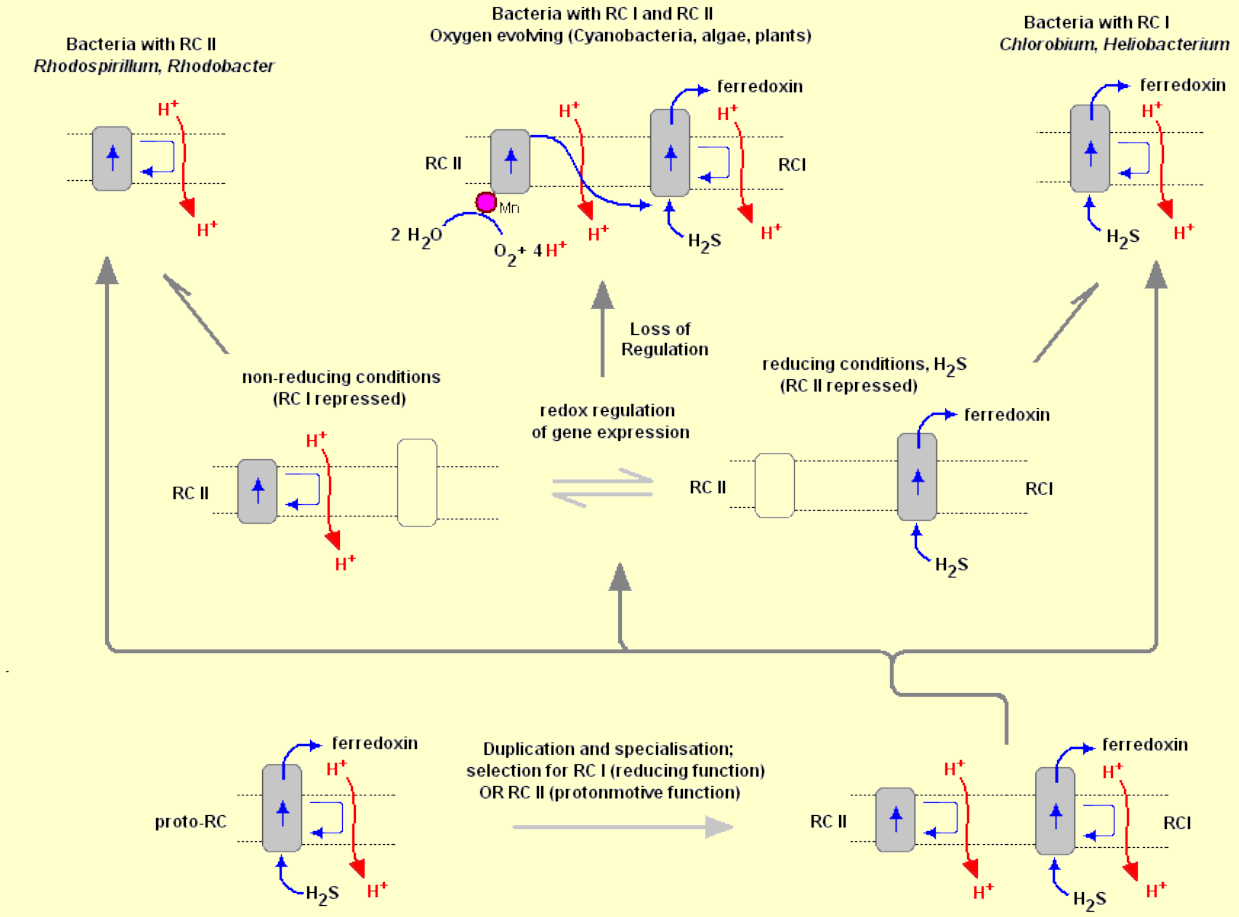
The Manganese-calcium oxide cluster of Photosystem II

The Oxygen Evolving Complex (OEC) is assimilated by Cyanobacteria (2.7 - 2.5 Ga)

The evolution of two Photosystems: The evolution of the oxygenic photosynthetic reaction center is of paramount importance in evolutionary biology. Evolution has produced two photosystems, known as Reaction Center Type I (RC1 or PSI) and Reaction Center Type II (RC II or PSII). The OEC operates on the oxidizing side of RC II, but we find that both Photosystems are used by prokaryotes with slightly differing functions and evolutionary adaptations. Cyanobacteria use the oxygenic PSII and PSI electron transport system as does algae and plants. Anoxygenic bacteria use either RC I, or a prototype of RC II, depending on species and environment. At least one organism, *Oscillatoria*, has both.

To understand the various distribution and functions of the two reaction centers, and how they might be related evolutionarily, **John F. Allen** (Dept of Biology, Queen Mary, University of London) recently published a model to explain the appearance of the two reaction centers throughout the prokaryotes and higher plants and algae. It is now generally accepted that PSII evolved from a primitive PSI, the two reaction centers are in fact homologous in both function and structure (determined from kinetic, X-Ray and genetic studies). Dr. Allen calls his model a "redox switch hypothesis" in which the redox levels in the environment (amount of available reductants, etc) may trigger a bacterium to switch from one reaction center to another. The associated light harvesting chlorophyll proteins, being integral within the bilipid membrane, are capable of serving as light harvesting complexes for both photosystems. Dr. Allen's model is presented below.



The original RC I is shown in the lower left, labeled as proto-RC. This reaction center operates with external reductants, such as hydrogen sulfide, and is capable of supplying electrons to a quinone pool, which can then recycle the energized electrons back to the reaction center while moving protons across the membrane into the periplasmic space thereby creating a protomotive force for the synthesis of ATP. In addition, the RC I provided electrons to soluble electron acceptors (ferredoxin) for use in organic synthesis.

Over time and changing conditions, a second independent photosynthetic reaction center emerged, under a scheme of genetic control that would favor one over the other. Allen argues that in this early stage the two reaction centers would have been cooperative, their expression genetically controlled by changing environmental conditions (alternatively, there may have been initial competition). The rise of the RC II enabled the bacteria, under conditions where reductant molecules were unavailable, to support photon-driven proton pumping for ATP synthesis. Single reaction center anaerobic phototropohs were either photolithotrophic (RC 1) or photoorganotrophic (RC II). Dr. Allen argues that metabolic flexibility may have selected those bacterial capable of providing one or both of the two types of photosystems, depending on environmental conditions.

Eventually bacteria appeared (such as in the present day *Oscillatoria*) in which both photosystems are genetically available, and are synthesized and distributed throughout

the membrane depending on environmental conditions encountered (Allen proposes this is carried out under redox control of gene expression). *Oscillatoria limnetica* has both RC I and RC II, and under conditions of low H_2S, switches to RC II and performs oxygenic photosynthesis (Oscillatoria is a member of the Cyanobacteria. Note that there are other conditions, such as the heterocyst of Nostoc, in which RC II is shut off, as oxygen interferes with and will chemically shut down nitrogen fixation. See also **C4 CAM plants** *et al.*).

As the image above shows, bacteria with RC II only are known (anoxygenic), as well as bacteria with RC I only (also anoxygenic) Dr. Allen proposes that at some point in time the genetic "redox switch" was not longer needed and the two photosystem began to specialize and work in cooperation. Genetic regulatory control can still take place by either lowering or increasing either the reaction centers themselves or distributing their associated light harvesting complexes between the two.

The Evolution of the Oxygen Evolving Complex: Olson (**29**) provides a working hypothesis to account for the evolution and rise of the Oxygen Evolving Complex, *q.v.*, that selective pressure, for the evolution of two populations of photoreactions with overlapping but non-identical redox spans, came into play as suitable electron donors (H_2, H_2S, simple organic molecules) were used up on the early Earth. An important aspect of this view is the appearence of a pool of quinones capable of being oxidized and/or reduced by either of the two reactions centers. Olson and Dismukes (29, 30) proposed that consumption of available oxidants in the environment forced the oxidizing end of proto-PSII to change to more oxidizing E_h values, oxidizing a **range of intermediate compounds** and finally able to utilized Mn to oxidize water.

