

Computational insights into the O₂-evolving complex of photosystem II

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Abstract Mechanistic investigations of the water-splitting reaction of the oxygen-evolving complex (OEC) of photosystem II (PSII) are fundamentally informed by important modifications due to structural refinement, hydration, and proteinaceous ligation which improve structural studies. Many physical techniques have provided agreement with a wide range of experimental data. The important insights into the OEC structure and function, computational models are useful for rationalizing spectroscopic and crystallographic results and for building a complete structure-based mechanism of water-splitting in PSII as described by the intermediate oxidation states of the OEC. This review summarizes these recent advances in spectroscopy applied in conjunction with mutagenesis studies. However, experimental studies have yet to yield consensus as to the exact configuration of the catalytic metal cluster and its ligation scheme. Computational modeling studies including density functional (DFT) theory combined with quantum mechanics/molecular mechanics (QM/MM) hybrid methods for explicitly including the influence of the surrounding protein, have proposed chemically satisfactory models of the fully ligated OEC within PSII that are maximally consistent with experimental results. The inorganic core of these models is similar to the crystallographic

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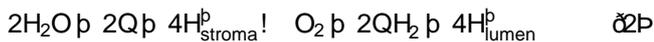
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The oxygen-evolving complex (OEC) of photosystem II (PSII) catalyzes the four-electron oxidation of water in the thylakoid membrane of green plant chloroplasts, producing dioxygen and releasing protons to the lumen as described by the following electrochemical half-reaction:



Our current understanding of the water-oxidation reaction introduced by Eq. (1) is based on the catalytic cycle proposed by Joliot and Kok (see Fig. 1) (Joliot et al. 1969; Kok et al. 1970). The cycle has been extensively investigated for several decades by a variety of biochemical, spectroscopic, and theoretical studies (Barber 2003; Diner and Babcock 1996; Renger 2001; Vrettos et al. 2001a; Yachandra et al. 1996). The solar

energy required for water oxidation is harvested by a system cycle and its ligation scheme remain poorly understood. of chlorophyll and carotenoid cofactors in PSII and Computational modeling studies (Blomberg et al. 1997, subsequently transmitted to the special chlorophyll moiety Lundberg et al. 2003, McEvoy and Brudvig 2004, Siegbahn 2002, Siegbahn and Crabtree 1999, Siegbahn and P₆₈₀ is oxidized, translocating electrons across the membrane through a number of redox cofactors (McEvoy based on density functional (DFT) theory and quantum and Brudvig 2006, Yachandra 2005). On the electron-acceptor side of PSII, plastoquinone (Q) is doubly reduced and doubly protonated to form plastoquinol (QH₂). This reaction takes place on the stromal side of the membrane and Q is displaced from its binding site by a fresh Q molecule. The overall reaction is:



On the electron-donor side of PSII, each highly oxidizing P₆₈₀⁺ radical ($E \approx +1.25$ V) (Grabolle and Dau 2005, Rappaport et al. 2002) abstracts an electron from substrate-water bound to the OEC. This is mediated by the tyrosine residue (Y_Z) that serves as a redox intermediary (Debus et al. 1988b, Diner and Britt 2005, Metz et al. 1989), probably alternating between the reduced and deprotonated Y_Z⁻ (oxidized) forms (Hoganson and Babcock 1997, Hoganson et al. 1995).

As shown in Fig. 1, the OEC undergoes four consecutive light-driven one-electron oxidations and thereby stores four oxidizing equivalents that are rapidly used in the S₄ → S₀ transition to oxidatively split two water molecules. Each redox state accumulates an oxidizing equivalent and is conventionally described as a S-state, with S₀ the most reduced state and S₄ the most oxidized. While the S-states are well established, the structure of the catalytic metal cluster along the

mechanism of the OEC of PSII.

Structure and mechanism of the OEC

The mechanism of the OEC-catalyzed water-splitting reaction has long been a subject of investigation (Barber 2003, Diner and Babcock 1996, Renger 2001, Vrettos et al. 2001a, Yachandra et al. 1996). Besides its intrinsic biological interest, efficient electrochemical water-oxidizing catalysts are urgently required for the development of artificial photosynthetic systems (Lewis and Nocera 2006, Whitesides and Crabtree 2007). Understanding the operation of the OEC is therefore important for the development of novel solar energy transduction and storage systems with the potential to revolutionize renewable energy technology. Much biochemical work has been pursued to elucidate the

