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# Photosynthetic water oxidation: binding and activation of substrate waters for O–O bond formation

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Photosynthetic water oxidation occurs at the oxygen-evolving complex (OEC) of Photosystem II (PSII). The OEC, which contains a  $\text{Mn}_4\text{CaO}_5$  inorganic cluster ligated by oxides, waters and amino-acid residues, cycles through five redox intermediates known as  $S_i$  states ( $i = 0-4$ ). The electronic and structural properties of the transient  $S_4$  intermediate that forms the O–O bond are not well understood. In order to gain insight into how water is activated for O–O bond formation in the  $S_4$  intermediate, we have performed a detailed analysis of S-state dependent substrate water binding kinetics taking into consideration data from Mn coordination complexes. This analysis supports a model in which the substrate waters are both bound as terminal ligands and react via a water-nucleophile attack mechanism.

## 1. Introduction

The climate, biology and geology of Earth were transformed by the evolution of oxygenic photosynthesis approximately three billion years ago.<sup>1</sup> All photosynthetic organisms, from the earliest cyanobacteria to modern vascular plants, use solar energy to oxidize water to molecular oxygen ( $\text{O}_2$ ), protons and electrons at the oxygen-evolving complex (OEC) of Photosystem II (PSII).<sup>2,3</sup> PSII is a membrane-bound pigment–protein complex that generates a solar light-induced charge separation in order to oxidize water and reduce plastoquinone. The charge separation is initiated by the primary chlorophyll-a electron donor,  $\text{P}_{680}$ , forming a powerful oxidant,  $\text{P}_{680}^+$ , that advances the oxidation state of the  $\text{Mn}_4\text{CaO}_5$  cluster in the OEC (Fig. 1) *via* a redox-active tyrosine,  $\text{Y}_Z$ , that mediates electron transfer from the OEC to  $\text{P}_{680}^+$ . The overall four-electron process leads to the formation of  $\text{O}_2$  that is released as a byproduct and has accumulated in the biosphere and the release of protons that contribute to the *trans*-membrane proton motive force.<sup>4</sup> The availability of  $\text{O}_2$  as an electron sink has since powered oxygenic respiration to give rise to Earth's current biological diversity.

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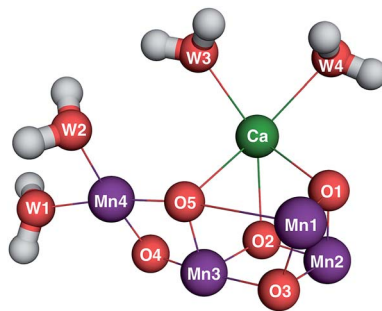


Fig. 1 Quantum mechanics/molecular mechanics (QM/MM) optimized  $S_1$  structure of the OEC<sup>5</sup> based on the 1.9 Å resolution crystal structure by Shen and coworkers.<sup>6</sup>

The catalytic cycle of the OEC involves five metastable redox intermediates known as  $S_i$  states ( $i = 0-4$ ), as first described by Kok and coworkers.<sup>7,8</sup> The dark-stable intermediate,  $S_1$ , contains the Mn oxidation state pattern (III,IV,IV,III)<sup>9</sup> (order of oxidation states is based on the numbering of the Mn ions in Fig. 1) and is converted to  $S_2$  upon the loss of one electron.  $S_2$  is present as two spin isomers with oxidation states of (III,IV,IV,IV) ( $S = 1/2$ ) or (IV,IV,IV,III) ( $S = 5/2$ ).<sup>10</sup> The next oxidation event results in the loss of one proton and one electron from the OEC to form  $S_3$ , in which all four Mn ions are in the +4 oxidation state ( $S = 3$ ).<sup>11,12</sup> Oxidation of  $Y_Z$  following the next charge separation causes release of a proton from  $S_3$  to form a modified  $Y_Z \cdot S_3'$  state,<sup>13</sup> which then forms the  $S_4$  state. The structures of  $S_3'$  and  $S_4$  are poorly understood.  $S_4$  spontaneously produces  $O_2$ , binds substrate water(s), and releases a proton to form  $S_0$ , thus resetting the catalytic cycle.

The general structure of the OEC has been revealed through a series of X-ray diffraction (XRD) studies.<sup>6,14-17</sup> However, the atomic structure of any single Kok cycle intermediate has not been determined through these experiments. Early XRD structures of the OEC suffered from X-ray induced reduction of the high-valent Mn centers.<sup>18</sup> Nevertheless, the availability of metal-metal distances from extended X-ray absorption fine structure (EXAFS) spectroscopy<sup>19,20</sup> allowed computational studies (QM/MM and density functional theory (DFT)) to refine XRD models to structures corrected for radiation damage.<sup>5,21</sup> At present, the PSII research community is in general (but not universal<sup>3</sup>) agreement on the structures of the  $S_0$ ,<sup>5</sup>  $S_1$ ,<sup>5,10</sup> and  $S_2$ <sup>10</sup> intermediates and the Mn oxidation states of all  $S$  states.<sup>9</sup>