As discussed by Larkam (**23**), three proposals have been put forward regarding compounds which may have served as precursors to the Manganese complex, *q.v.*, (1) formate (**29**), (2) hydrogen peroxide (**30**) and (3) bicarbonate (33). Bicarbonate is an ideal precursor because of its abundance in marine environments (curr. ~ 2mM). In the early oceans of the earth bicarbonate would have been at much higher levels due to the higher levels of carbon dioxide that existed then. Additional evidence supporting bicarbonate use is supported by the discovery of a putative bicarbonate active binding site at the level of the water oxidizing site, as well as bicarbonic anhydrase activity found in association with PSII (perhaps facilitating the movement of carbon dioxide to the stroma). The evolution of the manganese complex, its association with various extrinsic polypeptides (D1, CP43 and CP47), will become more attenable once the mechanism of oxygen evolution is understood (see references below for current schemes as well as one introduced here involving a single manganese atom at the site of the OEC).

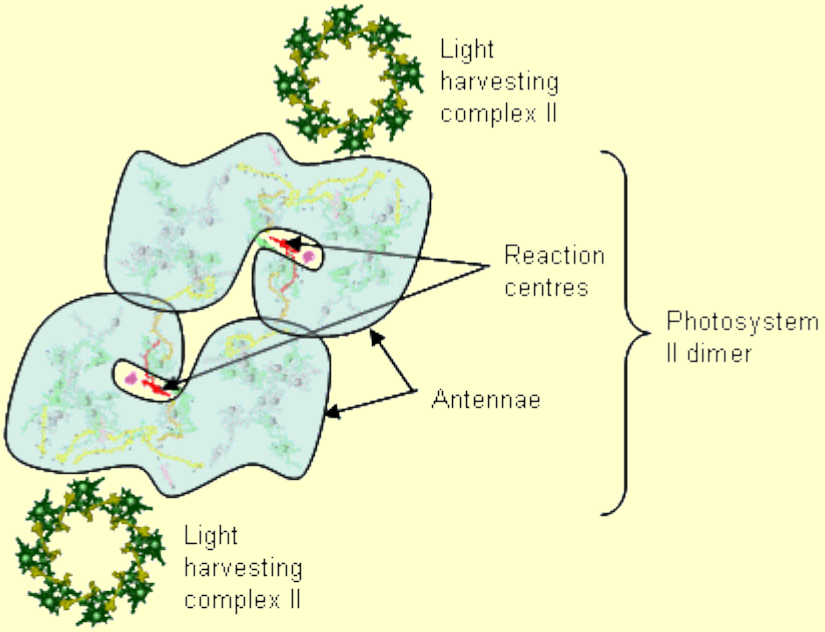
4. The molecular structure of the manganese-calcium oxide water splitting complex

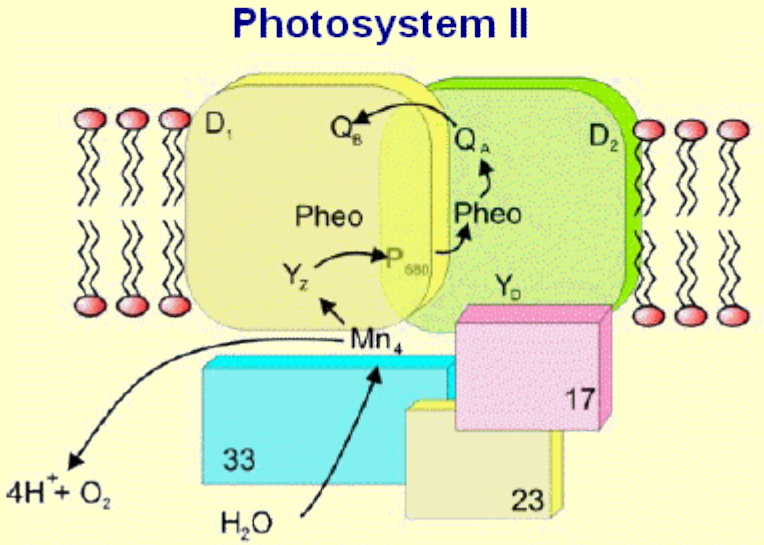
Resolution: The recent 2.5 Angstrom resolution of the OEC (31) has opened a black box that has been difficult to open for many years. Earlier kinetic and spectroscopic data are falling in line with the mechanism of this complex but the race is on to determine exactly how the oxygen evolving complex extracts electrons from water. We now know there are

4 manganese atoms (their respective redox states and behavior has not been determined with certainty), a calcium atom, now believed to be an essential component of the water oxidizing site, and a chloride (Cl^-) co-factor. Chloride does not appear to be essential and is believed to play a charge stabilizing role during water oxidation. An excellent animation of the Photosystem II complex, including the light harvesting chlorophylls, the reaction center and the OEC can be downloaded at **Dr. Johannes Messinger's** site (36 megs, Windows, RealTime) and is a beautiful rendition of PSII (highly recommended).

Dr. Messinger suggests that once the mechanism of water oxidation has been determined, it may be used to engineer a renewable man-made energy source by combining the oxidation of water with a hydrogenase (future solar hydrogen and oxygen production).

The mechanism of water oxidation has eluded scientists for decades. In the last 50 years much work has been carried out on the kinetics of PSII (OEC). Electrophoresis and related enzymatic techniques have identified most of the polypeptides associated with PSII. New X-Ray diffraction studies have identified the stereochemistry and atomic structure of the OEC and surrounding complexes, though there is still debate over the number of ligands involved, their types, and how many of the ligand positions may be occupied by water. Once the mechanism of water oxidation is elucidated, it will celebrated as one of bilogy's greatest discoveries since the discovery of DNA's 3D structure in 1954.



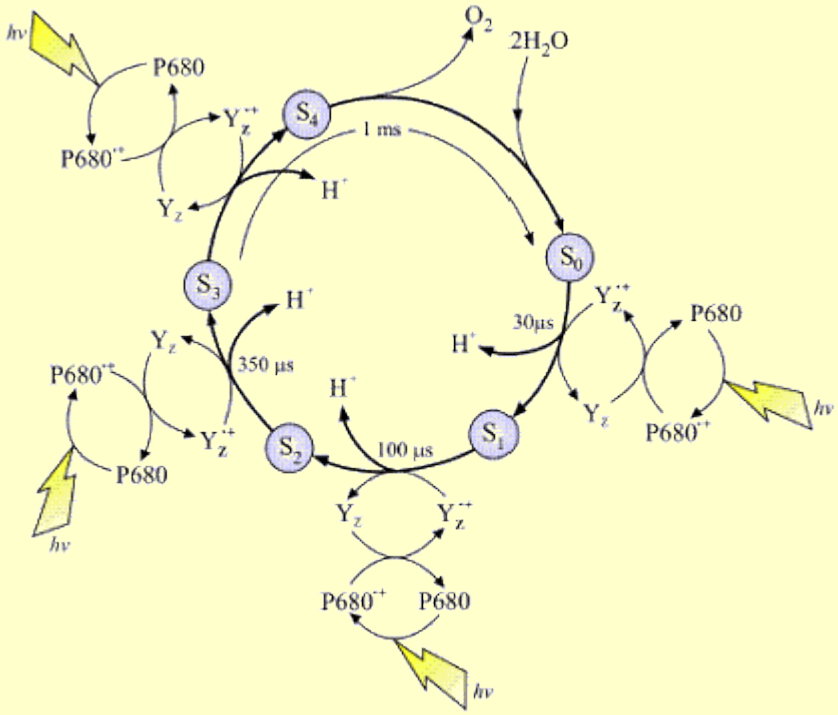


Photosystem II consists of an array of proteins, held together by numerous salt links, hydrogen bonds, water molecules et al. and therefore especially labile under conditions of high temperatures or high pH. The complex is also sensitive to photoinactivation if electron flow throw the "Z" scheme is not delicately balanced (ATP phosphorylation of the surrounding light harvesting chlorophyll proteins is part of this regulatory scheme). Other photo-oxidative events at the reaction site can cause damages (*e.g.*, the presence of carotenoids, etc. may server to prevent inactivating photo transfer events through quenching, etc).

