imidazole [52]. The involvement of a histidine residue as the proton acceptor from \( Y_Z \) is also mirrored in the D2 subunit, where \( Y_D \) oxidation by P680** proceeds by deprotonation of the phenol group to its acceptor base, which has been identified as D2-H189 [63]. Together, the pH effects on the oxidation/reduction of \( Y_Z \) in wild-type and D1-H190 mutant PSII suggest that H190 and \( Y_Z \) form a H-bonded pair in which H190 is the proton acceptor for \( Y_Z \).

As one would expect, \( Y_Z \) oxidation and S-state advancement exhibit kinetic deuterium isotope effects. The rate of oxidation of \( Y_Z \) (measured as the reduction of P680**) has a large kinetic deuterium isotope effect of \( k_{\mathrm{H}}/k_{\mathrm{D}} = 2.9–3.4 \) at pH 6.5 in Mn-depleted core complexes but is almost H/D insensitive in oxygen-evolving centers [60,64–66]. This suggests that, in Mn-containing PSII: (1) there exists a strong H-bond between \( Y_Z \) and its proton acceptor, D1-H190, which requires minimal proton movement during the S-state cycle to facilitate deprotonation of \( Y_Z \). This idea is supported by the observation that, in Mn-depleted centers, \( Y_Z^* \) has a disordered distribution of H-bonds (in contrast to \( Y_D^* \)) due to disruption of the hydrogen-bonding network [67]. Furthermore, it has been shown by FTIR that \( Y_Z \) is structurally coupled to the Mn4 cluster [68].

The kinetics of the oxidation of \( Y_Z \) consist of a fast nanosecond component and a slower microsecond component, while the reduction proceeds on a microsecond time-scale. There is good agreement among the reported values of \( k_{\mathrm{H}}/k_{\mathrm{D}} \) for the S-state transitions (reduction of \( Y_Z^* \)) in both oxygen-evolving thylakoids and PSII core complexes [64,65,69]. The values reflect the changes in the microsecond component of flash-induced absorbance changes at 355 nm. Careful analysis of the nanosecond component of \( Y_Z \) oxidation and the kinetics of \( Y_Z^* \) reduction reveals that the nanosecond component is H/D insensitive, while the microsecond component of the reduction reflects proton motion [70]. These components have been modeled as reflecting the kinetics of the initial charge separation followed by a slower relaxation of...
the product state [65]. The kinetic deuterium isotope effects on the microsecond phase are approximately 1.4 ($S_2 \rightarrow S_1$), 2.3 ($S_2 \rightarrow S_3$), 1.5 ($S_3 \rightarrow S_0$) and 1.4 ($S_0 \rightarrow S_1$).

The fate of the proton released by $Y_Z$ is not clear. It has been proposed that the proton released from $Y_Z$ during its oxidation resides on its acceptor base (D1-H190) during the lifetime of $Y_Z^*$ [62,64,71,72]. This has led to the proposal that chlorophyll absorbance band shifts [73] that persist during the lifetime of $Y_Z^*$ arise from the formation of a positive charge on the acceptor base due to its protonation [62,71,72]. In addition, it has been suggested that the phenolic proton returns to $Y^*$ each time it is reduced, in contrast to the H-atom abstraction model [71]. However, the accumulation of a positive charge on the base, D1-H190, could be avoided by deprotonation of the distal δ-nitrogen [53]. This is supported by the observation that proton release into the lumen is kinetically correlated with $Y_Z$ oxidation [74,75]. The chlorophyll band shifts have also been described as originating via another mechanism, such as through changes in the H-bond strength to the 9-keto group of P680 [50]. Furthermore, if the phenolic proton returned to $Y_Z^*$ with each reduction, then $Y_Z^*$ reduction upon $S_1 \rightarrow S_3$ may not be expected to exhibit a pronounced kinetic deuterium isotope effect ($k_H/k_D = 2.3$), but rather one of a magnitude similar to that for the low S-state transitions ($k_H/k_D = 1.4$) [69]. Also, in calcium- and chloride-depleted and acetate-treated samples, reduction of $Y_Z^*$ does not occur from the $S_2Y_Z^*$-state, but does in the prior S-states. The return of the phenolic proton to $Y_Z^*$ upon its reduction in all S-states is not consistent with these observations.

Additional evidence for the involvement of PCET in the water oxidation cycle arises from the temperature-dependence of the S-state transitions. In untreated PSII, the $S_1 \rightarrow S_2$ transition can proceed at temperatures as low as 140 K [76], which suggests that minimal structural rearrangements occur. In contrast, the $S_0 \rightarrow S_1$ and $S_2 \rightarrow S_3$ transitions advance only at temperatures greater than 220 K [77,78] and have significantly greater activation energies (59.4 kJ/mol for $S_0 \rightarrow S_1$ and 26.8 kJ/mol for $S_2 \rightarrow S_3$ versus 9.6 kJ/mol for $S_1 \rightarrow S_2$) [79]. The $S_2 \rightarrow S_1$ transition is also inhibited in samples that are depleted of Ca$^{2+}$ or Cl$^-$, resulting in the $S_2Y_Z^*$-state [1,27,29,31,37–41].

