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Towards understanding the chemistry of photosynthetic oxygen evolution: dynamic structural changes, redox states and substrate water binding of the Mn cluster in photosystem II

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This paper is dedicated to M.P. Klein

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Abstract

This mini-review summarizes my postdoctoral research in the labs of T. Wydrzynski/C.B. Osmond, J.H.A. Nugent/M.C.W. Evans and V.K. Yachandra/K. Sauer/M.P. Klein. The results are reported in the context of selected data from the literature. Special emphasis is given to the mode of substrate water binding, Mn oxidation states and the structures of the Mn cluster in the four (meta)stable redox states of the oxygen evolving complex. The paper concludes with a working model for the mechanism of photosynthetic water oxidation that combines μ-oxo bridge oxidation in the S_3 state (V.K. Yachandra, K. Sauer, M.P. Klein, Chem. Rev. 96 (1996) 2927–2950) with O-O bond formation between two terminal Mn-hydroxo ligands during the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition.

Keywords: Photosystem II; Water oxidation; Oxygen evolution; Manganese cluster

1. Introduction

Photosynthetic water oxidation takes place at a tetranuclear Mn cluster housed in photosystem II (PS II) (for reviews see [1–4]). The Mn cluster including its ligands, together possibly with Y_Z and the co-factors Ca and Cl, forms a functional unit that is referred to as the oxygen-evolving complex (OEC) or the water-oxidizing complex (WOC). The D1 and D2 polypeptides are assumed to provide most of the ligands for Mn binding, but few have been unambiguously identified. Based on EXAFS and ESEEM spectroscopy the first shell Mn ligands are mostly oxygens, one or two nitrogens and possibly one Cl.

A milestone for the understanding of photosynthetic water oxidation was the observation of a period four oscillation in flash induced oxygen evolution patterns of dark-adapted PS II preparations by Joliot and co-workers [5] and the interpretation of these data by Kok and co-workers [6]. The periodicity of four shows that the OEC functions as a unit that sequentially stores four oxidizing equivalents before molecular oxygen is released. In the Kok model these different redox states of the OEC are referred to as the S_0, S_1, S_2, S_3 and S_4 states, where the subscript gives the number of stored oxidizing equivalents. The S_4 state, which may be identical with $S_3Y_Z^{ox}$ [7–10],

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decays immediately into the S_0 state and molecular oxygen is released ($t_{1/2} \approx 1$ ms). In the dark S_3, S_2 and S_0 return to the stable S_1 state with half-times of seconds to tens of minutes depending on pH, temperature and the availability of endogenous electron donors (S_2, S_3) or acceptors (S_0) [11–14].

Each S state transition is driven by the redox potential generated through a light-induced charge separation between P680, a special Chl*a* component, and a pheophytin molecule. This primary charge separation is stabilized by electron transfer to the plastoquinone co-factors Q_A and Q_B and by reduction of $P680^+$ through Y_Z, a redox active tyrosine residue of the D1 polypeptide. The oxidized tyrosine (Y_Z^{ox}) in turn is reduced by the OEC which ultimately abstracts electrons from water. According to the most recent estimates the distance between Y_Z and the Mn cluster is about 8–11 Å [15–17]. Recently arguments have been put forward to suggest that Y_Z is not a simple electron carrier, but plays an active role in water oxidation by directly abstracting H atoms from substrate water [18–20].

To understand the basics of water chemistry during photosynthetic oxygen evolution one needs to know the structure of the Mn cluster, the Mn redox states and the mode of substrate water binding in the different S states. The current state of knowledge in these areas of research is summarized below.