From the image at left, PSII is diagrammatically depicted as a dimer, as it is found embedded in the thylakoid membranes (see Dr. Messinger's video, which clearly shows the central axis of this complex). The PSII reaction center complex is surrounded by additional light harvesting chlorophylls, shown here as LHCII (only 2 are shown, these would completely surround the complex in a natural state.

The various proteins that surround the PSII complex are shown in the diagram at left. They include the principal Light Harvesting Complexes (LHCI), D1, and D2, as well as associated peptides including the 33 kdal, 23 kdal and 17 kdal. These periphery proteins support the reaction center and associated cytochromes and other pigments.

The general reaction is also outline (detailed below) in which the reaction center chlorophyll dimer, P680, is initially oxidized by an incoming photon, the electron being passed to the nearby pheophytin and then transferred to the quinone pool. Manganese, bound to water, calcium, chloride and oxygen (in manganese oxo bridges) then rapidly reduces Yz (a local Tyrosine) which was oxidized as it reduced the oxidized P680+. 35 years ago Joliot and Kok (see Photosynthesis references below) worked out the mechanics of the four step cyclic process required for turning over PSII during its water oxidation event. Each PSII reaction center within the membrane works independently of one another and cycles through a set of 5 oxidation states, known as the "S" states ($S_o \rightarrow S_4$). Our current understanding of the mechanism of water oxidation proceeds through these four states known as the "Kok Scheme" (for more details on the structure of PSII proper see the references and links provided below.

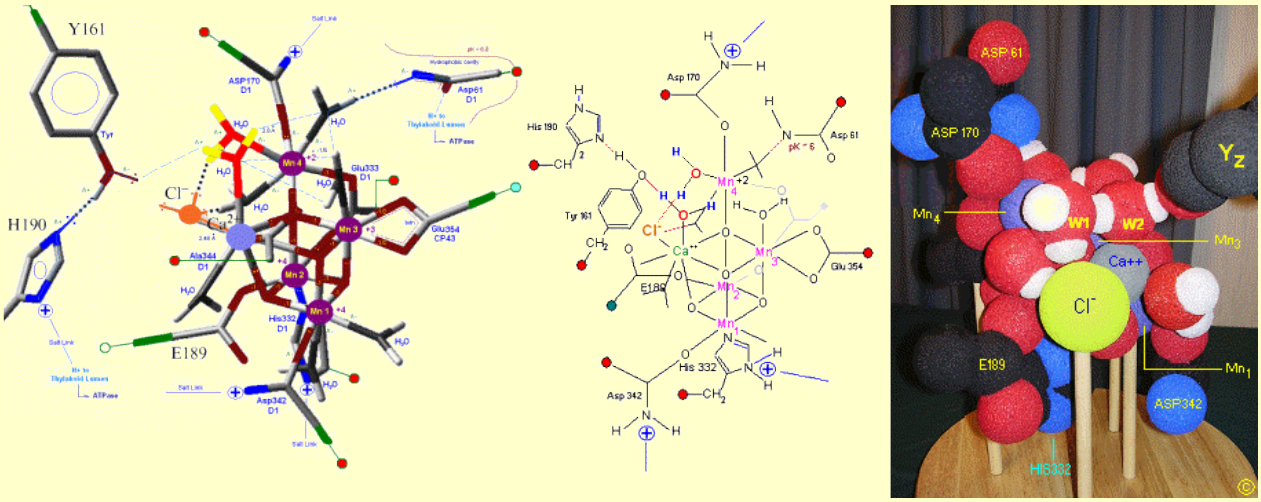


The above image (left), is a typical representation of the Joliot-Kok Cycle. This particular schematic is from an article published by **Dr. James Barber**, Imperial College of London, *International Journal of Photoenergy* **6**:43-51 (2004). (Take the time to visit Dr. Barber's web site to see his **Photosystem II** images.

The 4-step Kok Cycle is represented schematically in the image at left. Four incoming photons are required to complete the process. The OEC starts at State So, and then with

each incoming photon moves on to Sate S1, then S2, S3 and finally S4. There are a total of five oxidation states of the OEC during this process. As the OEC cycles through this process four electrons are removed from bound water (see mechanism below). The deposition of protons during this process varies but it is believed 2 protons are released during the final S3→S4 transition. The schematic at least depicts one proton released per photon absorbed. Yz is a nearby Tyrosine residue, and P680 the reaction center special chlorophyll dimer (as discussed).

X-Ray data and model of the OEC are shown below.



The original X-Ray at a resolution of 3.5 Angstroms, was reported by Ferreira et al in 2004. The image to the left, above, was taken from an article published by **James P. McEvoy**, Yale University. The image was colored coded and the middle image drawn directly from that. The model on the right was built using this X-Ray information. Since this publication the OEC has been resolved to a resolution of 2.5 Angstroms (x).

The salient features of the complex (not all natural ligands may be in place, and McEvoy added an additional water ligand to complete the complex) include the manganese-calcium oxo bridging of the cubane cuboidal structure. The manganese atoms are labeled 1, 2, 3 and 4, with manganese number 4 protruding outside the main cubical structure below it, which includes oxo binding between Mn 1, Mn 2 and Mn 3. Calcium is placed as one of the cuboidal corners (shown in blue on the left). Chloride is believed to reside on the outside of this cubane complex, and in our model on the right, appears to sit nicely (electrostatically) amongst the water molecules and calcium that surround it.

Yz, the primary electron acceptor of the OEC, sits to the left, approximately 3.4 Angstroms away from the chloride. Note the numerous ligands supporting the complex, which includes several Aspartates (contributed from the nearby D1 protein) as well as a histidine residue (near the bottom), also from D1. The substrate water molecules, labeled W1 and W2 in our model (right) is, at this time, an arbitrary assignment since the exact details of the mechanism are not yet known (we selected these water molecules after the model was built because of their proximity to Mn 4, and to illustrate a potential source of

oxygen in our mechanistic scheme below. A proposed water channel may be directed out from aspartate 170 (D1) since it is believed that Tyrosine and histidine (left of the manganese cube) reside in a hydrophobic environment.

In the mechanism reported here, protons are shunted away form the OEC via Yz's oxidation and HIS 190's resonance (see below). Though no experimental evidence was found to support this proposition, our proposed mechanism argues that Yz's location is in fact adjacent to an available water channel (Yz is already assumed in this model to be hydrogen bonded to one of the reactive waters and utilizes (is coupled to) HIS 190 to shunt protons away from the OEC via HIS 190 resonance. This is indicated below in the mechanism proposed by the dashed blue line between Yz and P680, that is, once Tyrosine is oxidized, HIS 190 resonates electrons inward toward the OEC, thereby passing a proton into a nearby water channel. See the reference for Dr. Johannes Messinger below for a review of currently proposed mechanism for water oxidation.

