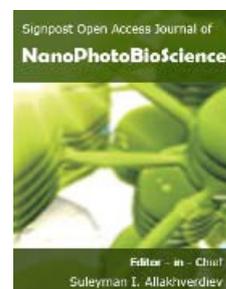


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## Recent Proposed Mechanisms for Biological Water Oxidation

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### Abstract

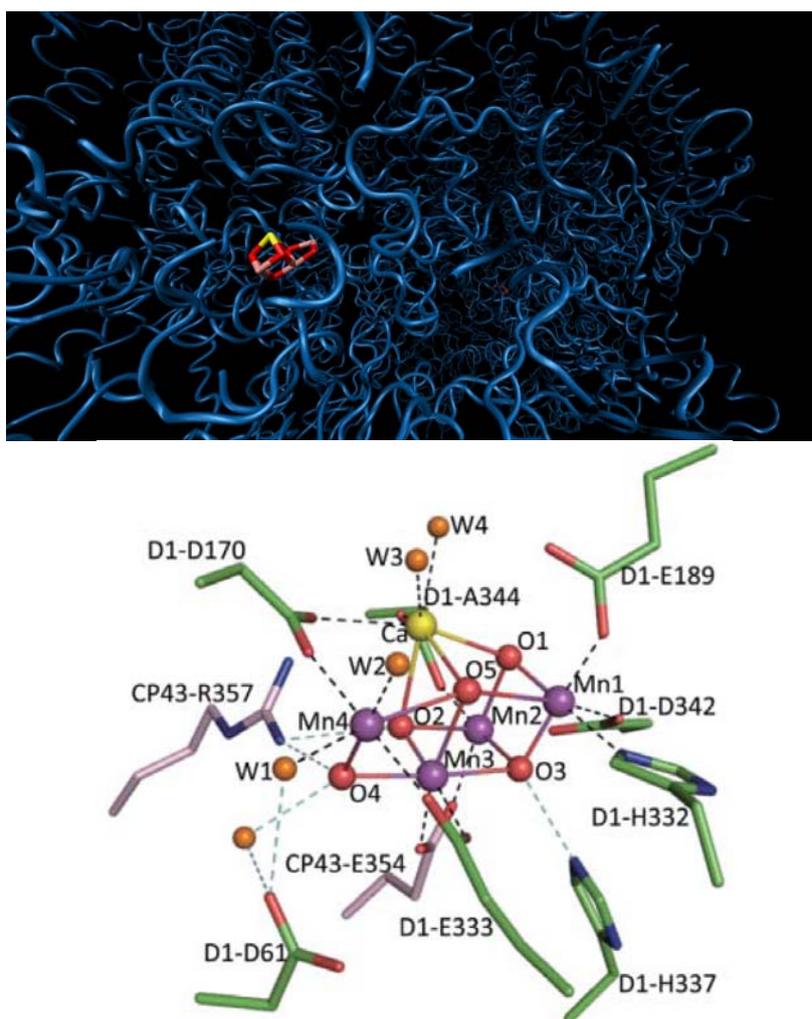
*Photosystem II (PSII) drives life on our planet by capturing sunlight and extracts electrons from H<sub>2</sub>O to reduce CO<sub>2</sub> into sugars while releasing the O<sub>2</sub> we breathe. In this review, the important proposed mechanisms of water oxidation by the water-oxidizing complex (WOC) of PSII are discussed and the structure of the WOC and its ligand environment are described with reference to the 1.9-Å resolution X-ray-derived crystallographic model of PSII from the cyanobacterium *Thermosynechococcus vulcanus*.*

## Introduction

A critical development in the first oxygenic photosynthetic organisms was the ability to store oxidizing equivalents because the water oxidation by the lowest activation energy is a four-electron process, whereas photochemistry is a one-electron process (Blankenship, 2002). Thus, oxygenic organisms need an enzyme to store oxidizing

equivalents and inorganic core of the enzyme is manganese-calcium cluster that charge accumulation is carried out by this tetranuclear manganese cluster in the WOC. The cluster is part of the PSII reaction center complex (Umena et al., 2011).

Pirson was the first one to find the importance of manganese in oxygenic photosynthesis (Pirson,



**Figure 1.**  $Mn_4O_5Ca$  cluster in PSII is housed in a special protein environment (shown in blue). The image was made with VMD and is owned by the Theoretical and Computational Biophysics Group, NIH Resource for Macromolecular Modeling and Bioinformatics, at the Beckman Institute, University of Illinois at Urbana-Champaign. The original data is from (Umena et al., 2011) (PDB: 3ARC) (a). Structure of the  $Mn_4CaO_5$  cluster (Umena et al., 2011) (b) (Image is from (Umena et al., 2011)).

1937). Since then, many groups used a variety of methods to give insight into the structure of the WOC. These methods were: 1) Electron paramagnetic resonance (EPR) and electron nuclear double-resonance (ENDOR) spectroscopy (Dismukes and Siderer, 1981; Evelo et al., 1989; Miller and Brudvig, 1991; Pace et al., 1991; Hasegawa et al., 1999; Ioannidis et al., 2002; Olesen and Andreasson, 2003; Britt et al., 2004; Pantazis et al., 2009; Teutloff et al., 2009). 2) Fourier-transform infrared (FTIR) spectroscopy (Hillier and Babcock, 2001; Yamanari et al., 2004; Debus, 2008; Noguchi, 2008; Chu, 2013). 3) X-ray absorption spectroscopy (XAS) (Yachandra et al., 1986; MacLachlan et al., 1992; Yachandra et al., 1993; Penner-Hahn, 1998; Dau et al., 2001; Sauer et al., 2008). 4) X-ray diffraction on PSII crystals (protein crystallography) (Zouni et al., 2001; Kamiya and Shen, 2003; Ferreira et al., 2004; Loll et al., 2005; Murray et al., 2008; Guskov et al., 2009; Kawakami et al., 2009; Broser et al., 2011; Umena et al., 2011).