2. Structure of the Mn cluster

So far the knowledge about the structure of the Mn cluster is almost exclusively based on EXAFS measurements on PS II and relevant model compounds (for a review see [4]). The Fourier transform EXAFS spectrum of PS II exhibits three well resolved peaks. The first peak corresponds to the first shell O/N ligands and the second peak arises in the S_1 and S_2 states from two 2.7 Å Mn-Mn distances. Based on comparisons with model complexes the 2.7 Å distances are universally interpreted to correspond to bis-μ-oxo bridged Mn-Mn units ('diamonds'). The fit of the third peak is more difficult and it has been proposed to reflect either Mn-Mn or Mn-Ca interactions or both. The latter assignment is most likely, because results from Sr EXAFS experiments imply Ca binding at 3.5 Å near the Mn cluster [21] and the 3.3 Å Mn-Mn distance is also observed in Ca-depleted samples [22]. The 3.3 Å Mn-Mn separation is consistent with mono-μ-oxo bis-carboxylato bridging [23]. Several different arrangements of the three Mn-Mn distances are possible [4,24], but the simplest and most discussed is the dimer of dimers model (or Berkeley C, see Fig. 1). Based on DFT calculations [25] and ^{55}Mn-ENDOR measurements [26] models with 'joined diamonds' (plus one Mn at 3.3 Å) have been discussed recently (options E or F in [4]). EXAFS spectroscopy on partially oriented PS II samples shows that the 3.3 Å Mn-Mn and 3.5 Å Mn-Ca vectors are almost parallel to the membrane normal, while the two 2.7 Å vectors have angles of 55° and 67°, respectively [4,27].

Several experiments indicate that a conformational change of the OEC occurs on the $S_2 \rightarrow S_3$ transition ([28] and references therein; [29]). The structure of the Mn cluster in the S_3 state has recently been characterized by EXAFS spectroscopy and significant changes compared to the S_2 state were found [30]. Both 2.7 Å Mn-Mn distances increase, one to 3.0 Å and the other to about 2.8 Å. Even the 3.3 Å distance is found to be 3.4 Å in S_3. This result shows that the four Mn centers are highly connected and is an additional argument against the assignment of the 3.3 Å distance to only Mn-Ca interactions as suggested e.g. in [31].

The S_0 EXAFS data are not completely analyzed, but it is clear that one of the two Mn-Mn distances is longer than in the S_1 state. This finding can be explained by a protonation of one μ-oxo bridge and/or the presence of Mn^{II} in S_0 [32].

One Cl is specifically bound to PS II probably in all S states [33]. Some experiments indicate that it may only be essential for the $S_2 \rightarrow S_3$ and the $S_3 \rightarrow S_0$ transitions ([34] and references therein), while others suggest that it may not be essential for water oxidation at all [33]. No direct physical evidence for Cl binding to the Mn cluster exists at present, but recently the first indications for a Cl ligand were reported based on Mn-EXAFS measurements of oriented PS II samples in the S_3 states [35].