It has been suggested that removal of these ions or treatment with acetate disrupts the hydrogen-bonding network in the OEC, thus retarding S-state transitions by obstructing PCET (Eqs. 1a and 1b) [40].

\[
\begin{align*}
S_2(OH_2) + ^\bullet O - Y_Z &\rightarrow S_3(OH) + HO - Y_Z & (1a) \\
S_2(\text{acetate}) + ^\bullet O - Y_Z &\rightarrow \text{No reaction} & (1b)
\end{align*}
\]

The observations described above can be explained by the PCET model shown in Fig. 2, which depicts two routes for $Y_Z^*$ reduction. As has been clearly demonstrated, $Y_Z$ oxidation proceeds by deprotonation of the phenolic proton to D1-H190, with little deuterium kinetic isotope effect. In our model, we suggest that the distal nitrogen of H190 deprotonates after accepting the phenolic proton. There are now two routes for $Y_Z^*$ protonation upon its reduction. In one case, reduction by the Mn$_4$ cluster can occur with simultaneous acceptance of the proton back from H190. This is a consecutive PCET, meaning proton transfer follows electron transfer, and so the electron transfer and proton transfer events are denoted as being separate (ET/PT) [80]. On the other hand, $Y_Z^*$ may accept both the electron and the proton together from the Mn$_4$ cluster (H-atom abstraction), which is a concerted PCET process (denoted ETPT). We propose that the consecutive PCET path is utilized in the low S-states, i.e. those that lose a proton not involved in $Y_Z^*$ reduction ($S_0 \rightarrow S_1$), are accessible at low temperature ($S_1 \rightarrow S_2$) and in samples depleted of Ca$^{2+}$ or Cl$^-$. However, oxidation of the Mn$_4$ cluster by $Y_Z^*$ beyond $S_2$ cannot occur in the latter samples. This led to our proposal that there is insufficient driving force for the reduction of $Y_Z^*$ in these samples in the absence of concerted PCET (H-atom abstraction) [40]. Thus, S-state advancement beyond $S_2$ requires the concerted ETPT pathway. At low temperatures or in samples where the required hydrogen-bonding network is disrupted, this cannot occur. A possible molecular basis for the switch between consecutive and concerted PCET will be discussed later, along with our proposed water oxidation mechanism. This model was inspired by and is further supported by estimates of the O–H bond strength of water bound to high-valent Mn as 78–89 kcal/mol, a decrease of about 30 kcal/mol from free water (118.4 kcal/mol) [49]. This range of
values is comparable to that for the tyrosine phenolic O-H bond (81.5 kcal/mol) [81]. It has been proposed that the reactivity of metal-oxo complexes in reactions involving H-atom abstractions is determined by the energies of the O-H bonds [82,83]. This makes H-atom abstraction from water bound to high-valent Mn by Y_Z a feasible and, possibly, a thermodynamically favorable process in the higher S-states.

These paths for PCET are illustrated in our proposed S-state cycle (Fig. 1). During the transitions S_0→S_1 and S_1→S_2, Y_Z is reduced via the consecutive ET/PT pathway. However, advancement beyond S_2 requires the abstraction of both an electron and a proton (an H-atom) from the Mn_4 cluster and follows the concerted ETPT path. Ultimately, this leads to the formation of a terminal Mn(V)=O species, the structure and reactivity of which we will discuss in the following sections.
3. Biophysical studies of the OEC

As stated, the components of the OEC include the Mn\(_4\) cluster, Y\(_2\), Ca\(^{2+}\), Cl\(^-\), and a number of amino acids. In this section, we focus on spectroscopic and biochemical studies of the OEC.

Despite numerous spectroscopic investigations, the exact structure of the Mn\(_4\) cluster remains unknown. A number of structural models of the Mn\(_4\) cluster exist [84,85], although most reproduce only certain aspects of the spectroscopic signatures of the OEC. XAS and EPR have been the most widely used spectroscopic techniques to study the structure of the complex [16,18,22], although UV-visible spectroscopy [86–90] and, more recently, vibrational spectroscopy [91,92] have been employed as well.