The problem of X-ray induced Mn reduction can be completely avoided by using femtosecond pulses from X-ray free electron laser (XFEL) radiation sources.<sup>22,23</sup> XFEL studies using PSII nanocrystals have produced structures of dark-adapted PSII at 6.5–4.9 Å resolution.<sup>24-27</sup> Structures of  $S_2$ ,<sup>25,26</sup>  $S_3$ ,<sup>26,27</sup> and  $S_0$ <sup>26</sup> have also been reported at low resolution, which limits their interpretation. However, QM/MM methods combined with electron density map analysis have shown that the  $S_1 \rightarrow S_2$  transition involves significant displacement (and oxidation) of Mn4 (also referred to as the “dangler” Mn).<sup>28</sup> A 1.95 Å resolution XFEL structure of dark-adapted PSII was recently reported by Shen and coworkers.<sup>29</sup> However, the resulting XRD model of the OEC does not agree well with EXAFS data of  $S_1$ ,<sup>19</sup> which is likely the result of inaccuracy in positioning of the bridging oxo ligands and significant accumulation of  $S_0$ .<sup>30</sup>



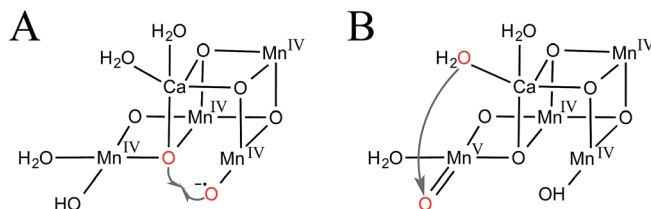


Fig. 2 Proposed mechanisms of O–O bond formation in the  $S_4$  intermediate. (A) Oxo-oxyl radical coupling and (B) water-nucleophile attack. Substrate waters are shown in red.

The  $S_4$  state decays faster than it is formed and, therefore, cannot be observed as a kinetic intermediate. Consequently, no direct experimental evidence for the nature of O–O bond formation in  $S_4$  has been produced. However, insights from inorganic and computational chemistry have produced two competing mechanisms: water-nucleophile attack and oxo-oxyl radical coupling.

In the oxo-oxyl radical coupling mechanism,<sup>31–33</sup> a Mn(IV)-oxyl radical reacts with a Mn-bridging oxo to generate  $O_2$  (Fig. 2A). Extensive calculations by Siegbahn suggest that one of the substrate waters first binds during the  $S_2 \rightarrow S_3$  transition and then is oxidized to an oxyl radical in  $S_4$  to carry out the reaction.<sup>32</sup>

A water-nucleophile attack mechanism for the OEC<sup>34–37</sup> is most consistent with synthetic water oxidation catalysts.<sup>38</sup> As shown in Fig. 2B, such a mechanism would involve a terminal water-nucleophile (preferably bound to calcium, see discussion below) and a formally Mn(V)-oxo electrophile.

These mechanisms can be distinguished by the positions of the substrate waters involved. Kinetics of substrate water exchange throughout the Kok cycle have been determined by membrane inlet mass spectrometry (MIMS) measurements following rapid mixing of PSII with  $H_2^{18}O$ . This technique, which was pioneered in the Wydrzynski laboratory, allows  $^{34}O_2$  and  $^{36}O_2$  release by PSII in specific S states (advanced by single-turnover flashes) to be monitored as a function of incubation time with  $H_2^{18}O$ .<sup>39,40</sup> The resulting rates of substrate water exchange are summarized in Table 1. In  $S_0$ , only one slowly exchanging water ( $W_s$ ) is resolved with a rate constant of approximately  $10\text{ s}^{-1}$ . The exchange rate of  $W_s$  is then dramatically slowed to  $0.02\text{ s}^{-1}$  in  $S_1$ . In  $S_2$  and  $S_3$ , two phases of substrate water exchange kinetics are resolved; the slower and faster exchanging waters are referred to as  $W_s$  and  $W_f$ , respectively.  $W_s$  exchanges at a nearly identical rate in  $S_2$  and  $S_3$ , but the rate is much faster than in  $S_1$ .  $W_f$  exchanges at decreasing rates in  $S_1$ ,  $S_2$ , and  $S_3$ .

Determining the molecular mechanism of photosynthetic water oxidation is crucial for applying the principles of Nature's design to synthetic systems for solar

Table 1 Substrate water exchange rates in spinach thylakoids at  $10\text{ }^\circ\text{C}$ .<sup>39,41</sup>

	$k_s, \text{s}^{-1}$	$k_f, \text{s}^{-1}$
$S_0$	$\sim 10$	$>120$
$S_1$	$\sim 0.02$	$>120$
$S_2$	$\sim 2$	$\sim 120$
$S_3$	$\sim 2$	$\sim 40$



fuel production. In the following analysis, we detail models for substrate binding and O–O bond formation in the OEC in light of experimental evidence and comparisons to well-studied inorganic systems.