3. Mn redox states

The Mn redox states in PS II have been studied by

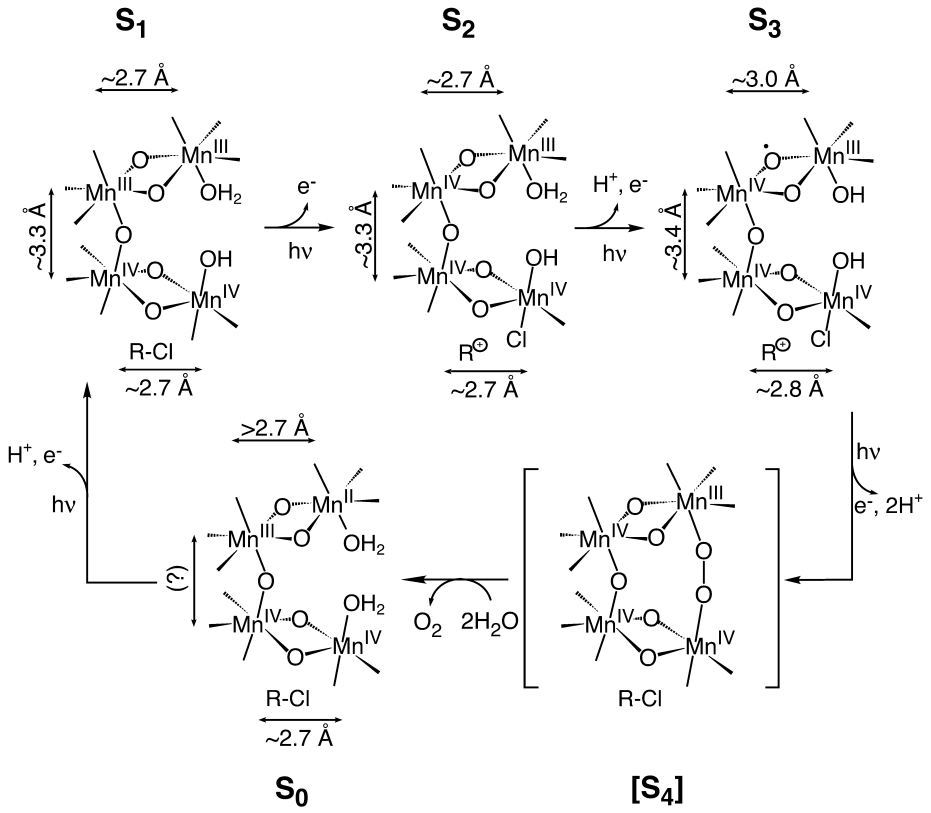

Fig. 1. Working model for the mechanism of photosynthetic water oxidation. The model accounts for X-ray absorption, EPR, substrate water exchange/binding, proton release and electrochromic measurements on PS II preparations. Mn redox states and distances as in [4]. The co-factor Ca is not shown for clarity of presentation (see [21]). R^+ is a positively charged residue (or co-factor) close to the Mn cluster, where Cl is proposed to bind in the S_0, S_1 and S_4 states. For further details see text.

a variety of techniques including UV absorption, NMR proton relaxation enhancement (NMR-PRE), EPR, XANES, Kβ spectroscopy and reduction of the Mn cluster with exogenous reductants like NH_2OH or NH_2NH_2 (reviewed e.g. in [36]). The most reduced redox state of the OEC that could be unambiguously identified so far is the S_{-3} state [37]. This sets a lower limit of Mn(III,III,III,III) for the Mn redox states in the S_1 state. The relative stability of the S_{-3} state makes, however, a Mn(III,III,IV,IV) assignment for S_1 more likely.

The S_2 state is paramagnetic and has an EPR signal with about 20 ^{55}Mn hyperfine lines [38,39]. With few exceptions it is assumed that this EPR multiline signal arises from an anti-ferromagnetically coupled Mn(III,IV) dimer that interacts with a second Mn dimer. Simulations have not yet arrived at a consistent picture about the redox states of the second dimer and either Mn(III,III) or Mn(IV,IV) has been used.

Because in each S state transition one electron is removed from the OEC, it was expected that the S_0 state should also be paramagnetic. The S_0 multiline signal was first discovered for the so-called S_0* state which can be generated from S_1 either in the dark by hydroxylamine incubation or with hydrazine incubation (to S_{-1}) and a single turnover illumination [40]. It was found that the presence of 0.5–3% methanol in the sample buffer is necessary to observe the S_0* multiline signal. Shortly afterwards it was shown that the physiological S_0 state has an identical EPR signal under these conditions [41,42]. The spectral width of the S_0 multiline signal is greater than for the S_2 multiline signal. This finding is consistent with the assumption that one Mn(II) is present in the S_0 state. However, current simulations do not allow an unambiguous decision between Mn(II,III,IV,IV) and Mn(III,III,III,IV) [42].

XANES and Kβ fluorescence measurements are element specific and can be applied with same sensi-

tivity to all S states. Based on the edge energies and shapes all groups report consistently Mn(III,III, IV,IV) and Mn(III,IV,IV,IV) as redox states for the S_1 and S_2 state, respectively [23,43–47]. No general agreement could, however, yet be reached for the Mn redox states in the S_3 state: some groups conclude that another Mn(III)-Mn(IV) oxidation occurs [46,48] while others favor a ligand centered oxidation of the OEC [45]. There are many factors that contribute to these differences and, over time, these complex experiments have been performed more and more carefully. Critical factors are: (a) the sample quality (no unspecific Mn), (b) a deep S_2 EPR multiline oscillation pattern that allows a unique S state deconvolution, (c) documentation of no (or minimal) radiation damage and (d) an excellent signal to noise ratio. Additional confusion comes from the use of several different methods to determine the edge positions: half-height, first zero crossing of second derivatives and integral method. The second derivative approach has several advantages: (a) it is insensitive to linear baseline subtraction and errors in normalization, (b) for a large number of Mn model compounds it has been established that, without exception, a shift of 1–2 eV is seen in the edge position per oxidized Mn, provided that compounds with similar structure and ligands are compared, and (c) in addition to edge positions, detailed information on the edge shapes is obtained. The latest study of the Berkeley group confirms their earlier result that a much smaller Mn K-edge shift occurs on the $S_2 \rightarrow S_3$ transition compared to the $S_1 \rightarrow S_2$ transition (Messinger et al., in preparation). Although a structural change accompanies this transition (see above) and the shape of the Mn K-edge is different for the S_2 and S_3 states, the most likely interpretation for the obtained XANES spectra is that a ligand centered oxidation and *not* a Mn centered oxidation occurs at the $S_2 \rightarrow S_3$ transition. In addition, in the same study, independent information on the Mn redox states was obtained on the same set of samples using Kβ fluorescence spectroscopy. These measurements fully confirm the lack of Mn oxidation for the $S_2 \rightarrow S_3$ transition and a comparison of the first moment value for the $K\beta_{1,3}$ peak of the S_3 state with those of several different Mn(IV) compounds shows that the S_3 state must contain at least one Mn(III) ion, thus arguing against Mn oxidation during the $S_2 \rightarrow S_3$ transition.