The Mn K-edge XANES [16,93–95] and the K\(_\beta\) X-ray fluorescence [96] spectra for the OEC are consistent with oxidation states of either Mn(III)\(_2\)-Mn(IV)\(_2\) or Mn(III)\(_4\) for the S\(_1\)-state, although the former assignment is most widely accepted. This assignment is supported by the fact that the OEC can be chemically reduced to an ‘S\(_{3-}\)’ state while remaining intact [97]. Reduction of the OEC by hydroxylamine causes Mn(II) to be released [98] and, therefore, it appears that an all Mn(II) oxidation state cluster would not be stable; this result argues for at least one Mn(III) in the ‘S\(_{3-}\)’ state and, consequently, a Mn(III)\(_2\)-Mn(IV)\(_2\) oxidation level for the S\(_1\)-state. The EXAFS spectrum of S\(_1\) indicates scatterers at 2.7, 3.3, and 4.2 Å [16,20,21,24,32,33,95]. There are 2–3 backscatterers at 2.7 Å, diagnostic of di-µ-oxo-bridged Mn units. However, there is not a consensus on the assignments of the 3.3 and 4.2 Å vectors. The 3.3 Å distance has been attributed to both Mn–Mn and Mn–Ca scattering, but the Mn–Ca assignment has been quite controversial [21,32–34]. A 3.3 Å distance is consistent with a mono-µ-oxo bridge between two Mn ions. However, the groups of Klein and Sauer have found the 3.3 Å distance to be sensitive to the replacement of Ca\(^{2+}\) and so assign this vector to Mn–Ca scattering [33,34]. On the other hand, Penner-Hahn and coworkers attributed the 4.2 Å distance to Mn–Ca scattering and proposed a structure similar to the heterodinuclear site in concanavalin A [21]. In this polysaccharide-binding protein, a Mn(II) ion is linked by two carboxylato groups to a Ca\(^{2+}\) ion, with a separation of 4.3 Å [99]. A Mn–Ca distance of 3.3 Å is very short. Such short Mn–Ca distances are only observed in oxo-bridged calcium oxide clusters [100,101]. Moreover, such a structure would not be stable at near-neutral pH. However, the 4.2 Å distance is compatible with a carboxylato or long single-atom bridge (e.g. a halide) between Mn and Ca\(^{2+}\), and we favor the latter interpretation. Such a structure is supported by FTIR data contrasting untreated and calcium-depleted PSII [102], although this result has been questioned [103]. Furthermore, Penner-Hahn and coworkers found the 3.3 Å distance to be insensitive to replacement of Ca\(^{2+}\) by Sr\(^{2+}\) or Dy\(^{3+}\), but the 2.7 Å Mn–Mn distance changed slightly in proportion to the Lewis basicity of the cation [32]. These results were explained by a model in which a Ca-bound water molecule is H-bonded to a µ-oxo bridge between two Mn ions. Because Dy\(^{3+}\) is a much stronger Lewis acid than either Ca\(^{2+}\) or Sr\(^{2+}\), the Dy-bound water may protonate the µ-oxo bridge; protonation of a bridging µ-oxo could influence the Mn–Mn distance. This model may explain why lanthanide-inhibited PSII cannot advance to the S\(_2\)-state [35]. Protonation of a µ-oxo...
bridge could change the reduction potential of the cluster [104,105], making its oxidation by $Y_Z^*$ no longer possible. We employ a similar structure in our OEC model (Fig. 3), and propose that the Ca-bound water molecule is one of the substrate waters. A more detailed description of this structure will be given below.

There is an increase in the Mn K-edge energy on forming both the multiline and $g = 4.1$ forms of the $S_2$-state from the $S_1$-state [106]. Together with EXAFS evidence that the structure of the Mn$_4$ cluster changes very little in the $S_1 \rightarrow S_2$(multiline) conversion (see below) [107], this result is a clear indication of Mn-centered oxidation in the $S_1 \rightarrow S_2$ transition. EXAFS data on oriented layers of PSII membranes were modeled with two slightly different isotropic Mn-Mn distances of 2.74 and 2.71 Å in $S_1$, which was interpreted as evidence for two Mn$_2$ units, a Mn(III)$_2$ dimer and a Mn(IV)$_2$ dimer [108]. This difference disappeared upon formation of $S_2$. As mentioned above, the longer scattering distance of 3.3 Å has also been modeled as a Mn-Mn interaction. The dichroism of both this vector and the 2.7 Å vector has been studied in oriented PSII samples [108]. The 3.3 Å vector is orientation-dependent and modeled as forming an angle of $43 \pm 10^\circ$ with the membrane normal, while the 2.7 Å vector is isotropic.

The $S_2 \rightarrow S_3$ transition, however, has been the cause of great controversy. Period-four oscillations at 355 nm in the UV-visible absorption spectrum have been observed [86–90]. These absorptions have been attributed to Mn oxidations and provide evidence for Mn-centered oxidations during each S-state advancement. In addition, XAS measurements of flash-induced S-states gave supporting evidence for Mn-centered oxidations [93]. However, oxidation of the Mn$_4$ cluster during $S_2 \rightarrow S_3$ was later questioned based on other XAS measurements [94]. Problems with analyzing data from the $S_0$-state [22] arise because $S_3$ is best prepared from $S_1$ by using two short saturating flashes of light and then quickly freezing the sample. S-state scrambling due to misses and double hits of centers introduces inhomogeneity such that only about 65% of the centers are thought to be in the $S_1$-state. In addition, the measured changes depend on the method employed to analyze the spectra. Some groups argue that there is no change in the edge energy upon formation of $S_3$ and that the oxidizing equivalent in this step resides on a protein residue or an oxo ligand of Mn [94]. On the other hand, other groups have measured an increase in edge energy of about 1 eV and attribute this to oxidation of Mn [93]. Recently, XAS of layered PSII membranes that were corrected for S-state dephasing, X-ray photoreduction, and analyzed by four different methods indicates that there is a significant increase in edge energy in the $S_2 \rightarrow S_3$ transition, in support of Mn oxidation [109].