## 2. Where are the substrate binding sites?

### Kinetics and mechanism of terminal water vs. $\mu$ -oxo ligand exchange from model chemistry

Given our current understanding of the structure of the OEC in the  $S_0$ ,  $S_1$ , and  $S_2$  states, we can predict that the substrate waters that give rise to  $O_2$  are bound either as terminal ligands to  $Mn^{IV}$  and  $Ca^{2+}$ , or as  $\mu$ -oxo/hydroxo bridges. In order to understand the substrate water exchange kinetics of the OEC, we first look to inorganic model systems.

The exchange rate of a terminal water ligand on Mn depends greatly on the oxidation state, protonation, ancillary ligands, and geometry. As shown in Table 2, both the water exchange rate and  $pK_a$  of a terminal aqua ligand decrease dramatically with increasing oxidation state from  $Mn^{2+}$  to  $Mn^{3+}$  to  $Mn^{4+}$ . The hexa-aqua  $Mn(IV)$  complex has an especially slow water ligand exchange rate ( $<10^{-4} s^{-1}$ ), as expected for a high-spin  $d^3$  ion with octahedral geometry for which the ligand field stabilization energy disfavors ligand dissociation leading to very slow ligand exchange. However, exchange of the terminal water ligands of the  $[Mn_4^{IV,IV,IV,IV}(\mu-O)_5(terpy)_4(H_2O)_2]^{6+}$  ( $terpy = 2,2':6',2''$ -terpyridine) complex occurs faster than the mixing time for the mass spectrometry measurement ( $\sim 10$  s,  $k_{ex} > 10^{-1} s^{-1}$ ), despite the high-spin  $d^3$  configuration of the  $Mn(IV)$  ions. This can be explained by the reduced symmetry of the  $Mn(IV)$  ions in the tetrameric oxomanganese-terpy complex. Deviations from octahedral geometry of the  $Mn^{IV}$  centers resulting from the  $<90^\circ$  bite angle of the tridentate terpy ligand and the asymmetry associated with oxo and pyridyl ligands result in loss of degeneracy of the  $t_{2g}$  and  $e_g$  orbitals, and can lower the barrier for exchange of the terminal

Table 2  $pK_a$  values and exchange rates of terminal waters. Calculated values appear in italics

	$pK_a$	Reference	$k_{ex}, s^{-1}$	Reference
$[Ca(H_2O)_6]^{2+}$	12.8	43	$\sim 10^8$	44
$[Sr(H_2O)_6]^{2+}$	13.2	43	$\sim 10^9$	44
$[Mn(H_2O)_6]^{2+}$	10.6	45	$2 \times 10^7$	46
			$1 \times 10^7$	47
$[Mn(H_2O)_6]^{3+}$	0.7	45	$2 \times 10^3$	47
			<i>0.01–1</i>	39 and 48
$[Mn(H_2O)_6]^{4+}$	<0		$10^{-6}$ to $10^{-4}$	39
			$10^{-7}$ to $10^{-8}$	48
$[Mn_2^{III,IV}(\mu-O)_2(mes-terpy)_2(H_2O)_2]^{3+a}$	nd <sup>d</sup>		$>10^{-1}$	49
$[Mn_4^{IV,IV,IV,IV}(\mu-O)_5(terpy)_4(H_2O)_2]^{6+b}$	nd		$>10^{-1}$	49
$[Mn_2^{IV,IV}(\mu-O)_2(terpy)_2(H_2O)_2]^{4+c}$	1.8	50	nd	
$[Mn_2^{III,III}(L)_2(H_2O)]^{+c}$	19–20	51	nd	
$[Mn_2^{III,IV}(L)_2(H_2O)]^{2+c}$	10–11	51	nd	

<sup>a</sup> mes-terpy = 4'-mesityl-2,2':6',2''-terpyridine. <sup>b</sup> terpy = 2,2':6',2''-terpyridine. <sup>c</sup> L = 2-hydroxy-1,3-bis(3,5-X<sub>2</sub>-salicylideneamino)propane. <sup>d</sup> nd = not determined.



water ligands. Indeed, computational studies have found low reaction barriers for the dissociative exchange of terminal waters in  $\mu$ -O bridged Mn(IV) dimers.<sup>42</sup>

Generally, ligand exchange for high-valent first-row transition ions involves a dissociative mechanism, especially for Mn(IV) which is normally six-coordinate and has a small ionic radius that makes an associative mechanism unfavorable. For terminal water ligands, dissociation involves removing a polar but neutral ligand from the cationic metal center, which has a modest barrier.<sup>52</sup> However, dissociative exchange of a  $\mu$ -oxo ligand involves removing an anionic ligand from the cationic metal center, which has a much higher barrier. Therefore, the exchange of  $\mu$ -oxo ligands with bulk water is thermodynamically more challenging than the exchange of terminal water ligands.