5. A proposed mechanism for the oxidation of water by the OEC of PSII

The following mechanism of water oxidation [outlined below - two alternative paths are given] is derived simply from the physical model above, and a few tips from the literature (see below). The purpose of this section of the MOTM page is to demonstrate to biochemistry students who have had one or two semesters in biochemistry that by using simple principles such as Lewis and Resonsance structures, potential mechanistic paths can be discovered through trial and error. While waiting for further research to emerge, a student may become familiar with an enzymatic reaction and get their feet wet simply by sitting down with pen and paper, perhaps a crude model, and make an attempt to deduce a pathway for catalysis. In this way a student may familiarize him or herself with the catalytic problem at hand.

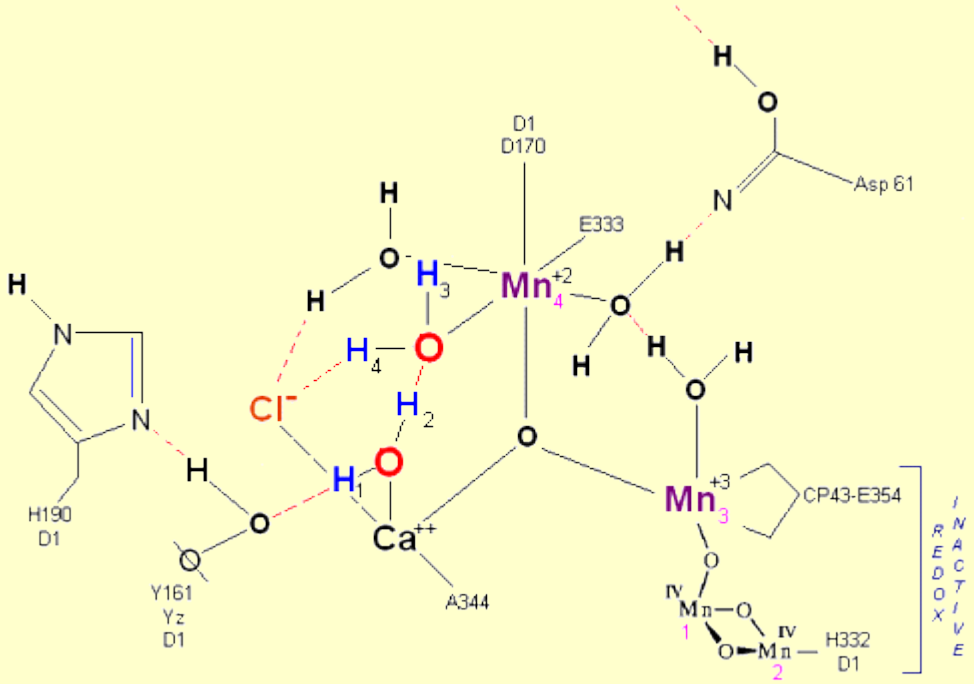
Before introducing an attempt to use simple Lewis structures for deducing a pathway for water oxidation it is important to note (see also note at the end of this section) that enzymatic catalysis may, and often is, much more complex and involved then what first meets the eye. In many cases a significant level of research and working knowledge is necessary to make educated deductions about any mechanistic path involved. In addition, genomic data may be required to interpret protein interactions and evolutionary relationships between active site participants. It is believed, and the evidence suggests, that in the case of water oxidation **bicarbonate** may be required as an **essential co-factor** (**31-33**).

The proposed reaction below is based entirely on the physical model built, along with the X-Ray image above. From the literature the fact that a potential attack on a Mn(V)=O (manganese oxo) and a proton release pattern of (So→S1) **1**, (S1→S2) **1**, (S2→S3) **0**, (S3→S4) **2**, is considered. Aside from this information, the following mechanism was derived simply by applying Lewis dot and Resonance structures to the catalytic event (reference to the catalase class of enzymes gives insight into the mechanism of oxo attack by water). Some of the leading mechanisms now proposed may be found in Johannes

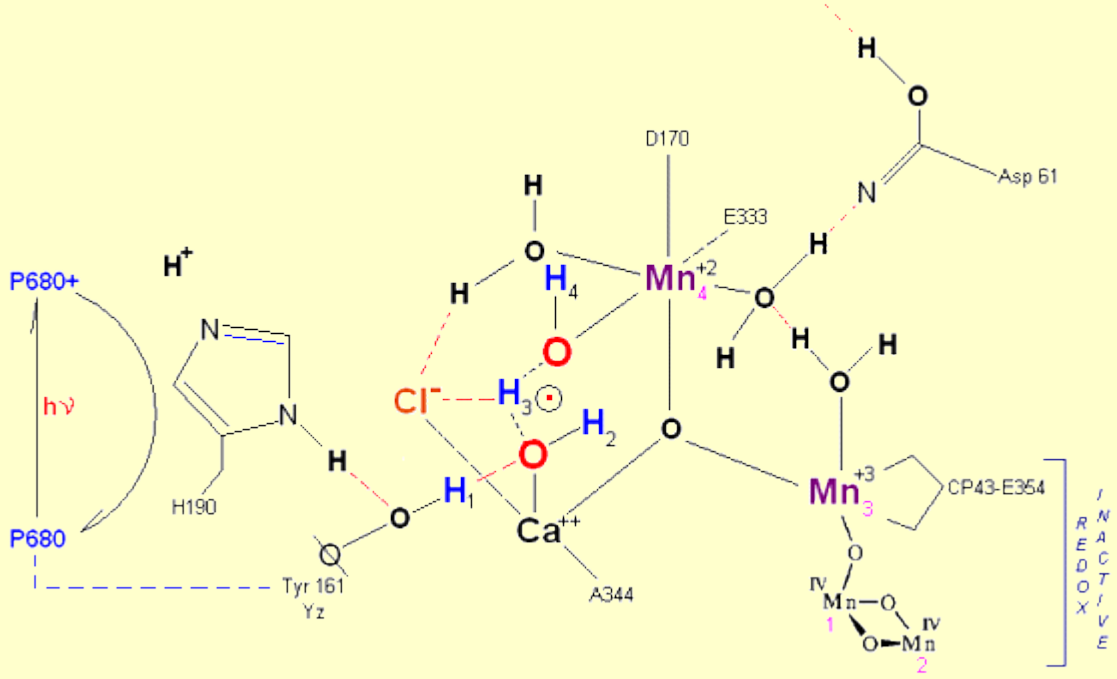
Messinger's review article, cited below, entitled Evaluation of different mechanistic proposals for water oxidation in photosynthesis on the basis of Mn4OxCa structures for the catalytic site and spectroscopic data, *Phys. Chem. Chem. Phys.*, **6**:4764-4771 (2004)

For the purposes of this exercise the following assumptions are made: (**1**) only 1 Manganese is redox active (Mn number 4 - most models involve at least two manganese and base their argument on the oxidative potential necessary to carry out the final step of water oxidation, but note too that the invovlement of Mn 3 or even Mn 1 and Mn 2, does not necessarily require their full oxidation for catalytic assistance; (**2**) Tyrosine 161 (Yz) removes protons from the reaction center at every step through the Kok S-state cycle (note that the proton release pattern, not shown in the Kok diagram above, is believed to be 1 proton from So $\rightarrow$S1, 1 proton from S1$\rightarrow$S2, o protons from S2$\rightarrow$S3, and 2 protons from S3$\rightarrow$S4; (**3**) there is an attack on a Mn=0 oxo linkage; [Note: although not reported, one might envision a Mn triple bond with oxygen - at least one metallo-oxygen evolving scheme uses this] (**4**) a peroxide is formed at the S3 state; (**5**) one substrate water forms a ligand to Ca^{++} (**6**) one substrate water forms a ligand to Mn 4 (5 and 6 based simply at looking at the physical model. Chloride is assumed to play a co-factor supporting role, and HIS 190 is coupled to Yz in a resonance proton shuttle (see below).