A Mn-centered oxidation in $S_2 \rightarrow S_3$ is also consistent with the observation that $S_3$ is the least reactive S-state towards reduction by hydrazine and hydroxylamine [110]. Supposing the Mn(III)$_2$Mn(IV)$_2$ assignment for $S_1$, then $S_3$ would be Mn(IV)$_4$. Octahedral Mn(IV) is expected to be slow to exchange ligands due to its crystal field stabilization energy. Therefore, an all Mn(IV) cluster is expected to be especially unreactive, assuming that the rate-limiting step in the reaction of hydrazine and hydroxylamine is binding to the Mn$_4$ cluster.

Magnetic resonance has also been invaluable in studying the structure of the OEC [1,18]. EPR signals have been observed for all the S-states except $S_4$. The $S_0$-state has a ground spin state of $S = 1/2$ and its spectrum consists of 24–26 peaks, centered at $g = 2$, spanning 2200–2400 G [111–113]. The broader width of this spectrum compared to the $S_2$ multiline may indicate the presence of a Mn(II) ion in $S_0$. The $S_1$ signal is detectable in parallel mode EPR and appears as a >18-line spectrum, 600 G wide, centered near $g = 12$ [114]. Based on the number of lines and the temperature-dependence of the spectrum, it was concluded that all four manganese in the cluster contribute to the integer spin state. The multiline ($S = 1/2$) form of the $S_2$-state is the best characterized of the EPR signals arising from the OEC [115]. It is formed in high yield by continuous illumination at 200 K or at ambient temperature in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyleurea), a competitive inhibitor of the Q$_B$ site which limits electron transfer to one turnover. The spectrum, 1900 G wide with 18–20 lines centered at $g = 2$, is indicative of an exchange-coupled cluster of mixed-valent Mn [19]. An analogous multiline spectrum was recently generated by the one-electron reduction of a linear tetrameric Mn(IV)$_4$ di-µ-oxo-bridged model complex,
which supports the oxidation state assignment Mn(III)Mn(IV)₃ for the S₂-state [116]. Simulations of the EPR and electron spin echo-ENDOR spectra indicate that the S₂ multiline signal originates from a tetranuclear cluster in which the spin is shared on all four Mn ions, and not a pair of isolated Mn₂ dimers nor a Mn monomer-plus-trimer configuration [17, 19]. In the models of Britt and coworkers [19,117] and Kusunoki and coworkers [17], three of the four Mn are strongly coupled (e.g. via di-μ-oxo bridges) with the fourth Mn more weakly coupled (e.g. via a mono-μ-oxo bridge).

An alternate form of the S₂-state can be prepared, having an S = 5/2 ground state and an EPR signal centered at g = 4.1, by illumination at 140 K [76, 118,119]. This ‘g = 4.1’ S₂-state is characterized by a 340 G wide spectrum with no resolved Mn-hyperfine lines (unless NH₃-treated and oriented on Mylar films [120]) and is a ground spin state of the tetrameric cluster with altered couplings between Mn ions versus the g = 2 multiline form of the S₂-state [121]. The g = 4.1 form of S₂ is formed in untreated samples from the multiline form by illumination with infrared light [121]; in some treated samples, such as chloride-depleted or -substituted PSII, it is the stable form of the S₂-state [122]. It may be that the two forms of the S₂-state arise from a structural difference that alters the magnitude of the weak coupling in the aforementioned models of Britt [19,117] and Kusunoki [17].

Depletion of Ca²⁺ or Cl⁻, or treatment with acetate, inhibits S-state advancement at the S₂Y₂-state [1,27,29,31,37–40]. The magnetic interactions between the Mn₄ cluster and Y₂ has been exploited to estimate the distance and angles between the two. The spectrum is best simulated with a point-dipole intershell distance of 7–10 Å [42–45]. This distance is short enough that a Mn-bound water molecule can directly H-bond with the phenol group of Y₂ [3,46]. We propose that having a single Mn proximal to Y₂ is key for the mechanism of water oxidation, as it is the water molecule bound to this Mn center that forms the Mn(V)=O through the ETPT pathway of Y₂ reduction.

Recently, EPR signals arising from the S₁-state have been reported [123,124]. The signals resonate at g = 8 and 12 in parallel mode detection and can be simulated by an integer (S = 1) spin state [123]. Furthermore, these signals occur with formation of a split g = 4 signal, and the signal amplitudes decrease in the presence of small alcohols in a manner similar to the g = 4.1 S₂-state signal [124]. It was suggested that the g = 4.1 form of the S₂-state may be the precursor of the low-field S₃ signals. The S₃-state shows a response to near-infrared illumination resulting in the formation of a broadened signal at g = 2, which may arise from the interaction of a modified form of the S₂-state with a radical.