The mechanism of  $\mu$ -oxo exchange has been investigated for di- $\mu$ -oxo di-Mn(III,IV) complexes.<sup>53</sup> These studies have shown that  $\mu$ -oxo exchange requires protonation of the oxo before dissociation and exchange; therefore,  $\mu$ -oxo  $pK_a$ s are good predictors of their rates of exchange. The  $\mu$ -oxo  $pK_a$  decreases dramatically with increasing Mn oxidation state in Mn model complexes (Table 3). The rate of  $\mu$ -oxo exchange is very fast for Mn(III,III) dimers, owing to the high  $pK_a$  of the  $\mu$ -oxo ligands. Indeed, di- $\mu$ -oxo di-Mn(III,III) complexes are generally not stable to hydrolysis. For di- $\mu$ -oxo di-Mn(III,IV) complexes, the rate of  $\mu$ -oxo exchange is on a timescale of minutes ( $t_{1/2} = 21$  min for the  $[\text{Mn}_2^{\text{III,IV}}(\mu\text{-O})_2(\text{bpy})_4]^{3+}$  complex, bpy = 2,2'-bipyridine).<sup>53</sup> However, no  $\mu$ -oxo exchange could be detected on a one-day timescale for all-Mn(IV) complexes, such as  $[\text{Mn}_4^{\text{IV,IV,IV,IV}}(\mu\text{-O})_5(\text{terpy})_4(\text{H}_2\text{O})_2]^{6+}$ , reflecting the very low  $pK_a$  of the  $\mu$ -oxo ligands in all-Mn(IV) complexes (Table 3).

The  $[\text{Mn}_2^{\text{III,IV}}(\mu\text{-O})_2(\text{terpy})_2(\text{H}_2\text{O})_2]^{3+}$  complex contains terminal water ligands adjacent to the  $\mu$ -oxo ligands. It has been found that the terminal water ligands accelerate the rate of  $\mu$ -oxo exchange.<sup>53</sup> Because the  $pK_a$  of the water ligand bound to a high-valent Mn center is lower than solvent water (Table 2), a  $\mu$ -oxo can be more readily protonated by ligated water, which opens up the  $\mu$ -oxo bridge and lowers the energetic barrier for dissociation.<sup>53</sup> In this mechanism, the terminal water and  $\mu$ -oxo are in dynamic equilibrium with bulk water. For Mn dimers without terminal water ligands, the exchange is much slower, such as in  $[\text{Mn}_2^{\text{III,IV}}(\mu\text{-O})_2(\text{bpy})_4]^{3+}$ , because intramolecular proton transfer between terminal-bound water and  $\mu$ -oxo bridges is eliminated.<sup>53</sup>

There have also been measurements of terminal water and  $\mu$ -oxo ligand exchange of the di-Mn center in Mn-catalase.<sup>54</sup> The active site of Mn-catalase contains a redox-active di-Mn center for which ligand exchange has been studied in the di- $\mu$ -oxo di-Mn(III,IV) state. It was found that the terminal water ligand exchanges within the mixing time of one minute, but the  $\mu$ -oxo ligands require over one hour for exchange. These results parallel those obtained for Mn model complexes in solution, although the exchange rate of the  $\mu$ -oxo ligands is much lower in the protein active site.

**Table 3**  $\mu$ -oxo  $pK_a$  values of  $[\text{Mn}_2(\mu\text{-O})_2(\text{bpy})_4]^{n+}$ . Calculated values appear in italics

	$pK_a$	Reference
$[\text{Mn}_2^{\text{III,III}}(\mu\text{-O})_2(\text{bpy})_4]^{2+}$	<i>12.4</i>	55
$[\text{Mn}_2^{\text{III,IV}}(\mu\text{-O})_2(\text{bpy})_4]^{3+}$	2.3	49
$[\text{Mn}_2^{\text{IV,IV}}(\mu\text{-O})_2(\text{bpy})_4]^{4+}$	−6.5	55



In summary, electrostatics play a dominant role in the dissociative ligand exchange reactions of high-valent oxo-manganese complexes. There is a large barrier for  $\mu$ -oxo exchange due to the high negative charge of the oxo ligand. Thus, protonation of the  $\mu$ -oxo ligand is required to promote ligand exchange. Because of the large  $pK_a$  shift of the  $\mu$ -oxo ligands between di- $\mu$ -oxo di-Mn(III,IV) and di- $\mu$ -oxo di-Mn(IV,IV) complexes,  $\mu$ -oxo ligands exchange at a modest rate for di- $\mu$ -oxo di-Mn(III,IV) complexes but do not exchange for di- $\mu$ -oxo di-Mn(IV,IV) centers. On the other hand, terminal water ligands exhibit fast rates of ligand exchange for both Mn(III) and Mn(IV) centers. Moreover, the rate of terminal water ligand exchange is similar for Mn(III) and Mn(IV) because both have similar electrostatic potential (ESP) charges.<sup>52</sup>

### Ammonia does not compete with substrate water

The water analog ammonia inhibits the OEC by binding to two sites in PSII. The first is in the outer coordination sphere of the OEC and upon binding of ammonia, alters the hydrogen-bonding networks in such a way that the  $S_2$  state  $S = 5/2$  isomer is favored.<sup>56</sup> The binding of ammonia to this site is competitive with chloride<sup>57,58</sup> and can be accessed by larger amines,<sup>56,58</sup> but its specific location has not been resolved.