In agreement with this conclusion, more indirect methods like Y_D^{ox} power saturation and T_1 relaxation time measurements also suggest that no Mn oxidation occurs on $S_2 \rightarrow S_3$ [49,50]. The only results that appear to be at variance with this conclusion are UV absorption changes connected with the S state transitions (for review see [36]). The interpretation of the rather structureless UV difference spectra is, however, not straightforward because PS II components like quinones and certain amino acid side chains can contribute significantly to the UV absorption changes.

The problem has been raised that near 10 K, where X-ray and EPR studies of PS II are performed, the Mn cluster may be in a different electronic and/or structural state than under in vivo conditions, because a temperature dependent redox equilibrium between ligand and Mn oxidation (or between different ligands) and/or temperature induced structural changes may exist [51]. This is an important concern that needs further attention, but some room temperature studies like NMR-PRE [52] and the differences in reactivity of S_2 and S_3 towards $NO^{\bullet}$ [53] support either the lack of Mn oxidation or indicate radical formation in the S_3 state. A structural change of the OEC during the $S_2 \rightarrow S_3$ transition had been previously invoked from the surprisingly low reactivity of the S_3 state towards NH_2OH/NH_2NH_2 [28]. In addition, density functional theory calculations are in agreement with radical formation and a structural change in the S_3 state [54]. Therefore, in Fig. 1 it is assumed that the low temperature data reflect the in vivo situation.

4. Substrate water binding

Because for PS II the substrate is identical to the solvent, the study of substrate binding kinetics and the determination of binding constants are more complicated than for other enzymes. The first investigations concerning the question of substrate water binding were performed by Radmer and Ollinger [55]. The experimental approach was to preflash the PS II samples into a certain S state in $H_2^{18}O$, then dilute the label with $H_2^{16}O$ and finally to give another

series of flashes after a variable mixing time. The isotopic distribution of evolved oxygen was then determined by mass spectrometry. They found that within their time resolution of about 30–60 s, there are no non-exchangeable water intermediates bound to the OEC. It was pointed out early on that the time resolution achieved in those experiments was not sufficient to exclude bound water intermediates [51]. Bader et al. have therefore attempted in another series of investigations to improve time resolution or circumvent the problem by certain flash trains [56]. A significant improvement of time resolution (factor of 1000) was achieved by Messinger et al. [57]. This allowed the detection of one slowly exchanging substrate water molecule in the S_3 state. A further improved time resolution (factor 4) and a mathematical correction for the increasing $H_2^{18}O$ and decreasing sample concentration during injection enable the resolution of the fast water exchange in the S_3 state [58]. The detection of two distinct exchange rates proves unambiguously that both substrate water molecules are bound to the OEC in the S_3 state. Meanwhile exchange data have also been collected for the S_2, S_1 and S_0 states [58–61]. The data listed in Table 1 show that at least one substrate water molecule is bound to the OEC in the S_0, S_1 and S_2 states and that both water molecules are bound in the S_3 state. The heterogeneity of the exchange rates, their different S state dependence and their different activation energies (78 ($\pm$10) kJ mol^{-1} and 39 ($\pm$5) kJ mol^{-1} for the slow and fast exchange, respectively [57,58]) imply that the binding sites for the two water molecules are not identical.