As mentioned, several amino acids have been identified as integral to the OEC. Obviously, Y₂ and D1-H190 are intimate components of the OEC. Site-directed mutagenesis has identified other amino acids that may be either ligands to the Mn₄ cluster or Ca²⁺, or proton acceptors involved in water oxidation. Mutations of D1-D59 and D1-D61, found in the luminal interhelical a-b loop, result in centers that exhibit impaired oxygen-evolution capability [125,126]. These amino acids are thought to ligate Ca²⁺. Ligands to the Mn₄ cluster may include the following D1 residues: D170 [51,57], H332 [55,56,127], E333 [55,127], H337 [55,127], D342 [55,127], and A344 (the C-terminus) [128]. Replacement of D170 with amino acids that are Lewis bases yields PSII complexes that can assemble the Mn₄ cluster but have greatly reduced O₂ evolution rates [57]. D1-H332 is particularly interesting in that H332E mutants assemble a Mn₄ cluster but evolve no O₂ [56]. These mutants exhibit altered S₂ multiline EPR signals and are inhibited at the S₂Y₂-state under multiple turnover conditions. The former strongly suggests that D1-H332 ligates Mn, while the latter suggests that D1-H332 is involved in the PCET of the high S-states, either as a Lewis base or by modulating the reduction potential of the OEC.

4. Proposed models for the structure and reactivity of the OEC

The electron crystallography [8,9] results confirm that there is considerable homology in the arrangement of the transmembrane helices of D1 and D2 of PSII and the L and M subunits of the bacterial photosynthetic reaction center (bRC). A structural model of the transmembrane helices of PSII has been calculated by overlaying the PSII amino acid se-
quence on the crystal structure of the bRC [10,11]. Based on this structure, the aforementioned spectroscopic data, and analogy to other proteins with oxo-bridged metal clusters, we have developed a structural model of the OEC. The model includes one of the μ-oxo-bridged di-manganese units, Ca$^{2+}$, Cl$^-$, and the substrate waters together with Y$_Z$, H190, E189, and D170. We also suggest possible roles for H332 and H337. An S-state cycle which involves the formation of a terminal Mn(V)\(\rightarrow\)O moiety ensues from the structural model.

4.1. Structural model of the OEC

Our proposed structure of the OEC as it appears in $S_1$ is shown in Fig. 3. All of the bond lengths and angles are based on analogy to known structures. Metrical parameters for the models were obtained from the Cambridge Structural Data Base (CSDB). A survey of bond lengths and angles was taken from applicable inorganic model compounds and the averages of these values were used to build the structures in the model [46]. The models were constructed using Chem3D (CambridgeSoft Corp.) constraining the parameters to those obtained from the CSDB. Based on the oxidation state assignments from the EXAFS and EPR results, we infer that two of the four Mn ions do not change oxidation state, but rather remain Mn(IV) throughout the S-state cycle; we refer to these as the [Mn$^{IV}$OX] unit in Figs. 1, 3–5. In our model, only two of the four Mn ions are shown in detail, because we propose that these two are the only Mn that undergo redox changes during the S-state cycle and participate directly in the water oxidation process. In addition, because of the limits of the theoretical protein structure, these Mn are the only two that can be placed in the protein structural model with spatial constraints. They are separated at a distance of 3.3 Å by a μ-oxo-μ-carboxylato bridge, where the carboxylato group is D1-D170. This structure is modeled after the dinuclear metal sites of hemerythrin, manganese catalase, ribonucleotide reductase, and arginase [129] and has a Mn–O bond length of 1.8 Å and a Mn–O–Mn angle of 133°. We employ this μ-oxo-μ-carboxylato bridge based on simulations of the $S_2$ multiline EPR [17] and $^{55}$Mn ENDOR [19] spectra, which require that one of the Mn ions is less strongly coupled to the Mn$_4$ cluster than the other three Mn [17,19,117]. Thus, we propose that it is the Mn$_3$ unit containing the more weakly coupled terminal Mn which is the catalytic site of water oxidation in the Mn$_4$ cluster, with the Mn proximal to Y$_Z$ being the one that binds one of the substrate water molecules.

We propose that oxidation of the di-manganese unit, together with concerted PCET, results in a Mn(V)$\rightarrow$O moiety formed on the proximal Mn (explained below). A Mn(V)$\rightarrow$O has been invoked in other water-oxidizing mechanisms as well [4,5]. Results from density functional theory calculations predict that a Mn(V)$\rightarrow$O may be too unreactive for water oxidation and that a spin state change to a Mn(IV)$\rightarrow$O$^*$ is required for reactivity [4]. Spin state changes may be quite relevant to the mechanism of water oxidation particularly because of the metallo-radical nature of the OEC. However, in the absence of a detailed structure of the OEC, it is difficult to draw straightforward conclusions from DFT. Pecararo and coworkers proposed a water oxidation mechanism in which PCET results in the formation of a Mn(V)$\rightarrow$O that undergoes nucleophilic attack by a calcium-bound hydroxide [5]. They also demonstrated that the coupling of proton transfer to electron transfer will significantly lower the activation energy for S-state advancement by preventing the accumulation of positive charge on the Mn$_4$ cluster. We also propose that O–O bond formation involves nucleophilic attack, but of a Ca-bound water on the electrophilic Mn$\rightarrow$O in a step mediated by Cl$^-$. The role of Ca$^{2+}$ is both structural and to serve as a Lewis acid to aid in the deprotonation of the second substrate water. The inequivalency of the substrate water-binding sites is supported by measurements of the rates of exchange of water in the $S_1$-state and the affinities of the substrate waters under turnover conditions [26]. It has been shown by mass spectrometry that in the $S_1$-state, the substrate waters exchange with solvent with two quite different rates, and that the affinities of the two substrate waters vary independently during turnover (discussed in further detail below) [26,130,131]. We will now consider this structural model with regard to the geometric constraints determined by biophysical studies of PSII and inorganic modeling of the OEC.