Ammonia also binds directly to Mn in the OEC in the  $S_2$  state resulting in the formation of an altered multiline  $g = 2$  EPR signal.<sup>59</sup> However, the substrate water exchange kinetics of  $S_2$  do not change when ammonia is bound.<sup>60</sup> Therefore, the binding site of ammonia *cannot* be the binding site of a substrate water.

Lubitz and coworkers have proposed that ammonia binds to Mn4 *trans* to O5 based on  $^{17}\text{O}$ -ELDOR-detected NMR (EDNMR) measurements.<sup>60</sup> In these experiments, dark-adapted PSII (poised in  $S_1$ ) is incubated with  $\text{H}_2^{17}\text{O}$  for tens of minutes and perturbations in the  $^{17}\text{O}$ -EDNMR signal are detected when ammonia (as  $\text{NH}_4\text{Cl}$  at pH 7.6) is present following advancement to the  $S_2$  state by 200 K illumination and subsequent annealing at 260 K. In accompanying  $^1\text{H}$ -ENDOR measurements, no change in the proton environment around the OEC was observed.<sup>60</sup>

Other lines of evidence have suggested that ammonia binds as a bridging ligand between two Mn ions. This motif was first proposed by Britt and coworkers based on ESEEM measurements of the ammonia-bound  $S_2$  state in higher plant PSII.<sup>61</sup> The quadrupole coupling between the  $S = 1/2$   $S_2$  state of the OEC and bound  $^{14}\text{NH}_3$  was resolved as 1.61 MHz with  $\eta = 0.59$ . A very similar coupling was observed in cyanobacterial PSII (1.52 MHz,  $\eta = 0.47$ ).<sup>60</sup> For comparison, a nitrogen nucleus with purely axial symmetry is characterized by  $\eta = 0$ , while ammonia

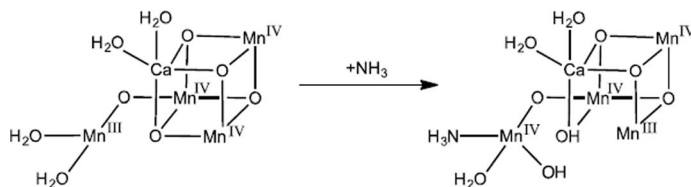


Fig. 3 Proposed mechanism of ammonia binding in the  $S_2$  intermediate. Ammonia binds as an additional ligand to the dangler Mn in the  $S = 5/2$   $S_2$  state, which induces a redox switch to the  $S = 1/2$  state. Adapted from ref. 63.



(amino) ligands display  $\eta \leq 0.3$ . Therefore, the measured quadrupole coupling of ammonia to  $S_2$  is highly deviant from axial symmetry and could represent a deprotonated bridging ligand such as an imido ( $\text{NH}^{2-}$ ) or nitrido ( $\text{N}^{3-}$ ). However, Britt and coworkers have very recently revealed the origin of this anisotropy. In wild type cyanobacterial PSII, they measured the  $^{14}\text{NH}_3$  coupling as 1.62 MHz with  $\eta = 0.40$ , but in a D1-D61A mutant, the interaction was nearly completely axial (1.54 MHz,  $\eta = 0.04$ ).<sup>62</sup> This work provides convincing evidence that bound ammonia has a strong hydrogen bond to Asp61 and is, therefore, a terminal ligand to Mn4.

In a recent study using QM/MM methods and EXAFS simulations, we have proposed that instead of replacing a terminal water or  $\mu$ -oxo group, ammonia binds as a sixth ligand to Mn4 resulting in a complete octahedral coordination sphere.<sup>63</sup> In this model (Fig. 3), ammonia binds to the  $S = 5/2$   $S_2$  state but then induces a redox switch stabilizing the  $S = 1/2$   $S_2$  state by protonating O5 and deprotonating W2. This additional ligand would lower the reduction potential of ammonia-bound  $S_2$  as has been observed by both thermoluminescence<sup>64</sup> and flash  $\text{O}_2$  measurements.<sup>65</sup>

While both substrate waters are already bound in  $S_2$ ,<sup>41</sup> an additional water may bind during the  $S_2 \rightarrow S_3$  transition as computationally predicted.<sup>32</sup> We have proposed that ammonia, a “harder” Lewis base than water, binds in  $S_2$  to the dangler Mn4 in an analogous site as water binds in  $S_3$  to the dangler Mn4.<sup>63</sup> The addition of a ligand to Mn4 in either  $S_2$  or  $S_3$  *trans* to O5 would cause the other terminal water ligands (W1 and W2) to shift their positions towards the cuboidal core of the OEC. In this model of ammonia binding, both water–nucleophile attack and oxo–oxyl radical coupling O–O bond formation mechanisms are feasible.