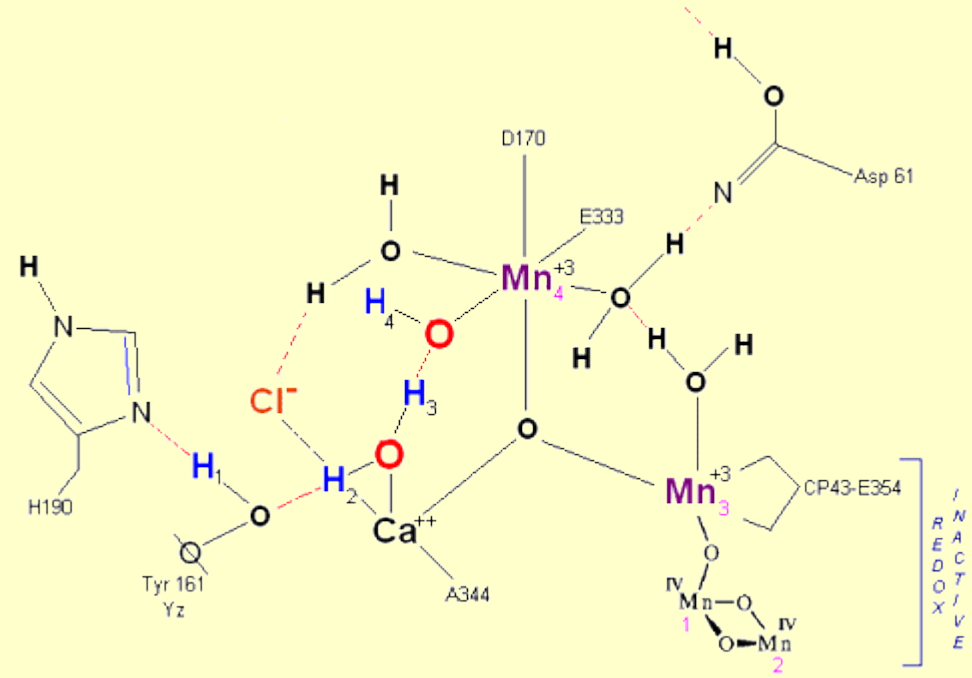
Recent X-Ray data suggests that protons are removed (via a water channel) on the Asp 61 side of the OEC (the argument being that Yz (Y161) is in a hydrophobic pocket and removed from what appears to be a channel to the lumen on the Asp 61 side of the complex). Since the mechanism proposed below requires Yz to extract protons with incoming photons, the protons are modeled here as being removed on the Yz side of the OEC complex in cooperation with HIS 190 (based on Yz being hydrogen bonded to a reactive water molecule, it is assumed here that HIS 190 is capable of rotation and deposition into a nearby water channel.



State So: Resting state. The two substrate water are colored. Mn 4 begins in a +2 state. Mn 3, Mn 2 and Mn 1 are in a +3, +4, +4 state (and remain that way being redox inactive for this scheme). Only Mn 1 is redox active. Protons are numbered 1-4. Note that the two substrate water molecules were chosen simply based on their suggestive positions from the physical model above. No argument is made here as to D1 ligand changes (for example, Mn in its lower oxidation state may prefer a hexagonal arrangement and may move to pentagonal coordination as its oxidation step increases but this is not considered here). Photon arrives at P680, and an electron is extracted from Yz (Tyrosine 161 - as indicated by the dashed line). Proton release occurs at the level of HIS 190.

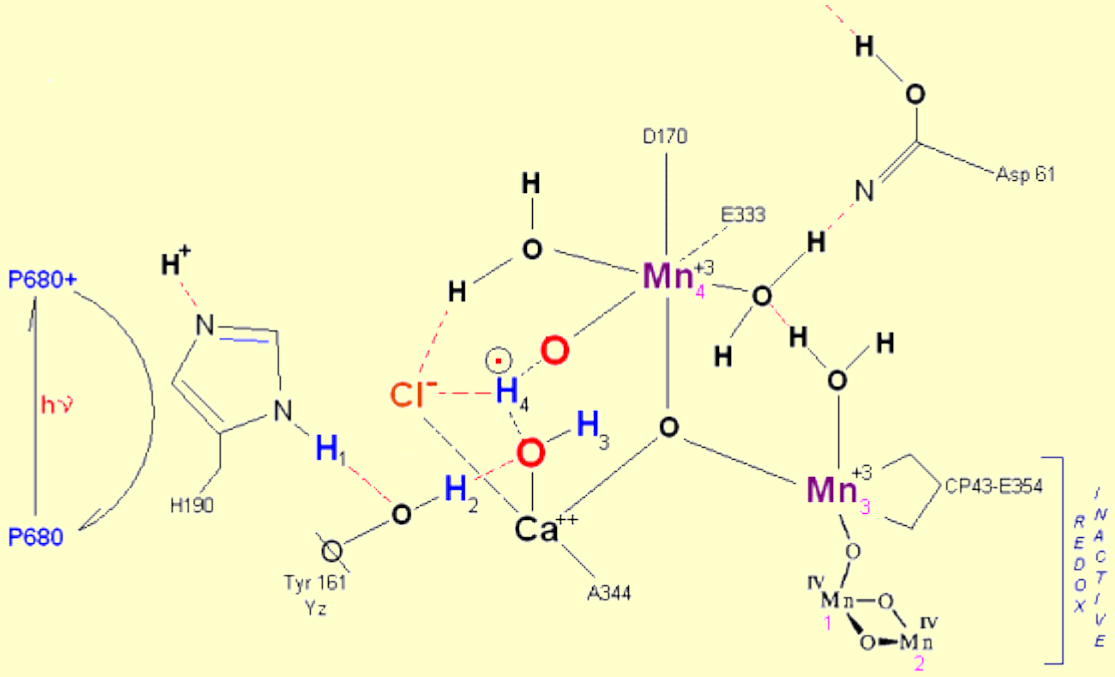


State So': First photon arrives. P680+ reduced by Yz. Initial + charged carried by hydrogen bridge between Mn 2 and Ca^{++} (note that in the physical model this transitory bridge would be linear). Cl^- helps to stabilize the bridged hydrogen radical. Notice in the proton extraction event HIS 190 rotates 180 degrees (in the process depositing a proton into a nearby water channel) before resting in the S1 state.

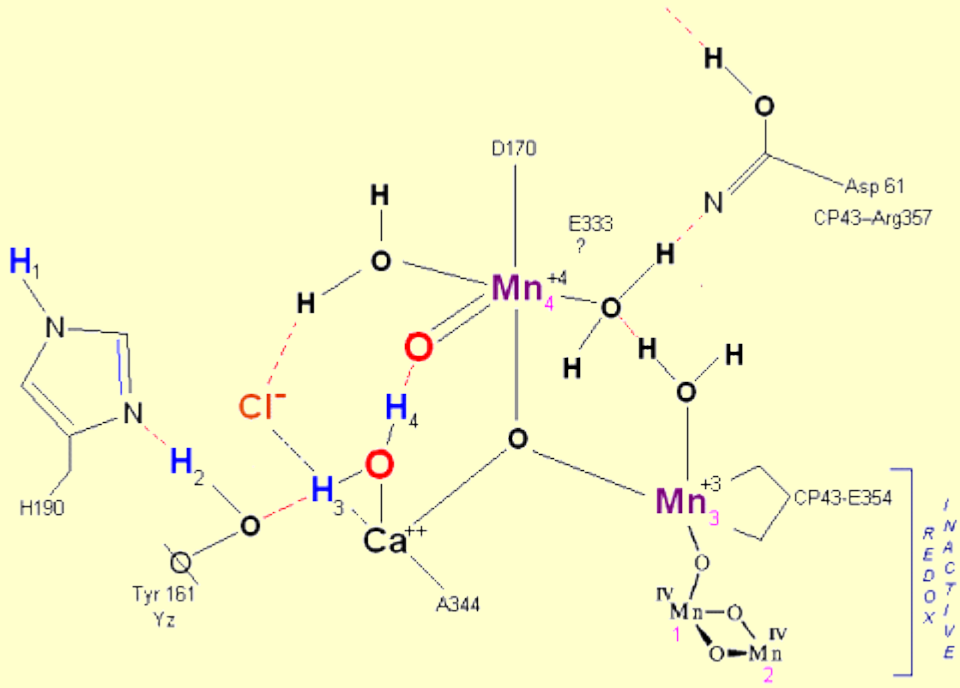


State S1: S1 resting state. H3 transfers from substrate H_2O #2 to H_2O #1. Redox reactive Mn 4 is now in the Mn(III) oxidation state. Note that after HIS 190 rotates 180 degrees it is now 'reloaded' and ready for the next proton extraction event. Next photon arrives at

P680.