For many years, it was accepted that the WOC contained four manganese, one calcium and likely also one chloride ion (Debus, 1992; Yachandra, et al., 1996). Based on the available data a number of structures for the WOC were proposed including symmetric  $Mn_4O_x$  structures such as cubane–adamantine or the butterfly model (Brudvig and Crabtree, 1986; Vincent and Christou, 1987; Rüttinger and Dismukes, 1997).

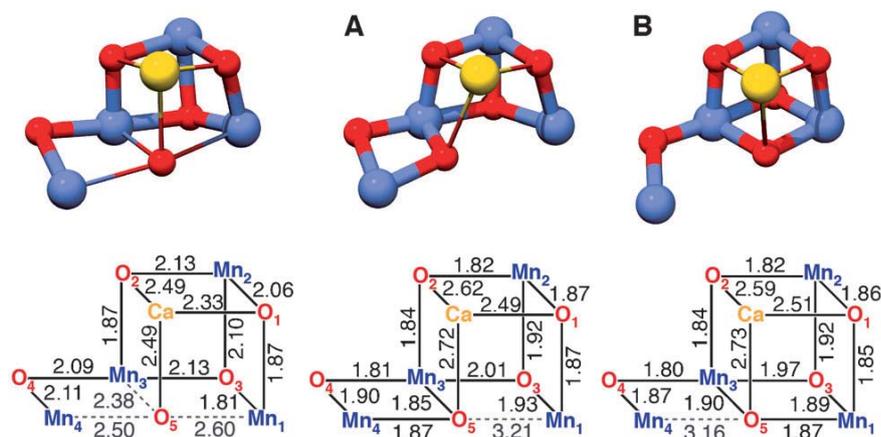
However, these structures were rejected by EXAFS experiments (Penner-Hahn, 1998). R. Britt and colleagues regarding electron spin echo electron nuclear double resonance experiments proposed a “dangler model” for manganese-calcium cluster (Peloquin et al., 2000). The first crystallographic model, with four Mn ions bound to PSII proteins, was presented at 3.8-Å resolution in 2001 (Zouni et al., 2001). Some more protein ligands were identified by Shen’ group in 2003 (Kamiya and Shen, 2003). In 2004, the research groups of J. Barber and S. Iwata provided, for the first time,

information on calcium: the WOC was a  $Mn_3Ca$ -cubane with the 4<sup>th</sup> Mn attached separately (Ferreira et al., 2004). They proposed that three manganese and one calcium ion form an elongated  $CaMn_3(\mu-O)_4$  cubane together with four bridging oxygen atoms. The fourth manganese ion was connected to the cube by binding to one of the bridging oxygen atoms, and was thereby positioned as a ‘dangler’. EXAFS data collected on protein crystals and EXAFS simulations in 2006 (Yano et al., 2006) led to the idea of four similar models for the  $Mn_4(\mu-O)_n$  core of the WOC. Batista, G. Brudvig and E. Sproviero developed structural models of the WOC in different states by means of Density Functional Theory (DFT) calculations (Sproviero et al., 2007). In 2011, the research groups of J.-R. Shen and N. Kamiya improved the resolution of the PSII crystals down to a high resolution of 1.9 Å and they analyzed the structure of the WOC in detail. This recent investigation has provided much more details of the structure of the WOC revealing the number and location of the bridged oxygens, the location of putative substrate water molecules and the precise arrangement of the amino-acid side chains (Umena et al., 2011). In the structure, four manganese ions, one calcium ion, and five oxygen atoms that serve as oxo bridges linking the five metal ions are found in the structure presented in Umena et al. (2011). In addition, four terminal water ligands were found, two of which were coordinated to Ca and two to the dangling Mn (Mn(4)). The structure suggests that the manganese-calcium cluster could be described as  $Mn_4CaO_5(H_2O)_4$  (Fig. 1). Of these five metals and five oxygen atoms, the calcium and three manganese ions occupy four corners while four oxygen atoms form the other four corners of the cubane-like structure. The fourth manganese ion is located outside the cubane and is linked to two manganese ions within the cubane by one oxygen of the cubane and the fifth oxygen by a di- $\mu$ -oxo bridge (an oxygen atom bridged between two or three metal ions) (Umena et al., 2011). However, recent experiments (Grundmeier and Dau, 2012; Lubner et al., 2011; Ames et al., 2011; Galstyan et al., 2012) showed that the Mn-Ca was damaged by radiation-induced Mn reduction. Long Mn-O bond

distances, which are chemically unreasonable for Mn(III/IV) oxidation states and the odd nonbonding position of a putative  $\mu_4$ -oxo bridge (O5) are two evidences in this regards (Robertazzi et al., 2011). Thus, some groups have concluded that this XRD model is mostly representative of the reduced  $S_3$  state (Galstyan et al., 2012; Grundmeier and Dau, 2012).