### A model for substrate water exchange in the OEC

**W2 exchanges rapidly.** In agreement with previous analyses,<sup>66,67</sup> we assign the kinetic features of  $W_f$  primarily to the terminal water W2. This water is already bound in  $S_2$  as resolved in measurements at reduced temperatures<sup>41</sup> and in PSII cores from the red alga *Cyanidioschyzon merolae*.<sup>68</sup> In  $S_0$  and  $S_1$ , W2 is present as a  $\text{Mn}^{3+}$ -aqua species and would be rapidly exchanged ( $>120 \text{ s}^{-1}$ ), consistent with the predicted exchange kinetics of  $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$  (Fig. 4, Table 2).

For the water–nucleophile attack mechanism, the substrate waters are W2 and W3. W3, which is bound to  $\text{Ca}^{2+}$ , is also expected to exchange rapidly (specifically in  $S_0$  and  $S_1$ , see discussion below). Therefore, the  $W_f$  kinetic (measured by monitoring  $^{34}\text{O}_2$ ) may reflect contributions from both W2 and W3.

The exchange rate of W2 is first resolved after the  $S_1 \rightarrow S_2$  transition with a value in the  $S_2$  state of  $k_f \sim 120 \text{ s}^{-1}$ . As discussed above,  $S_2$  is present as two spin isomers. Given the changes seen in XFEL experiments at the Mn4 position measured at physiological temperature, we have asserted that the  $S = 1/2$  isomer is dominant.<sup>28</sup> Therefore,  $W_f$  would be bound as a terminal  $\text{Mn}^{4+}$ -aqua species in the  $S_2$  state and exchanges at a rate comparable to those measured in model complexes (Table 2).

As modeled in Fig. 4, the formation of  $S_3$  involves the release of a proton from W2 and a 3-fold slowing of its exchange rate (Table 1). This change is qualitatively consistent with the differences expected between  $\text{Mn}^{4+}$ -aqua and  $\text{Mn}^{4+}$ -hydroxo species.





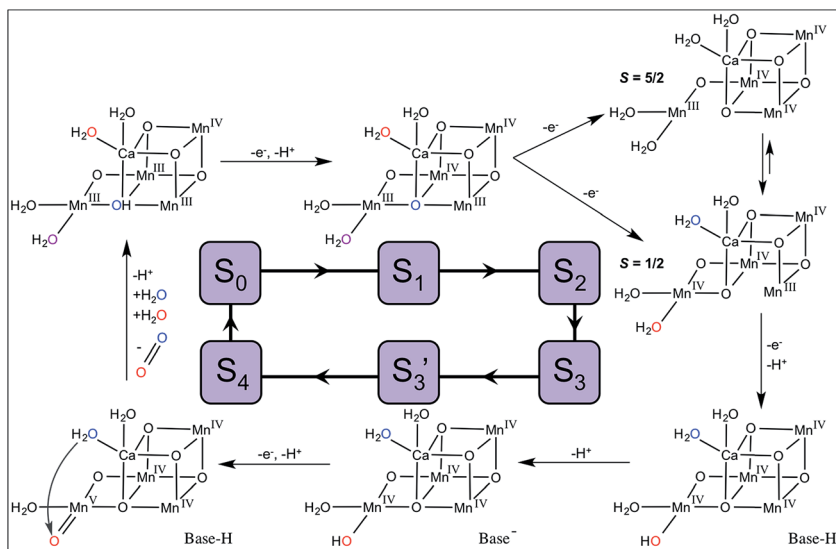


Fig. 4 Proposed mechanism of OEC turnover and substrate water exchange.  $W_s$  is shown in blue and  $W_f$  in red (in  $S_0$  and  $S_1$ ,  $W_2$  is shown in purple as it contributes to both the  $W_s$  and  $W_f$  kinetics).

**$W_s$  in  $S_0$  and  $S_1$  involves O5/ $W_2$  exchange.** Identifying the origin of the substrate water exchange rates for the  $W_s$  kinetic phase is more complex. The water exchanges fastest in  $S_0$ , dramatically slows down in  $S_1$ , and then dramatically speeds up again in  $S_2$  and  $S_3$ . These observations are difficult to explain based on exchange at a single site. Herein, we propose a new model for  $W_s$  exchange that involves contributions from both terminal and bridging ligands ( $W_2$  and O5, respectively) whose interconversion is possible only in the  $S_0$  and  $S_1$  states owing to the decreasing  $pK_a$  of the  $\mu$ -oxo species, O5, with increasing Mn oxidation states.