In addition to these direct conclusions, the S state dependence and the observed activation energies strongly support the idea that both substrate water molecules are bound directly to Mn [57,58,61]. The identification of the bound water species (terminal, bridging; oxo, hydroxo, water) and the determination of the redox states of the Mn to which they bind is very difficult, because exchange rates can be modified significantly by ligands, H bridging and charge of the complex. At present, terminal hydroxo groups bound to Mn(III) or Mn(IV) seem most probable [61].

Table 1
Rate constants for the substrate water exchange in spinach thylakoids as a function of S states at 10°C

S state	Slow exchange (s^{-1})	Fast exchange (s^{-1})
S_3	2.0 ± 0.2	37 ± 2
S_2	2.1 ± 0.2	> 175
S_1	0.022 ± 0.002	–
S_0	≈ 13 ± 5	–

Adapted from [61].

For the paramagnetic S_2 and S_0 states EPR techniques like ESEEM and ENDOR can be used to detect the binding of ^1H, ^2H or e.g. ^{17}O near the Mn cluster. With the exception of a few studies [62–64], where line broadening or other effects may have prevented the detection of bound water, these types of data are in agreement with one or two bound water molecules in the S_2 state [65–67] and the S_0 state ([32]; Peloquin, personal communication). Although unique interpretations are difficult, the detected proton (deuteron) couplings favor the binding of substrate water as hydroxo or water rather than as terminal oxo or μ-oxo for these S states. This conclusion is further supported by NMR-PRE experiments which require that at least one partly protonated water molecule is bound to the Mn cluster up to the S_3 state [52].

Another way of collecting information on the nature of the bound water species in the different S states are measurements of proton release patterns and electrochromic shifts (for review see [36,68]). The principal difficulty is that the internal proton release pattern from the bound substrate water is modified by the protonatable groups of the protein matrix as outlined in [51]. Based on the assumption that the internal pattern is pH independent and by the use of a highly purified PS II preparation from *Synechococcus elongatus*, an internal pattern of 1:0:1:2 for the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow (S_4) \rightarrow S_0$ transitions is reported, which is modified by one amino acid with a pK_a of 5.7 [69]. The same group reports net charges of 0, 0, 1, 1 for the OEC in the S_0, S_1, S_2 and S_3 states [70]. Although still controversial, these results are adopted for the mechanism in Fig. 1.

5. Working hypothesis for the mechanism of photosynthetic water oxidation

As a structural template for this proposal the Berkeley C is used (Fig. 1), but the proposed water

chemistry can be transferred to other possible configurations of the Mn cluster, like the joined diamond (or 'dangler') model (not shown). From the data presented about the Mn redox states it is clear that the assignment for the S_0 state is least known, but most data are in better agreement with Mn(II,III,IV,IV) than with Mn(III,III,IV,IV). There is little doubt about the other S states being Mn(III,III,IV,IV), Mn(III,IV,IV,IV) and Mn(III,IV, IV,IV)$^{\bullet}$ for S_1, S_2 and S_3, respectively (the dot represents a radical from an oxidized ligand). The structural change observed for the $S_2 \rightarrow S_3$ transition, i.e. the lengthening of all Mn-Mn distances and particularly of one 2.7 Å distance to 3.0 Å, argues clearly against a Mn oxidation during the $S_2 \rightarrow S_3$ transition and also makes the oxidation of a terminal ligand very unlikely. To increase the Mn-Mn distance in bis-μ-oxo bridged Mn(III,III), Mn(III,IV) or Mn(IV, IV) dimers their bridges need to be modified, e.g. by protonation or oxidation. Both events reduce the electron density in the bridges. In Mn model compounds it was shown that the protonation of one bridge increases the Mn-Mn distance from 2.7 Å to about 2.8 Å, protonation of both bridges leads to a lengthening to approx. 2.9 Å [71]. Because protonation is unlikely to accompany an oxidation of a complex, an oxidation of the bridge is the most likely possibility [4]. This will drastically lower the strength of the μ-oxo bridge and could explain the increase to 3.0 Å. The reason for the increase of the other Mn-Mn distances is unclear at the moment, but may be explained e.g. by a weak 'delocalization' of the radical character over the bridges of the Mn cluster. For the joined diamond model, DFT calculations show that a *trans* effect leads to an increase of the second 2.7 Å distance [25].