It is important to note that the two forms of  $S_2$  can be interconverted by near-infrared illumination (Boussac et al., 1996; Boussac et al., 1998b; Boussac et al., 1998a) (Fig. 2). On the other hand, theoretical calculations showed that for the WOC could be in two isoenergetic structures. Model A is consistently predicted to be more stable by a small margin of the order of  $1 \text{ kcal.mol}^{-1}$ . Regarding new data for the WOC, Kusunoki suggestion that several structures can in principle exist in the  $S_1$  state, which are close in energy and are related by proton migration (Kusunoki, 2011). Pantazis et al. with refinement of XRD structural models with analysis of their magnetic properties, showed that two

interconvertible structural forms of the WOC core exist in the  $S_2$  state. This finding provides a firm structural basis for rationalizing not only the spectroscopy of the  $S_2$  state, but also most of the related phenomenology for the site (Fig. 2). The group concluded that the isoenergetic nature of Model B with respect to Model A implies that the  $S_2$ - $S_3$  transition of the Kok cycle can potentially proceed through more than one pathway. This would be compatible with mechanism' Siegbahn for the  $S_2$  to  $S_3$  transition (Siegbahn, 2012) that involves an additional  $\text{H}_2\text{O}$  binding at Mn1 of structure A, but allows in principle for other possibilities if structure B also advances to  $S_3$  (Fig. 2). As Pantazis et al. believe the presence of an open coordination site along the Mn(III) pseudo Jahn–Teller axis is an inescapable result of the optimization of the photoreduced XRD structure and appears as a fundamental structural element of the  $S_2$ -state (Fig. 2).



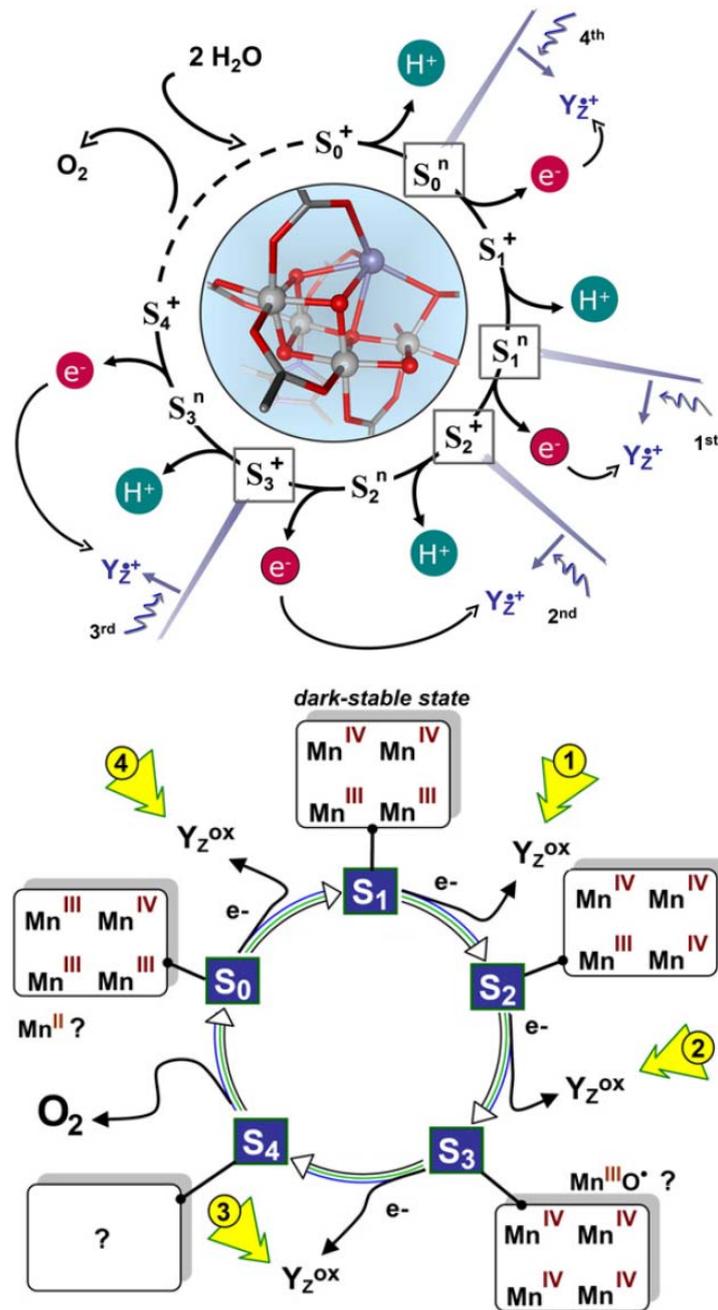
**Figure 2.** Two interconvertible structures could explain the spectroscopic properties of the WOC of PSII in the  $S_2$  state (Pantazis et al., 2012) (Images are from (Pantazis et al., 2012)).