It has been determined that only one of the  $\mu$ -oxo bridges denoted O5 slowly exchanges with  $H_2^{17}O$  in the dark-stable  $S_1$  state over tens of seconds.<sup>54,69</sup> We assume that O5 also exchanges with bulk water in  $S_0$  (oxidation states  $Mn^{III,IV,Mn,III}$ ). QM/MM models of the  $S_0$  state<sup>5</sup> predict that O5 is protonated, as expected if its  $pK_a$  is compared to  $Mn^{III,III}$  dimers (Table 3). Consequently, O5 exchange would be expected to occur relatively rapidly in  $S_0$  ( $10\ s^{-1}$ ) because it is already protonated at physiological pH. During exchange, protonated O5 may dissociate from Mn3 becoming a terminal water ligand to Mn4. Thus, in the  $S_0$  state,  $W_2$  (purple, Fig. 4) and O5 (blue, Fig. 4) are expected to be in rapid equilibrium with each other.

In  $S_1$  (oxidation states  $Mn^{III,IV,Mn,IV}$ ), O5 is present in a chemical environment analogous to  $Mn^{III,IV}$  dimer complexes and the  $pK_a$  of O5 in  $S_1$  is predicted to be much lower than in  $S_0$  (Table 3). Based on QM/MM models, O5 is not protonated in the  $S_1$  state<sup>5,21</sup> and, thus, would have a higher energetic barrier for exchange. This change is proposed to be the reason for the 500-fold decrease in the exchange rate of  $W_s$  observed in  $S_1$  as compared to  $S_0$ .





$W_f$  is not resolved in  $S_0$  and  $S_1$  and reflects the rapid exchange of  $W_2$  (and  $W_3$ , discussed below). Because  $\mu$ -oxo exchange involves subsequent dissociation, binding, and association steps,<sup>53</sup> isotopically labeled substrate will appear in both the O5 and  $W_2$  positions. Therefore,  $W_2$  is labeled with two rates. The first is rapid ( $>120\text{ s}^{-1}$ ) and reflects direct exchange with bulk water. The second kinetic is slow ( $0.02\text{--}10\text{ s}^{-1}$ ) and reflects the interconversion of labeled O5 with  $W_2$ . *Crucially, this mechanism implies that the rate of  $W_s$  exchange is controlled by the rate of O5 exchange in  $S_0$  and  $S_1$ , but that O5 is not necessarily a substrate water.*

For the dominant  $S_2$  state  $S = 1/2$  spin isomer (oxidation states IV,IV,IV,III), the O5 chemical environment is analogous to Mn(IV,IV) dimer complexes in which the  $\mu$ -oxo  $pK_a$  is very low (Table 3). Given the large energetic barrier for  $\mu$ -oxo exchange, the measured rate of  $W_s$  in  $S_2$  ( $2\text{ s}^{-1}$ ) is too fast to be assigned to O5.

**$W_s$  in  $S_2$  and  $S_3$  involves a terminal water bound to  $Ca^{2+}$ .** In  $S_0$  and  $S_1$ ,  $W_3$ , which is a substrate water in the proposed water-nucleophile attack mechanism (Fig. 2B and 4), exchanges at a rate  $>120\text{ s}^{-1}$  and its kinetic features are merged with those of  $W_2$ . However, the  $S_1 \rightarrow S_2$  transition does not involve the release of a proton to the bulk, but does involve an oxidation. The result is the build-up of a positive charge and a significant contraction of the OEC as recently observed in photothermal beam deflection experiments.<sup>13,70</sup> This accumulated charge (and contraction) is not released until the  $S_0$  state is reformed after  $O_2$  release.<sup>70</sup> As a result, we hypothesize that the  $Ca^{2+}$ – $W_3$  electrostatic interaction, as well as the surrounding hydrogen-bonding network, is stronger in  $S_2$  and  $S_3$  compared to  $S_0$  and  $S_1$ . This effect of the increased charge would have to be dramatic to explain the  $10^6$ -fold decrease in  $W_3$  exchange compared to the  $Ca^{2+}$ -aqua ion in bulk water (Table 2).

When  $Ca^{2+}$  is substituted with  $Sr^{2+}$ , only  $W_s$  is significantly affected:  $k_s$  is three- to four-fold faster in  $S_1$ ,  $S_2$ , and  $S_3$  when  $Sr^{2+}$  is present.<sup>71</sup> Both O5 and  $W_3$  are adjacent to  $Ca^{2+}/Sr^{2+}$  and are, therefore, reasonable candidates for  $W_s$ . Because  $Ca^{2+}$  and  $Sr^{2+}$  have very similar Lewis acidities (similar  $pK_a$ s of the aqua ion, Table 2),  $W_3$  is expected to be fully protonated throughout the Kok cycle providing an adequate nucleophile in the water-nucleophile attack mechanism.<sup>36</sup> However,  $Sr^{2+}$  is considerably larger than  $Ca^{2+}$  (ionic radii are  $1.12\text{ \AA}$  and  $0.99\text{ \AA}$ , respectively<sup>72</sup>) resulting in a weaker electrostatic interaction between  $Sr^{2+}$  and its ligands. This difference is reflected in the faster exchange rate of the water ligands bound to  $[Sr(H_2O)_6]^{2+}$  compared to  $[Ca(H_2O)_6]^{2+}$  (Table 2), and is also consistent with the faster exchange of  $W_s$  when  $Sr^{2+}$  is substituted for  $Ca^{2+}$  in the OEC.