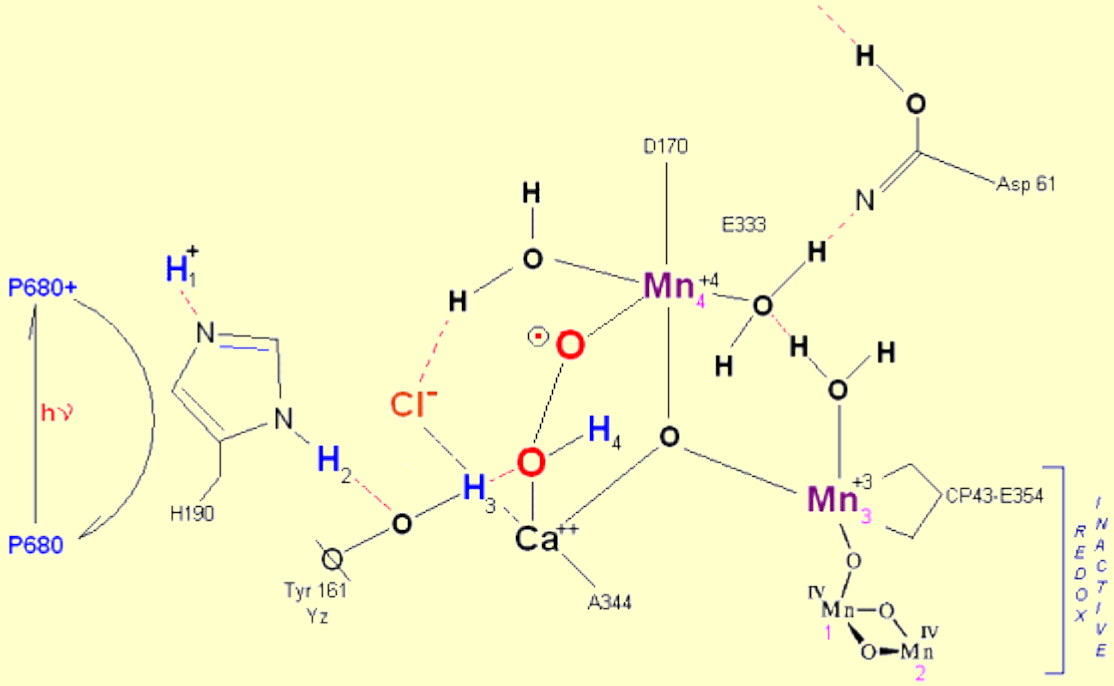


State S1': P680+ is reduced by Yz. Hydrogen bridge forms as in So →S1, charged stabilized by Cl-. Note that both hydrogens on the reactive Water molecule number 1 has transferred to reactive water molecule number 2 via a hydronium radical bridge transfer mechanism.

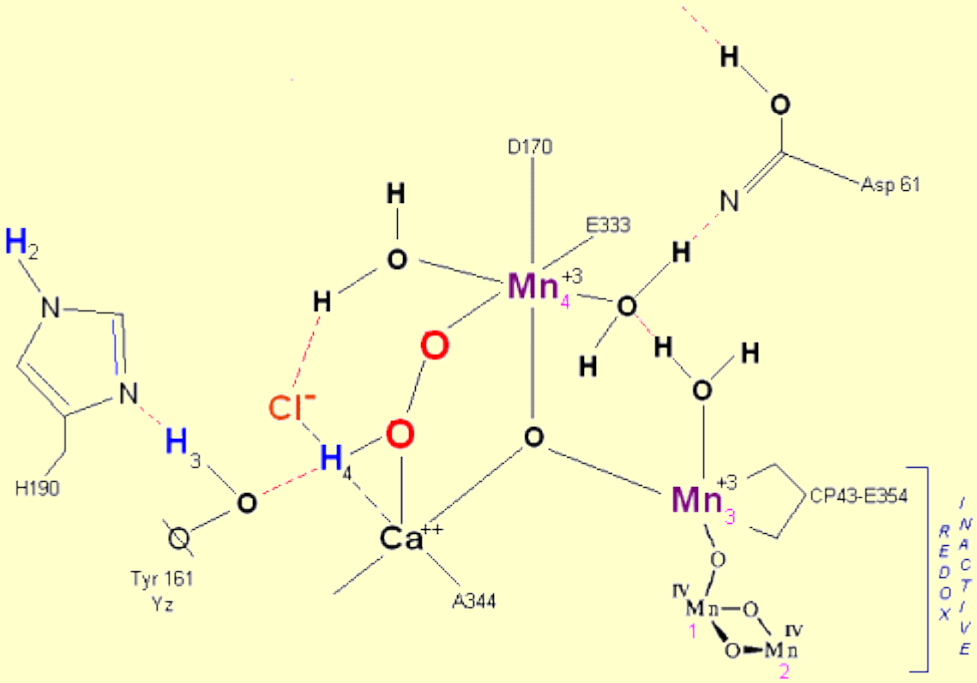


Note: See alternative S2 -> S2' below which diverges from this mechanism in order to accomodate an attack by water on a Mn(V)=O oxo in keeping with no proton release between S2 -> S3 and a 2 proton release in the final step (both mechanisms use a single redox active manganese.)