## Mechanism of water oxidation

### Kok cycle

Pierre Joliot's experiments in 1969 showed that flash illumination produced an oscillating pattern for oxygen evolution and a maximum occurred on

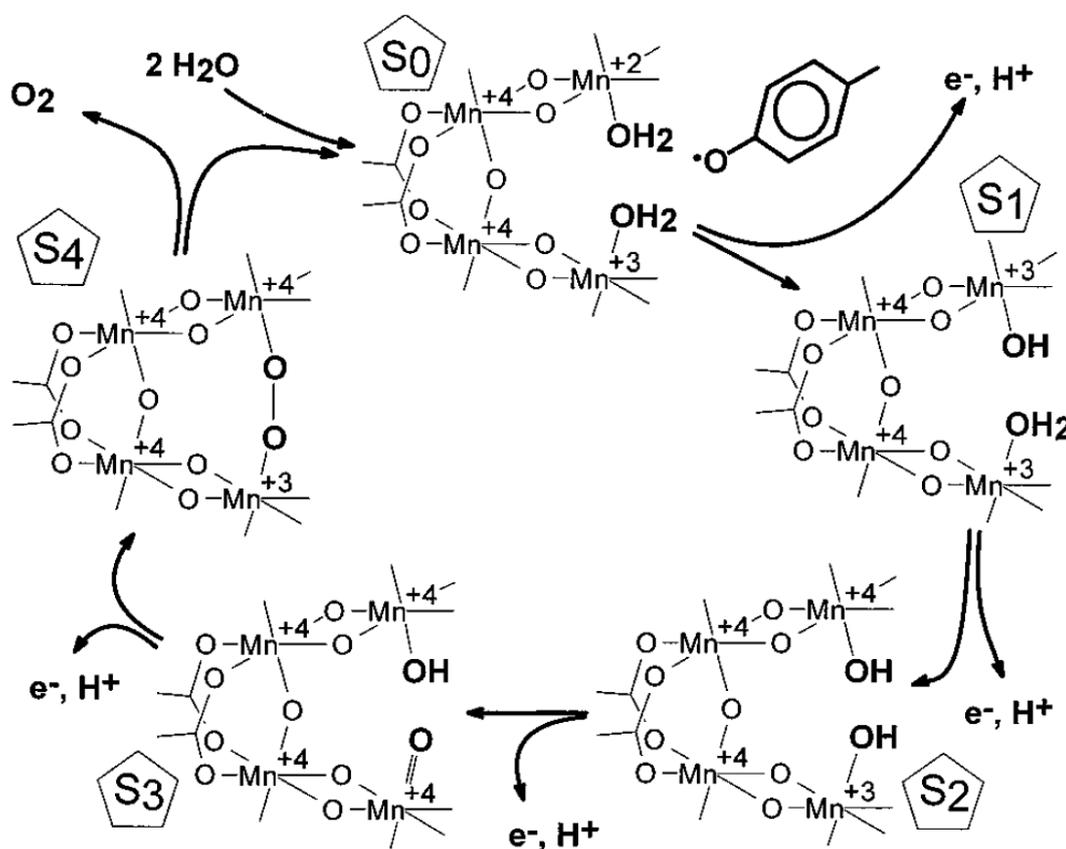
every *fourth* flash (Joliot et al., 1969) (Fig. 3). The conclusion that yields of oxygen is a function of the flash number and maximum occurred on every *fourth* flash are very interesting because splitting of two water molecules to produce one oxygen



**Figure 3.** Classical S-state cycle of photosynthetic water oxidation. Absorption of a photon causes charge separation at the reaction center  $P_{680}$  of PSII that leads to the formation of  $Y_Z^+$  (oxidized tyrosine-161 on the D1-protein) within less than one  $\mu s$ . Reduction of  $Y_Z^+$  by electron transfer (ET) from the manganese complex results in  $S_i \rightarrow S_{i+1}$  transition; typical time constants of the ET step are indicated in the diagram. There are several similar S-state cycle schemes. Here we show plausible oxidation-states of the four Mn ions in the different S-states (a). The extended S-state cycle including not only four oxidation but also four deprotonation steps is also shown (Figures are from (Grundmeier and Dau, 2012) (b)).

molecule requires the removal of *four* electrons. The experiments could show that each PSII complex operates independently. In 1970, B. Kok and colleagues (Forbush et al., 1971) proposed an explanation for the observed oscillation of the oxygen evolution pattern. In their model, each photochemical state removes a single electron from the WOC, which advances PSII to the next higher state ( $S_n$ ) state until there are four oxidizing equivalents in the complex, leading to the oxidation of two molecules of water. Each oxidation state of the WOC was termed an “S-state”, with  $S_0$  being the most reduced state and  $S_4$  the most oxidized state in the catalytic cycle (Scheme 1; also known as the “Kok cycle”) (McEvoy and Brudvig, 2006).

Identifying the chemical steps leading to water oxidation has proven to be a challenging problem. Each oxidation state of the WOC was termed an “S-state”, with  $S_0$  being the most reduced state and  $S_4$  the most oxidized state in the catalytic cycle (Scheme 1; also known as the “Kok cycle”) (McEvoy and Brudvig, 2006). All other S-state transitions are induced by the photochemical oxidation of oxidized chlorophyll ( $P_{680}^+$ ) (Forbush et al., 1971). Regarding the Kok cycle, recent results show proton transfer from the WOC into the lumen occurs with a 1:0:1:2 pattern within a strictly alternate sequence of electron and proton transfer steps (Dau and Haumann, 2007, 2008; Pushkar et al., 2008; Suzuki et al., 2009). The  $S_1$   $S_2$



**Figure 4.** A model for the S state cycle of the manganese cluster of PSII. For clarity,  $Y_Z$  is shown only in the  $S_0$  state. Calcium and chloride ions are required for oxygen evolution activity, particularly for the formation of the  $S_2$  state, and their specific functions within the context of the metalloradical mechanism are considered in detail elsewhere. Image and caption are from (Hoganson et al., 1997).