mechanism of water splitting at the OEC of PSII (Allahverdiyeva et al. 2004, Babcock et al. 1989, Bergmann et al. 1998, Berthomieu et al. 1998, Britt et al. 1994, Chu et al. 2001, Clausen et al. 2004, Debus 1992, 2001, 2003, 2005, Diner 2001, Diner and Babcock 1996, Diner et al. 1998, 2004, Diner and Nixon 1998, Diner and Rappaport 2002, Faller et al. 2002, Hays et al. 1998, 1999, Hillier and Messinger 2005, Hoganson et al. 1995, McEvoy and Brudvig 2006, Messinger 2004, Metz et al. 1989, Nixon and Diner 1992, Nixon et al. 1992, Rappaport et al. 2002, Strickler et al. 2005, Tang et al. 1994, 1996, Tommos et al. 1995, Wydrzynski and Satoh 2005). A variety of techniques have been applied, including electron paramagnetic resonance (EPR) spectroscopy (Boussac et al. 1989, 1998, Britt et al. 2000, 2004, Kulik et al. 2005a, b, c; Matsukawa et al. 1999, Messinger et al. 1997a, b; Miller and Brudvig 1991; Mino and Kawamori 2001; Nugent et al. 1997, Peloquin and Britt 2001; Peloquin et al. 1998, 2000, Poluektov et al. 2005; Razeghifard and Pace 1999), X-ray absorption spectroscopy (XAS) (Dau et al. 2001, 2003, 2004, Grabolle et al. 2006, Hasegawa et al. 1999, Haumann et al. 2005a, b; Iuzzolino et al. 1998,

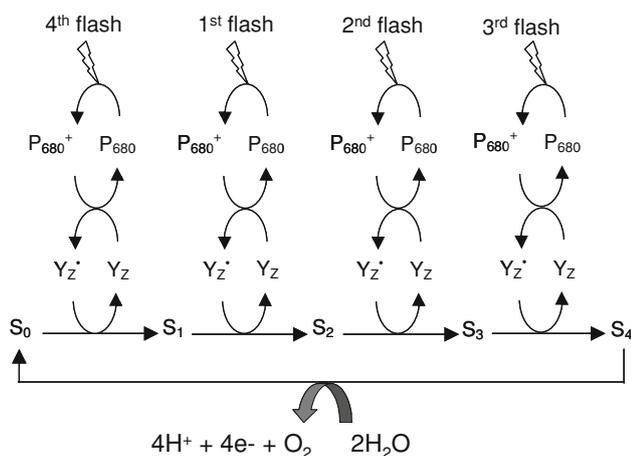


Fig. 1 Schematic description of the catalytic cycle of the OEC in PSII. P₆₈₀ is repeatedly photo-oxidized, in turn oxidizing tyrosine Z (Y_Z), which is the immediate oxidant of the OEC. Four one-electron oxidations of the OEC generate the S₄ state, which rapidly catalyzes the four-electron oxidation of water to produce O₂ and reform the S₀ state of the OEC

Liang et al. 2000; Mishra et al. 2007; Riggs-Gelasco et al. 1996; Robblee et al. 2002; Sauer and Yachandra 2004; Sauer et al. 2005; Stemmler et al. 1997; Yachandra 2002; Yachandra et al. 1986; Yachandra et al. 1987; Yano et al. 2005b, 2006), Fourier transform infrared (FTIR) spectroscopy (Chu et al. 2004; Debus et al. 2005; Kimura et al. 2005b; Strickler et al. 2005), and mass spectrometry (MS) (Hillier et al. 1998; Hillier and Wydrzynski 2000, 2001, 2004; Messinger et al. 1995) that have all particularly played important roles. In recent years, mechanistic investigations have been allied with a progressively clearer idea of the structure of the OEC, supported by X-ray diffraction studies (Biesiadka et al. 2004; Ferreira et al. 2004; Kamiya and Shen 2003; Loll et al. 2005; Zouni et al. 2001) and extended X-ray absorption fine structure (EXAFS) spectroscopy (Haumann et al. 2005a, b; Sauer et al. 2005; Yachandra 2005; Yano et al. 2005b). It appears that the OEC comprises a cluster of four manganese ions and one calcium ion, connected by bridging oxide ions. Surrounding amino-acids provide further carboxylic acid (oxygen) and histidine (nitrogen) ligation of the metals. However, there is still little agreement on the detailed configuration of the OEC Mn₄Ca cluster. Current X-ray crystallographic results are available only at a moderate resolution (3.0–3.5 Å) (Biesiadka et al. 2004; Ferreira et al. 2004; Loll et al. 2005) and have been called into question because of the possibility of reductive damage of the cluster due to the high doses of X-ray required for data collection (Grabolle et al. 2006; Yano et al. 2005a). EXAFS measurements, although able to provide accurate bond distances within the intact cluster, have not provided a complete picture of the OEC architecture, or the description of the arrangement of ligands (Yano et al. 2006). Therefore, fundamental aspects of structure/function relations in the OEC remain poorly understood, including the structure of the catalytic metal cluster, the coordination of substrate water molecules, the nature and functional role of structural rearrangements in the metal cluster along the catalytic cycle, and the role played by surrounding protein environment and cofactors on the catalytic mechanism.