In our model, the proximal Mn ion forms the Mn(V)$\rightarrow$O in which the terminal oxo is trans to an
imidazole moiety, which we suggest to be from D1-H332. This proximal Mn is bridged to Ca$^{2+}$ by Cl$^{-}$ with a Mn$^{2+}$Ca separation of 4.2 Å ($\text{Mn}^{2+}\text{Cl} 2.6$ Å, Cl$^{-}\text{Ca} 2.7$ Å, Mn$^{2+}$Cl$^{-}$Ca $110^\circ$). A calcium-bound substrate water molecule (Ca$^{2+}$O 2.4 Å) is positioned such that it forms a H-bond to the bridging W-oxo (OCa$^{2+}$H$^-$Oxo $175^\circ$) with the oxygen lone pair pointed towards the Mn$^{2+}$substrate water molecule (OCa$^{2+}$OMn 2.9 Å). Though not illustrated in Fig. 3, we propose that the second proton of the Ca-bound water is H-bonded to an amino acid acting as a Lewis base (see Fig. 1), which we suggest is D1-H337. This structure is consistent with the XAS data and incorporates amino acids that are thought to be ligands to the Mn$_4$ cluster or are implicated in S-state turnover. As mentioned previously, substitution of Ca$^{2+}$ by a lanthanide may favor a protonation state of the bridging oxo that inhibits S-state advancement by changing the reduction potential of the Mn$_4$ cluster.

As both calcium- and chloride-depleted samples give S$_2$Y$_Z$ spectra that are very similar, their roles, structural or functional, are likely connected. Acetate is a competitive inhibitor of Cl$^{-}$-binding [37], and ENDOR measurements have shown that acetate binds quite close to Y$_Z$ [47]; therefore, Cl$^{-}$ must as well. It is possible that acetate binds in place of Cl$^{-}$, bridges a Mn ion and Ca$^{2+}$ in a manner similar to that of D10 in concanavalin A [21,99], and inhibits water oxidation by blocking substrate water-binding. In our model for the acetate-inhibited state of the enzyme, one carboxylate oxygen is a monodentate ligand that occupies the substrate water-binding site on Mn, and the other oxygen bridges between Mn and Ca$^{2+}$ in place of Cl$^{-}$ (Fig. 4). Disruption of the hydrogen-bonding network would inhibit reduction of Y$_Z^*$ beyond S$_2$, resulting in blockage of advance past the S$_2$Y$_Z^*$-state [40].

Fig. 5 illustrates how the OEC model is incorporated into the theoretical structure of PSII [11] with minimal movement of the modeled residues. Included are Y$_Z$, D170, H190 and E189. Y$_Z$ was moved laterally 1 Å to bring the phenoxyl group to within H-bonding distance of the ε-N of H190; an adjustment in the position of Y$_Z$ is justified on the grounds that the C helix is tilted slightly differently in D1 relative to the L subunit of the bRC [8]. It should be noted that caveats of the calculated D1/D2 structural model include [11]: (1) the absence of metal ions during the energy minimization, so basic residues thought to ligate metals were protonated; (2)
the loop regions were not included; (3) differences in the number and position of proline residues between PSII and the bRC affect the backbone conformation; and (4) the theoretical structure was estimated to be accurate to within \( \sim 1 \) Å or so. With the pitch of \( Y_Z \) unchanged (22° relative to the membrane normal) [132,133] but rotating the carboxylate group of D170 (thus rotating the orientation of the Mn\(_3\)O unit), the Mn-bound water molecule can be brought to within H-bonding distance of \( Y_Z \) (2.6 Å). The orientation of the 3.3 Å Mn–Mn vector is then \( \sim 30° \) with respect to the membrane normal, which is within the range of the angle estimated from polarized EXAFS measurements (43 ± 10°) [108]. The distance between the center of \( Y_Z \) and the distal Mn is 7.5 Å; these distances and angles are in good agreement with the XAS and EPR data [45,108]. E189 was rotated from its minimized structure (participating in a salt bridge with D170) to the space behind H190 to avoid van der Waals contact with the Ca–Cl moiety of the OEC. It is possible that E189 is involved in proton transport [53] or, alternatively, E189 could be a ligand to Ca\(^{2+}\) or Mn. We do not comment on the structure of the remainder of the OEC (i.e. the other Mn\(^{IV}\)O\(_X\) unit) as this is probably ligated in the loop region, for which there is no available structure for modeling.