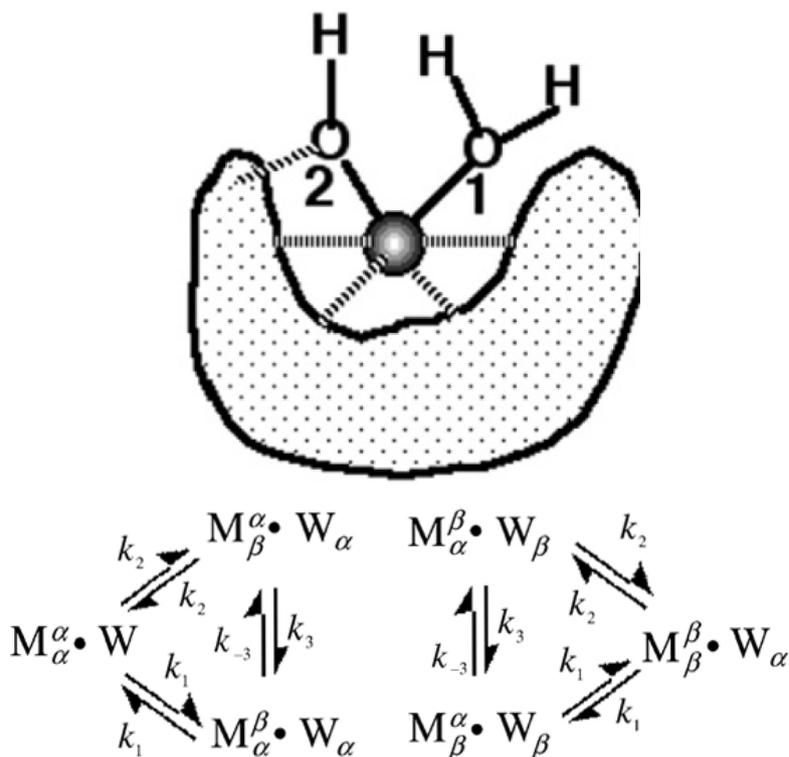
transition is the only oxidation step that is not accompanied by a concomitant proton transfer.

Mechanism of water oxidation by the WOC of PSII is an enigma for bio(ionorganic)chemists and it is not still resolved. There are several proposed mechanisms for biological water oxidation in Nature. G. Babcock and coworkers proposed that two different Mn ions are successively deprotonated until the O-O is formed between the two terminally coordinated oxides or oxyl radicals (Fig. 4) (Hoganson and Babcock, 1997).

The group proposed that the tyrosyl radical may act as a H abstractor. This enables the manganese

cluster to remain electrically neutral. Upon its oxidation, Y<sub>Z</sub> releases a proton to His190, and this proton is ultimately delivered to the bulk aqueous phase. In this mechanism, oxygen is postulated to form from water bound as terminal ligands to the manganese. Although, it is found that the distance between the tyrosine 161 and manganese ions in the WOC is too large for direct abstraction of a hydrogen atom from a water species coordinated to these ions. This means that two cornerstones of the model was no longer tenable in this form but it is inspired others to new models.

Membrane-inlet mass spectrometry showed that two substrate water molecules can be distinguished



**Figure 5.** Mono-metallic mechanism. In the mechanism, the O-O bond formation between the oxygens of the two water molecules coordinated to one Mn ion (a). A generalized scheme of the isotopic <sup>18</sup>O exchange reactions on the mono-manganese substrate binding sites in OEC: and stand for <sup>16</sup>O- and <sup>18</sup>O-isotope substrate; the suffix and superfix position on M implies the strong and weak binding site with the substrate exchange rates,  $k_1$  and  $k_2$ ;  $k_3$  and  $k_{-3}$  represents the forward and backward cross exchange rates; W and W are H<sub>2</sub><sup>16</sup>O and H<sub>2</sub><sup>18</sup>O, respectively (b). Images and caption from (Kusunoki, 2007).

by their exchange rates, they are commonly described as the fast ( $W_f$ ) and slowly ( $W_s$ ) exchanging substrate waters (Hillier and Wydrzynski, 2008).

The O-O bond formation between the oxygens of the two water molecules coordinated to one Mn ion was another idea (Fig. 5a) (Kusunoki, 2007). The kinetic data for  $^{34}\text{O}_2$  and  $^{36}\text{O}_2$  have been provided evidences for the separate substrate binding sites (Fig. 5b). However, Kusunoki has suggested that two substrate water molecules are bound to asymmetric cis-positions on the terminal Mn ion being triply bridged ( $\mu$ -oxo,  $\mu$ -carboxylato, and a hydrogen bond) to the Mn-Ca cluster, by developing a generalized theory of  $^{18}\text{O}$  exchange kinetics in the WOC to obtain an experimental evidence for the cross exchange pathway from the slow to the fast exchange process (Kusunoki, 2007).