In our model for OEC turnover and substrate water exchange shown in Fig. 4, the observed  $W_s$  kinetics reflect O5 exchanging with  $W_2$  in  $S_0$  and  $S_1$ , and  $W_3$  exchange in  $S_2$  and  $S_3$ . The  $W_f$  kinetic reflects both  $W_2$  and  $W_3$  exchange in  $S_0$  and  $S_1$ , and only  $W_2$  in  $S_2$  and  $S_3$ . This new substrate exchange model provides a complete description of all of the kinetic phases of substrate exchange throughout the S-state cycle and is consistent with data from model high-valent oxomanganese complexes. It, thus, provides a framework from which to design additional experimental measurements to test and validate this hypothesis.

### 3. Mechanism of O–O bond formation

The structure of the  $S_4$  intermediate will determine if the mechanism of photo-synthetic water oxidation involves water-nucleophile attack or oxo–oxyl radical



coupling. Unfortunately, few experimental clues about  $S_4$  are available. Given that the Mn oxidation states in  $S_3$  are (iv,iv,iv,iv), the  $S_4$  state is predicted to contain either a  $Mn^{IV}$ -oxyl radical or  $Mn^V$ -oxo species.

A  $Mn^{IV}$ -oxyl radical species has been suggested through computational studies by Siegbahn and supports an oxo-oxyl radical coupling mechanism. On the other hand, a high-spin  $Mn^V$ -oxo is predicted to serve as the electrophile in a water-nucleophile attack mechanism. Borovik and coworkers have recently reported experimental support for the latter option through studies of  $[Mn^V H_3 \text{buea}(\text{O})]$  (where *buea* = *tris*[(*N'*-*tert*-butylureaylato)-*N*-ethylene]aminato).<sup>73,74</sup> The *buea* ligand imposes  $C_3$  symmetry around Mn and stabilizes the Mn-oxo group through intramolecular hydrogen bonding.  $[Mn^V H_3 \text{buea}(\text{O})]$  is a high-spin  $S = 1$  system as confirmed by EPR spectroscopy and supported by DFT calculations.<sup>73</sup> Therefore, the  $d_{xz}$  and  $d_{yz}$  orbitals in  $[Mn^V H_3 \text{buea}(\text{O})]$  are degenerate and the Mn-oxo bond is likely to be weaker (and more reactive) than corresponding  $Mn^V$ -oxo species with tetragonal symmetry ( $S = 0$ ). By quantifying the hyperfine contributions from  $^{17}\text{O}$ -labeled  $[Mn^V H_3 \text{buea}(\text{O})]$  using EPR spectroscopy, Borovik and coworkers determined that 0.45 spins reside on the oxo group. Therefore, the  $Mn^V$ -oxo group is strongly covalent, but does not contain an oxyl radical. For  $[Mn^{III} H_3 \text{buea}(\text{O})]^{2-}$ , only 0.30 spins reside on the oxo group, suggesting that the Mn-O bond becomes more strongly covalent (and the unit more electrophilic) with increasing Mn oxidation state.<sup>74</sup>

To date, no experimental evidence of a  $Mn^{IV}$ -oxyl radical species has been found, calling into question the accuracy of the oxo-oxyl radical coupling mechanism. However, if a reactive high-spin  $Mn^V=O$  species is present in the  $S_4$  state, analogous to that found in  $[Mn^V H_3 \text{buea}(\text{O})]$ , a water-nucleophile attack mechanism would lead to O-O bond formation.

## 4. Conclusions

Determining the molecular mechanism of O-O bond formation in photosynthetic water oxidation remains one of the great challenges in bioinorganic chemistry. In the absence of direct experimental evidence of the  $S_4$  intermediate, we can predict its structure and, therefore, the mechanism of O-O bond formation by comparing the OEC to model Mn complexes and by determining where the substrate waters bind throughout the catalytic cycle.

In our proposed model of OEC turnover shown in Fig. 4, the substrate waters are W2 (terminal water on Mn4) and W3 (terminal water on  $\text{Ca}^{2+}$ ). The substrate water exchange kinetics in  $S_0$  and  $S_1$  are complicated by the dynamic equilibrium between W2 and the slowly exchanging  $\mu$ -oxo/hydroxo bridge, O5. This assignment of substrates dictates that O-O bond formation occurs *via* a water-nucleophilic attack of W3 (as water) on W2 (as a high-spin  $Mn^V=O$  species).

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