4.2. Mechanism of water oxidation

Our proposed S-state cycle is shown in Fig. 1. Based on the EPR and XANES data described above, we assign the Mn oxidation states in S\(_0\) as Mn(I)Mn(II)Mn(III)Mn(IV). The di-µ-oxo-bridged Mn(IV)\(_2\) unit does not change oxidation state during turnover, so we omit it from the rest of the figure. Because of the low oxidation states of Mn in the Mn(II)Mn(III) dimer, we suppose that a µ-hydroxo is present in S\(_0\). This follows the trends observed for other low-valent dinuclear metal sites in proteins, such as hemerythrin [Fe(II)\(_2\)(µ-OH)] and manganese catalase [Mn(II)\(_2\)(µ-OH)] [129] and also model Mn complexes [104,105]. Oxidation to S\(_1\) involves the ET/PT pathway for \( Y_Z \) reduction and deprotonation of the µ-OH bridge to form a [Mn(III)\(_2\)(µ-O)] dimer. Likewise, advance to S\(_2\) also utilizes the ET/PT pathway for \( Y_Z^* \) reduction. Because no proton is lost from the OEC in this step, one may expect a net increase in positive charge to accumulate; such a net charge increase is observed to persist through S\(_2\) and S\(_3\) as an electrochromic shift in the chlorophyll UV-visible absorbance spectra [62,71–73]. We hypothesize that this charge increase is what acts as the molecular switch between concerted and concerted PCET. The net positive charge now favors removal of protons from the OEC with each subsequent oxidation to prevent further charge increase and also because of electrostatic repulsion of protons which would re-protonate H190. This causes a change from ET/PT to the concerted ETPT pathway for \( Y_Z^* \) reduction. While the aforementioned study by Pecoraro and co-workers demonstrates that a net positive charge increase results in an energetic penalty of several hundred mV for the subsequent oxidation of a metal cluster, this analysis requires that no structural rearrangements occur in the oxidized complex [5]. It has been shown by EXAFS that there is a structural change of the Mn\(_4\) cluster in the \( S_2 \rightarrow S_3 \) transition [16], which coupled with factors such as hydrogen-bonding could significantly lower the increase in reduction potential.

Oxidation beyond S\(_2\) requires H-atom abstraction, resulting in a Mn(IV)–OH in S\(_3\). (Although we favor Mn-centered oxidation in the \( S_2 \rightarrow S_3 \) transition, movement of the oxidizing equivalent onto a ligand, as proposed based on XANES measurements [94], does not affect the subsequent chemistry of dioxygen production.) We propose that O–O bond formation does not occur until after the S\(_2\)-state, as is suggested by H\(^{18}\)O exchange studies [26]. Advancement to S\(_4\) forms an electrophilic Mn(V)=O species. The involvement of a Mn(V)=O species in O\(_2\) formation has been postulated for Mn complexes that are homogeneous water-oxidation catalysts [134–136]. The reactivity of the oxo may be enhanced through the occupation of a Mn=O LUMO by the lone pair of the trans imidazole (D1-H332 in our model), decreasing the Mn=O bond order and making the oxo more electrophilic (Fig. 6A). This supposition was formulated based on the observation that π-donor bases binding trans to the oxo group in Mn-porphyrin systems increase their reactivity as oxidation catalysts [137,138]. Furthermore, an increase in the net positive charge of a metal–oxo system has been shown to greatly increase its reactivity [139,140]; again, this is due to an increase in the electrophilicity.
of the oxo. Such a net positive charge may form in $S_2$, as mentioned above [62,71–73].

$O-O$ bond formation begins by bringing the second substrate water closer to the Mn(V)$\equiv O$ in an $S_N2$-like reaction (Fig. 6B). We propose that this occurs through contraction of the Mn$\equiv Cl$ bond upon formation of the high-valent Mn(V)$\equiv O$ moiety. Shortening of the Mn(V)$\equiv Cl$ bond would also increase the Lewis acidity of Ca$^{2+}$ because of a lengthening of the Ca$\equiv Cl$ bond. This would have the effect of enhancing the nucleophilicity of the Ca-bound water concomitant with formation of an electrophilic Mn(V)-bound oxo. In order for the O-O bond to form, there must be overlap between the oxygen lone pair of the water molecule with an empty (non- or anti-bonding) Mn$\equiv O$ orbital, in accordance with Woodward–Hoffman rules of symmetry. The orientation of the Ca-bound water is optimized for a nucleophilic attack by H-bonding to the $W-O$ bridge and possibly to a Lewis base; we suggest D1-H337 for this role. This base would also aid to deprotonate the water, as the $pK_a$ of Ca-bound water (12.7 for the aqua ion) is still too high to be deprotonated in vivo.

The resultant proton release pattern of the proposed cycle is in agreement with the experimentally observed 1:0:1:2 pattern [72,141,142], which is based on measurements of protons released to the bulk in thylakoid membrane preparations and also in accordance with the chlorophyll electrochromic band shifts [62,71–73]. However, the measurements of proton release have been shown to be dependent on the pH and the type of PSII preparation [142]. Because the proton release patterns represent release of protons into the bulk medium which includes both the protons released from the OEC and those arising from $pK_a$ changes of nearby amino acid residues, the pattern of protons released into the bulk medium will not necessarily reflect the pattern of protons released from the OEC.