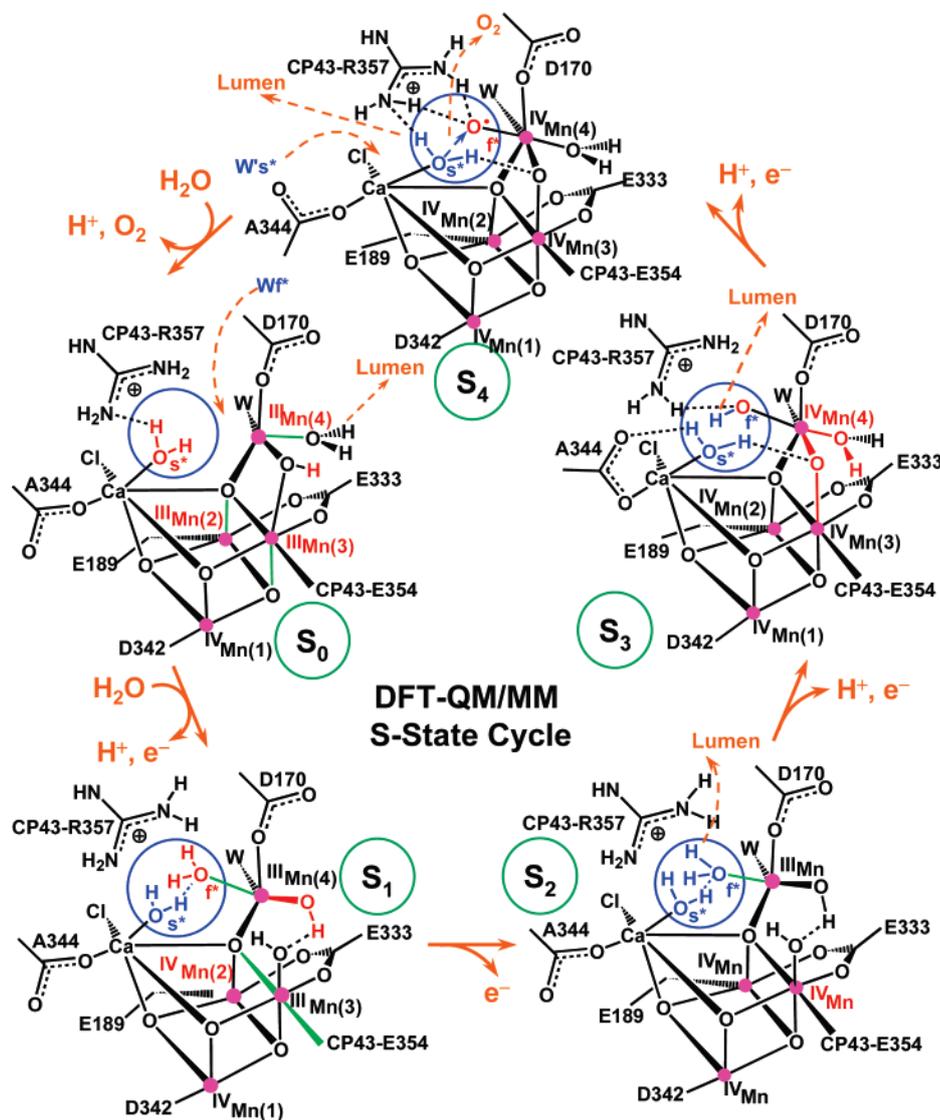
The theory suggests:

- $W_f$  is already bound in the  $S_1$  state
- $W_f$  is likely bound to the same metal as  $W_s$

A few mechanisms for water oxidation are based on O-O bond formation by the nucleophilic attack of a water or hydroxide that either is positioned by coordination to the Ca ion or stems from outer-sphere water (Fig. 6) (Pecoraro et al., 1998; Limburg et al., 1999).

This idea has been elaborated by Brudvig's group and co-workers (Lee et al., 2007; Brudvig, 2008). Pecoraro's group has proposed that a terminal  $\text{Mn(V)=O}$  undergoes a nucleophilic attack by a  $\text{Ca(II)}$  bound hydroxide ligand to form a Mn-bound hydroperoxide. Brudvig's group have also proposed a mechanism in which a  $\text{Ca(II)}$  ion plays a role as a weak Lewis acid. In the mechanism a water bound to calcium reacts with a  $\text{Mn(V)=O}$  species to form the O-O bond through a nucleophilic attack. The idea has been developed by Brudvig and co-workers to generate a mechanism that accounts for the detailed of oxygen evolution by PSII. In 2008, Brudvig and