The water exchange/binding data show that at least one, possibly both water molecules are bound (at least partly protonated up to the S_3 state) to the Mn cluster in all the S states. Now one problem remains: what kind of bound water species and mechanisms can be proposed to rationalize the S state dependence of the substrate water exchange rates? Only the slow water exchange rate is resolved for all the S states, so these data will be discussed first. In general, Mn oxidation and substrate water deprotonation will lead to a significant decrease in the water exchange rates (for a detailed discussion see [72]). It is therefore interesting that a 500–1000-fold decrease of the slow exchange rate is only observed for the $S_0 \rightarrow S_1$ transition and that the exchange in the S_2 state is actually 100 times faster compared to the S_1 state. Essentially no change is observed between S_2 and S_3 ([61] and Table 1). The large change between S_0 and S_1 would be consistent with water binding to the Mn ion that is oxidized during this transition. However, as most probably a Mn(II) to Mn(III) oxidation occurs and because the measured exchange rate is too slow to account for Mn(II) binding [61], this interpretation is unlikely. It is therefore more probable that the exchange rate decreases, because one water molecule (bound to a Mn(III) or Mn(IV) ion) is deprotonated as a consequence of a pK shift due to the oxidation of a Mn(II) ion to Mn(III). Such a deprotonation is in line with the proton release pattern. For the 100-fold increase of the slow exchange rate on the $S_1 \rightarrow S_2$ transition several different mechanism can be invoked: (a) change in H bonding of the hydroxo group, (b) deprotonation of a ligand to Mn [61] or (c) ligand exchange or binding to Mn. In the last case Cl binding is an attractive possibility, because it gives this cofactor a functional role and it is in line with the internal proton release pattern, the postulate for electroneutrality of the Mn cluster on each S state transition [18] and with the net charge of 1 in the S_2 and S_3 states, provided that Cl is bound to a positively charged group near the Mn cluster (R^+ in Fig. 1) that does not get neutralized in S_2 and S_3. This possibility is shown in Fig. 1. Binding of the slow exchanging substrate water molecule to Mn(IV) in all S states is favored in this proposal to account for the following findings: (a) the above discussed Mn oxidation states, (b) the S state dependent changes of the slow exchange rate and (c) the about 20-fold difference in the fast and slow exchange rates in the S_3 state. This is in contrast to a mechanism suggested by Hillier and Wydrzynski [61], where based on a detailed comparison of the PS II exchange rates to those of different model compounds arguments have been put forward to suggest water binding to Mn(III) and Mn(III,III,III,III) as redox states for the S_1 state.

The fast exchanging substrate water molecule is only observed in the S_3 state. It seems likely, however, that it is also bound to the Mn cluster in the

other S states, but exchanges too fast to be detected with the current setup. It is therefore assumed that the fast exchanging water binds to Mn(II) in S_0 and Mn(III) in S_1, S_2 and S_3. The at least 5-fold decrease in exchange rate upon the $S_2 \rightarrow S_3$ transition and the only 20-fold difference to the slow exchange in the S_3 state (assigned to Mn(IV)-OH) are rationalized by the oxidation of the nearby μ-oxo bridge that also triggers the deprotonation of this water molecule during the $S_2 \rightarrow S_3$ transition.

Starting from the proposed configuration of the S_3 state, a possible mechanism for the O-O bond formation would be as follows: Y_Z^{ox} oxidizes the slow substrate water molecule which is bound as a terminal Mn(IV)-OH group, then the hole of the bridge migrates to the fast exchanging terminal Mn(III)-OH group and the O-O bond is formed under the release of two protons. After the Mn cluster has accepted two more electrons, O_2 is released and two new water molecules bind. Based on kinetic arguments a preformation of the O-O bond in the S_3 state in form of an equilibrium between Mn (here possibly Mn and μ-oxo bridge) and substrate water oxidation has been proposed [1]. This is a viable option providing this equilibrium is temperature dependent and at cryogenic temperatures the redox states are as indicated in Fig. 1.

This proposal is certainly not unique and several assumptions made to explain the substrate water exchange data need to be tested. Important factors like the energies of each transition and the influence of the protein matrix e.g. on proton or O_2 release and H_2O binding have been ignored for simplicity or lack of data, but will have to be taken into account for a full understanding of this fascinating and unique process.

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