The rate-limiting step of $O_2$ production is reduction of $Y_Z^*$ upon advancement from the $S_2$-state. Because $Y_Z^*$ reduction and $O_2$ release follow the same kinetics, $O-O$ bond formation and $O_2$ release occur very quickly once $Y_Z^*$ is reduced [143]. Thus, the $O_2$ formation and release depicted in Fig. 1 must occur from the entatic state on a rapid time-scale. Considering the high reactivity of Mn(III)$\equiv OOH$ species [144], our mechanism is in agreement with these kinetics.

This mechanism also agrees with the reported rates of exchange of the substrate waters [26]. In these experiments, the rate of $H_{18}^2O$ incorporation into the product $O_2$ as a function of $S$-state was measured by mass spectrometry. The slow phase rate constants were 8, 0.021, 2.2, and 1.9 s$^{-1}$ for $S_0$, $S_1$, $S_2$ and $S_3$, respectively, while the fast phase exhibited rates >100 s$^{-1}$ for $S_0$, $S_1$ and $S_2$ and 36.8 s$^{-1}$ in $S_3$. There is difficulty in comparing water ligand exchange rates of metal complexes in proteins versus free ions in solution as the former are not all well-characterized [145]. Some investigations show that the rate of water exchange from a metal site may decrease by many orders of magnitude due to contributions from steric and H-bonding [146]. Bearing this in mind, the relative changes in the rates fit into our mechanism. The fast-exchanging substrate water, the rate of which does not vary much with $S$-state, would be the water bound to calcium. The other water is bound to Mn and exchanges more slowly. Its rate of exchange is fastest in $S_0$, as one would expect for Mn(II). In $S_1$, the rate decreases dramatically, as the water can now H-bond to the rather basic $\mu-O$ bridge that is formed. Advancement to $S_2$ oxidizes Mn(III) to Mn(IV) and greatly decreases the
basicity of the bridging μ-O, which weakens the H-bond to the water, so exchange is faster. In advancing to S₃, a hydroxide ligand is formed, which is a better ligand than water but loses the ability to H-bond to the μ-O; the exchange rate remains about the same. At the same time, the H-bond between the μ-oxo bridge and the Ca-bound water can strengthen which slows the exchange in S₁. These changes in H-bonding to the bridging μ-oxo may also relate to the structural changes that occur in the Mn₄ cluster in the S₂ → S₃ transition as observed by EXAFS [16]. It is the proton of the Mn-bound water that is not H-bonded to the bridging μ-O that is part of the H-bond network to YZ. When the first proton is removed, the second takes its place.

Nucleophilic attack by the Ca-bound water on the Mn(V)N moiety, concomitant with its deprotonation, results in a transiently formed hydroperoxide species (denoted S₄* in Fig. 1), in which the OOH moiety is H-bonded to the bridging μ-O. This structure is analogous to that of reversibly bound O₂ in hemerythrin [147,148]. The active site of hemerythrin consists of an Fe(II) dimer connected by a mono-μ-hydroxo-di-μ-carboxylato bridge. O₂ binds as a hydroperoxide by oxidizing the dimer to Fe(III)₂ and deprotonating the μ-OH to a μ-O; the proton remains H-bonded to the bridging μ-O. The Fe(III) ions are separated by 3.3 Å with an Fe–O–Fe angle of 125°. The release of O₂ is simply the reverse of its binding, in which the proton is transferred back to the μ-O and the iron dimer is reduced to the diferrous state. By analogy, release of O₂ from the OEC is proposed to proceed by a similar mechanism. In hemerythrin, reversible O₂-binding is allowed energetically because the reduction potentials for the [Fe(II)₂/Fe(III)₂] couple in hemerythrin [149] and [HOO⁻/O₂] [150] are nearly equal, approximately 500 mV. However, in the case of the Mn₄ cluster, the reduction potential for the S₀-state is substantially higher than that of hydroperoxide, and so oxidation of hydroperoxide is energetically favored. The reduction of the terminal Mn(III)–OOH to Mn(II) results in the release of O₂ and the protonation of the μ-oxo bridge, resetting the OEC to S₀.

5. Conclusions

In this minireview, we have developed a structural and functional model of the OEC that incorporates elements of the protein environment and is in good agreement with both the spectroscopic and biochemical data. The important aspects of the model are: (1) a Mn₂O unit can be ligated by D1-D170 in such a way that a Mn-bound substrate water molecule can H-bond to YZ, which is required for H-atom abstraction of substrate protons by Y₂*; (2) the orientations of the Mn are in agreement with EXAFS results and the distances and angles between Mn and YZ reflect those measured by EPR; (3) the reduction of Y₂* proceeds via the ET/PT pathway in S₀ → S₂ but requires ET/PT for S₂ → S₄ advancement, with ultimate formation of an electrophilic Mn(V) = O moiety; (4) the second substrate water molecule binds to Ca and is oriented properly for a nucleophilic attack on the Mn(V) = O moiety by H-bonding to the μ-oxo bridge and possibly an amino acid residue functioning as a base; (5) the O–O bond-forming step involves a transient hydroperoxide species that releases O₂ in analogy to the O₂ release reaction of oxyhemerythrin.

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