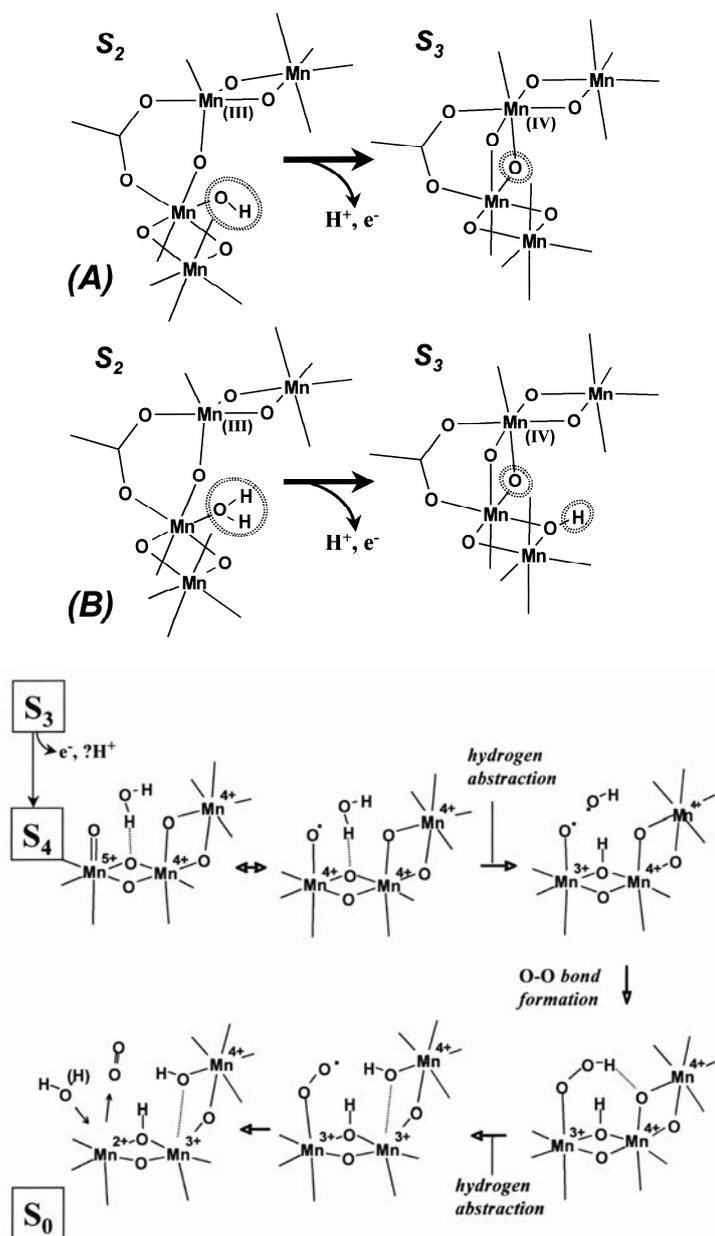
Batista's group developed sensible structural models of the WOC with complete ligation of the  $3 + 1$  Mn tetramer by amino acid residues, water, hydroxide, chloride, and calcium ions. Manganese and calcium ions are ligated consistently with standard coordination chemistry assumptions, supported by much biochemical and spectroscopic data (Sproviero et al., 2008) (Fig. 6). The proposed models were consistent with available mechanistic data as well as in good qualitative agreement with X-ray diffraction models of PSII and EXAFS measurements. The DFT QM/MM structures also provided new fundamental insight that is relevant to the elucidation of the detailed mechanistic aspects of photosynthetic water splitting and the structure of the WOC. In this model in the  $S_3$   $S_4$ , deprotonated substrate water molecule (bound to the dangling  $\text{Mn(4)}$  and hydrogen bonded to CP43-R357), releasing another proton to the lumen and changing the charge of the complex as suggested by electrochromic bandshift measurements (Fowler, 1977). The deprotonation of the substrate water molecule first forms the oxyl radical  $\text{Mn(III)-O}\cdot$  transient species that is subsequently oxidized by  $Y_Z$  to form the  $\text{Mn(IV)-O}\cdot$  species. Thus, regarding the method, these groups concluded that the  $S_4$  state accumulates the fourth oxidizing equivalent in the oxidized substrate water, as a high-spin oxyl radical species  $\text{Mn(IV)-O}\cdot$ , not in the dangling  $\text{Mn(4)}$  as the low-spin state  $\text{Mn(V) O}$  species. The  $S_3$   $S_4$  transition forms dioxygen, by nucleophilic attack of the calcium-bound water molecule on the electrophilic oxyl radical  $\text{Mn(IV)-O}\cdot$ . The reaction is promoted by substitution of the calcium-bound water molecule by a water molecule in the second coordination sphere of calcium. This process involves deprotonation of the displaced water, releasing a proton to the lumen via CP43-R357 and transferring the other proton to the basic  $\mu$ -oxo bridge linking  $\text{Mn(4)}$  and  $\text{Mn(3)}$ . The new proposed reaction between an water molecule bound to  $\text{Ca(II)}$  is similar to earlier proposals by Pecoraro et al. and Brudvig and co-workers but in the new model the nucleophilic water attacks an oxyl radical, rather than an  $\text{Mn(V) O}$  species.



**Figure 6.** Catalytic cycle of water splitting suggested by DFT QM/MM models of the OEC of PSII. Dashed arrows indicate transformations leading to the following S state in the cycle. Changes caused by an S-state transition are highlighted in red. The blue circles highlight substrate water molecules. Coordination bonds elongated by the Jahn-Teller distortion are marked in green. Image and caption from (Sproviero et al., 2008).

Dau's EXAFS data implied that upon the  $S_2 \rightarrow S_3$  transition, the mono- $\mu$ -oxo bridge is transformed to a di- $\mu$ -oxo bridge and they hypothesized that this transformation involves deprotonation of a terminally ligated hydroxide or water, and that could be facilitated by the transition from five-coordinated Mn(III) to six-coordinated Mn(IV) (Dau et al., 2001).

In addition to this, they concluded: 1) The  $S_2 \rightarrow S_3$  transition is significant modification of the WOC. 2) The transition is closely related to a deprotonation event. 3) The Mn oxidation upon the  $S_2 \rightarrow S_3$  transition is coupled to an increase in the coordination number of one Mn from 5 to 6. 4) The number of  $\mu$ -oxo bridges is increased in  $S_2 \rightarrow S_3$  metal complex.