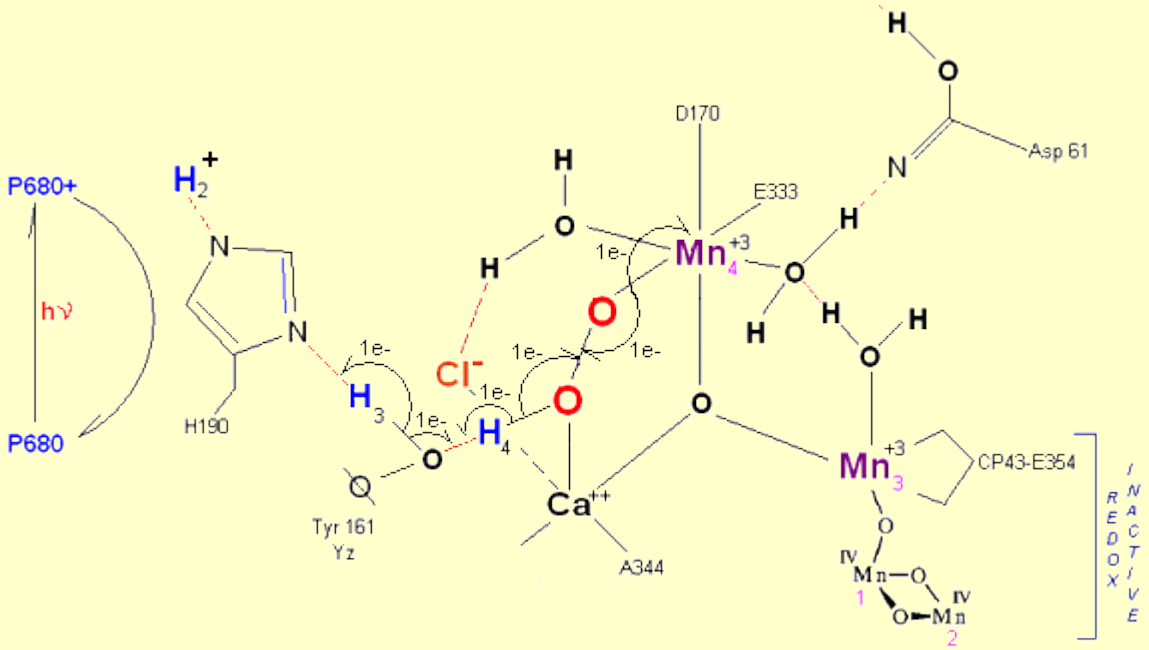
State S2: S2 resting state. Mn 4 is now an oxo-manganese (carbonyl) as Mn(IV), with a double bond to the original substrate H_20 #2. Ligands around Mn 4 may shift here. Cl- still serving in as a stabilizing co-factor. Note that the Tyrosine-Histidine system is moving protons out one at a time. Next photon arrives at P680.



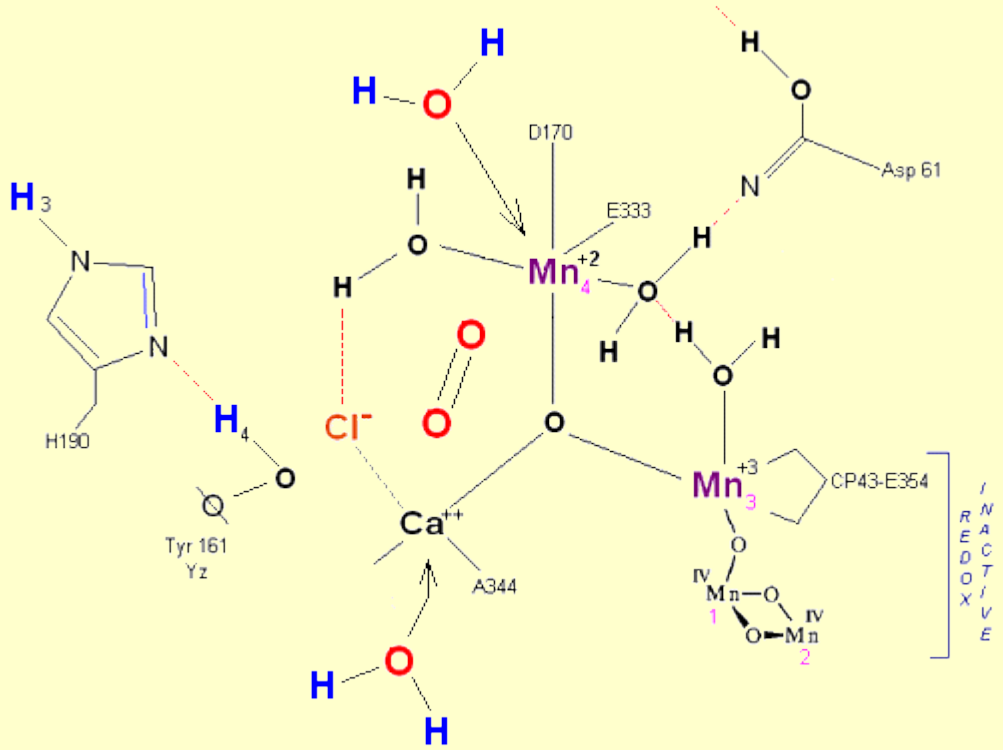
State S2': P680+ is reduced. This time the intermediate is a peroxo-radical. The move to the resting S3 state involves the reduction of Mn 4 from Mn(IV) to Mn(III) It is possible that H4 may bridge the two oxygens to stabilize the radical. [Note: See alternative mechanism below in which a Mn(V)=O is created which is attacked by water in the S3 --> S4 transition (this accommodates the proton release pattern of 1,0,1,2 as referenced in the literature)].



State S3: S3 resting state is a stable peroxide. Mn 4 is now back to a Mn(III) oxidation state. Only one of the original Hydrogens remains bound. Next photon arrives at P680.



State S3' --> S4: Finally in a series of 1 electron transfers, following the reduction of P680, Mn(III) returns to its ground state Mn(II), oxygen is formed, and the system prepares itself to be reset to the initial State So.

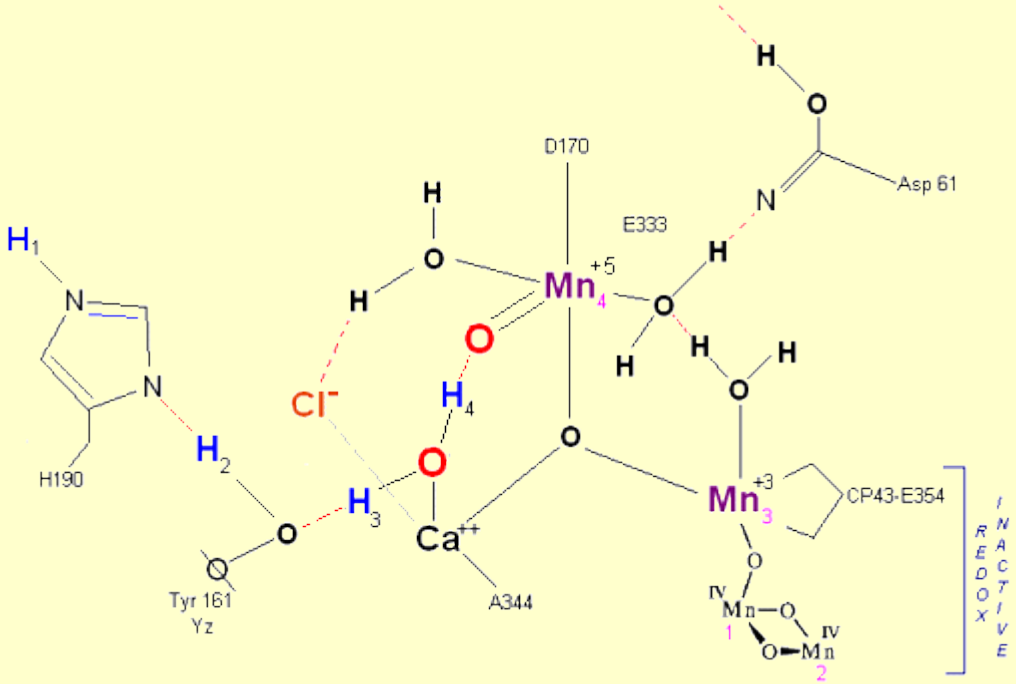


State S4 --> S4':

Incoming water molecules resets the OEC to State So.

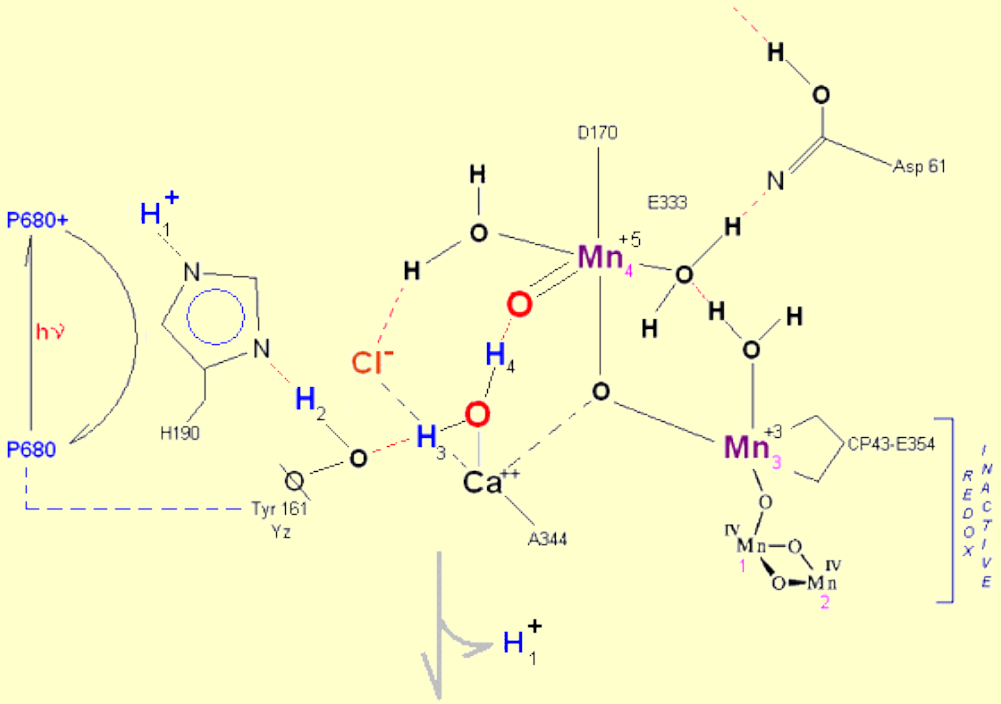
Alternative route (water attack on Mn(V)=O releasing 2H+ from S3 --> S4). In this scenario S2' releases an electron form Mn 4 but no protons, the resultant S3 state from S2 is shown below.

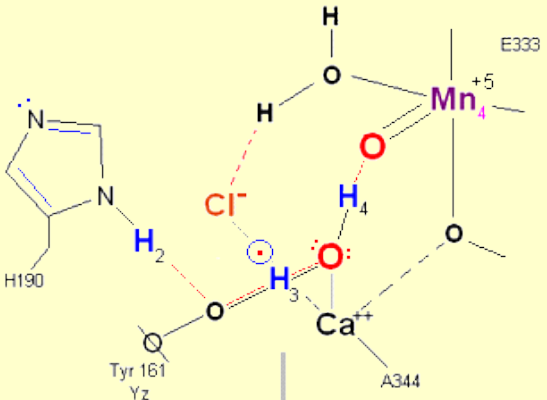
State S2 --> S3:

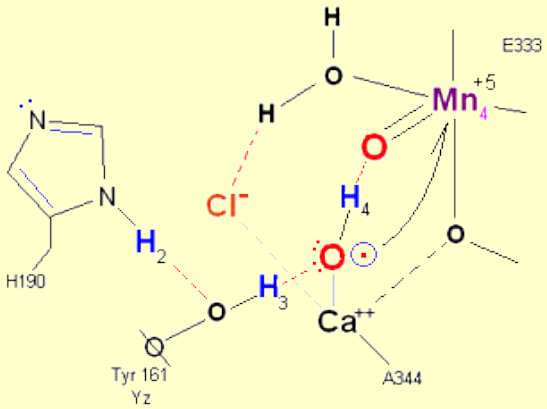


In this alternative mechanism a single electron is removed in the S2 --> S3 transition accommodating the argument that no proton is released. Mn 4 is oxidized to Mn(V) thereby forming an oxo with reactive water molecule number 1. The final step involving a direct attack by water on a Mn(V)=O complex (this mechanism is found in several oxygen evolving enzymatic scenarios).

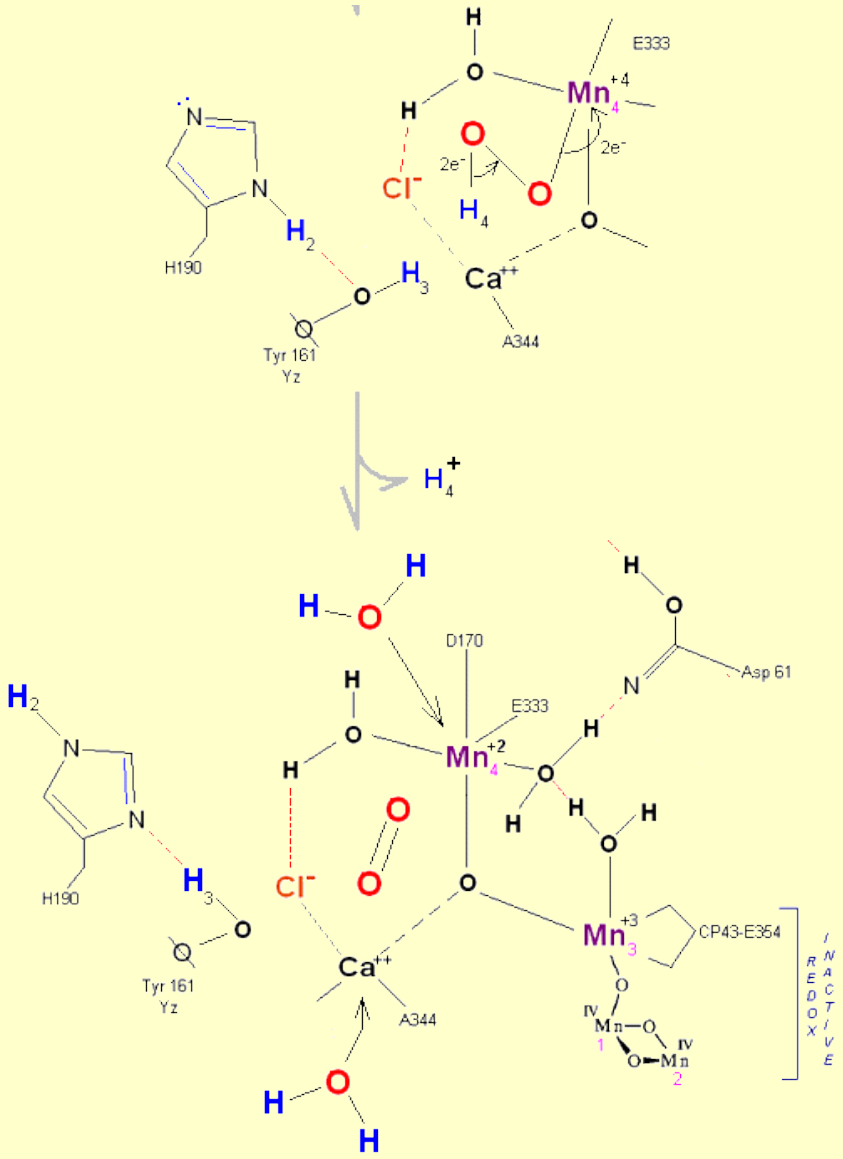
For a more detailed analytical evaluation of the Oxygen Evolving Complex and associated chemistry see Dismukes *et al.* (**31-33**) Note that in the above scheme a simple mechanism is proposed from the model built for this exercise. No attempt was made to scrutinize the mechanism for energetic and more detailed evaluation - the proposal is simply an exercise in Lewis dot structures provided as a suggestive exercise for undergraduate biochemistry students.







hydroxyl radical attack


E333
Mn+4
2e-
2e-
Cl-
H4
Ca++
A344
H190
H2
H3
Tyr 161
Yz
H4+
D170
Asp 61
E333
Mn+2
Cl-
Ca++
A344
H190
H3
Tyr 161
Yz
Mn+3
CP43-E354
REDOX INACTIVE