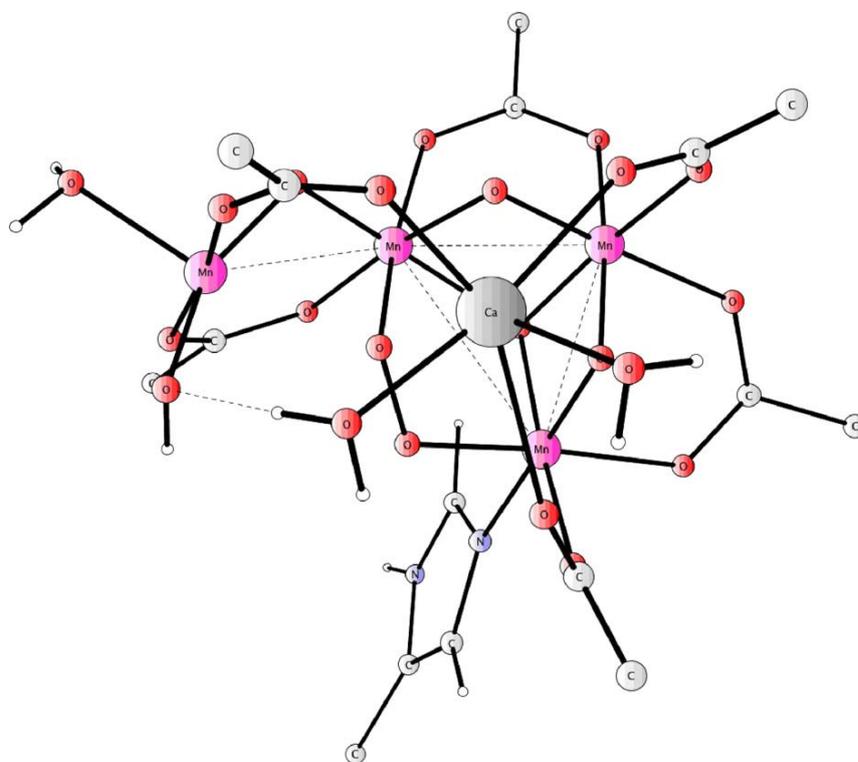


**Figure 7.** Hypothesis on the S<sub>2</sub> – S<sub>3</sub> transition. In (A), the manganese complex in its S<sub>3</sub>-state contains three unprotonated di-W-oxo bridges; in (B), one of the three di-W-oxo bridges is protonated. In (A) and (B), the oxidation of Mn(III) is linked to deprotonation of one water-derived ligand (marked by dotted circles) and formation of an additional bridge between manganese ions. In this scheme, an arrangement of the four Mn ions and six bridging ligands has been chosen for the Mn complex in its S<sub>2</sub> state; also other structural models for the Mn complex in its S<sub>2</sub> state models are in agreement with the EXAFS results (a). Hypothetical scheme on the mechanism of water oxidation and dioxygen formation upon the S<sub>3</sub> – S<sub>0</sub> transition. Only three Mn ions are depicted because the proposed mechanism does not require that four manganese ions are actively involved. Image and caption are from (Dau et al., 2001).

Holger Dau and coworkers also proposed that the O-O bond could be formed by nucleophilic attack of an outer-sphere water to a Mn=O that may be facilitated by the transfer of a proton from an outer-sphere substrate water to a bridging oxygen (Dau et al., 2001) (Fig. 7).

There are also a few proposed mechanisms for oxygen evolution by the WOC on the basis of theoretical calculations. In 2013, the group used the high-resolution (1.9 Å) structure by Shen et al. (2011) 200 atoms near the Mn-Ca cluster used in these calculations. The amino acids included in the model are first the directly binding amino acids,

Asp170, Glu189, His332, Glu333, Asp342, Ala1344 and Glu354. The second shell residues Asp61, His337 and Arg357 and the region around the chloride are also included. Lys317 and three water molecules, forming a hydrogen bonding network were also considered in this calculation. In the proposed mechanism, the  $S_2 \rightarrow S_3$  and the  $S_3 \rightarrow S_4$  transitions start with an electron transfer from  $Y_Z$  to  $P_{680}^+$  followed by an exergonic release of a proton of the WOC. The group suggested that similar steps might be observable also for the  $S_2 \rightarrow S_3$  transition. The next step in the  $S_2 \rightarrow S_3$  transition is the oxidation of Mn1.



**Figure 8.** Optimized transition state for O-O bond formation. Only most essential atoms are shown (a). Optimized peroxide product. Only most essential atoms are shown (b). Images and captions from (Siegbahn, 2012).

In this state, the substrate water becomes more strongly bound to Mn1 and the Jahn–Teller -axis on that center disappears. In the  $S_3 \rightarrow S_4$  transition, there is a proton transfer, which unlike the previous

$S$ -transition precedes the oxidation step (Fig. 8). The proposed reason is that since all manganese atoms are in the Mn(IV) oxidation state, the next oxidation has to be for an oxygen derived ligand

(Siegbahn, 2012). Deprotonation is very important for the oxygen oxidation and oxyl radical formation. After oxyl radical formation, there is another proton transfer before the S<sub>4</sub>-state is reached at which O-O bond formation occurs. In this mechanism proposed by Per E.M. Siegbahn at Stockholm University, the O-O bond is formed between an oxyl radical in the center of the cluster and a Mn-bridging μ-oxo ligand (Fig. 8).

In conclusion, the real mechanism for water oxidation in *Nature* is an enigma in science. Here, we reviewed the most important mechanisms for biological water oxidation. New experiments should be performed to find real mechanism among of them.

### Acknowledgments

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