QM/MM studies have developed chemically sensible models of the OEC compatible with XRD and EXAFS measurements of PSII (Sproviero et al. 2006b, 2007, 2008b). Starting from the empirical XRD structure at 3.5 Å resolution (Ferreira et al. 2004), computational models were developed by DFT QM/MM structural refinement of PSII in oxidation states consistent with EPR (Peloquin and Britt 2001; Peloquin et al. 2000) and X-ray spectroscopy (Dau et al. 2001; Yachandra et al. 1993) as well as recent XANES (Haumann et al. 2005b) and ⁵⁵Mn ENDOR (Kulik et al. 2005a, b, c) experiments. Therefore, the resulting models circumvent the effect of radiation damage caused by reduction of the Mn centers in the description of the OEC configuration. The implemented computational methodology has been rigorously tested with well-characterized manganese model compounds, verifying its ability to predict highly accurate XRD geometries (Sproviero et al. 2006a). Therefore, the resulting DFT-QM/MM structures are expected to benefit the construction of a complete model of the water-splitting catalytic cycle in terms of detailed molecular structures of the OEC catalytic intermediates (Sproviero et al. 2008b).

DFT benchmark studies have analyzed the ability of the Becke-3-Lee-Yang-Parr (B3LYP) hybrid density functional to predict the geometric, electronic, and magnetic properties of well-characterized polynuclear high-valent oxomanganese complexes (Sproviero et al. 2006a). This work has been essential for validating the DFT level of theory, as applied to Mn complexes, since hybrid density functionals were known to exhibit shortcomings in the description of low-lying spin states of open-shell transition-metal complexes (Ghosh et al. 2003; Ghosh and Steen 2001; Ghosh and Taylor 2003, 2005; Koch and Holthausen 2001; Lundberg and Siegbahn 2005b; Reiher et al. 2001a, 2001b). Spin-polarized calculations on benchmark model compounds included the synthetic di-μ-oxo Mn^{III}Mn^{IV} complexes [Mn^{III}Mn^{IV}(μ-XO)₂(H₂O)₂(terpy)₂]³⁺ (terpy = 2,2',6,2''-terpyridine) and [Mn^{III}Mn^{IV}(μ-O)₂(phen)₄]³⁺ (phen = 1,10-phenanthroline), the [Mn₃O₄(bpy)₄(H₂O)₂]⁴⁺ (bpy = 2,2'-bipyridine) trimer, and the [Mn₄O₄L₆]⁺ tetramer, with L = Ph₂PO⁻² (Sproviero et al. 2006a). These complexes involve unpaired spins with frustrated antiferromagnetic couplings that required to be addressed in terms of the broken symmetry (BS) DFT method (Noodleman 1981; Noodleman and Case 1992; Noodleman and Davidson 1986; Noodleman et al. 1995). The BS-DFT approach is also useful for calculations of exchange magnetic coupling constants and the ligand field analysis for metal d → d, charge transfer (ligand → metal, metal → ligand), and intervalence charge transfer (metal → metal or ligand → ligand) transitions (Sproviero et al. 2006a; Sproviero et al. 2006b; Sproviero et al. 2008b). Earlier computational studies of oxomanganese complexes had found that the DFT/B3LYP methodology typically overestimated Mn–Mn distances, with errors in the range of 0.10–0.15 Å (Lundberg and Siegbahn 2004; Petrie and Strange 2004), when comparing fully relaxed configurations of model complexes with oxo bridges and XRD data. In addition, it was reported that B3LYP overestimated Mn–ligand distances along the Jahn–Teller axis by as much as 0.25 Å (Lundberg and Siegbahn 2004; Petrie and Strange 2004). These errors were comparable to typical structural rearrangements in the OEC metal cluster, induced by oxidation of the constituent ions

(i.e., changes of Mn ligand bond-lengths in the 0.1–0.2 Å embedding (EE) at the (UHF B3LYP/lacvp,6-31G(2df),6- and therefore required further analysis. In addition, hybrid B3LYP/AMBER) level of theory. These computations functionals were known to overestimate the relative stability of high-spin over low-spin states of transition metal complexes (Koch and Holthausen 2001; Reiher et al. 2004), including both the link-hydrogen atom scheme for 2001a, a difficulty that could be critical in the process of efficient and flexible definitions of QM layers and the identifying the nature of ground electronic states, or impossibility of modeling open-shell systems by performing studies of spin-crossover phenomena in transition metal complexes (Harvey 2001; Harvey et al. 2003; Holthausen 2005; Poli and Harvey 2003; Reiher et al. 2001a; Schroder et al. 2000; Shaik et al. 2002). Unrestricted-DFT (e.g., UB3LYP) calculations, and the efficient generation of high-quality initial-guess spin-electronic states generated with Jaguar 5.5 (Jaguar 5.5, Schrodinger 1991–2003).

Findings of the most recent benchmark studies can be summarized as follows (Sproviero et al. 2006a): (1) The reduced system, including the metal cluster, the protein-ligands (E333, CP43-E354, D342, D170, E189, and D1332), water and hydroxo ligated to metals and chloride. The molecular structure beyond the QM layer is the same as when applied in conjunction with rather modest basis sets. The molecular structure beyond the QM layer is the same as long as oxo-bridges include sufficiently expanded basis set extended region that has been described by the Amber force-field (Cornell et al. 1995, 1996). Region Y includes all amino-acid residues with carbons within 4.5 Å from any atom in the OEC metal ion cluster and an additional buffer shell of amino-acid residues with carbons within 2.0 Å from any atom in the OEC ion cluster, with harmonic constraints to preserve the natural shape of the system (McEvoy et al. 2005a, b; Sproviero et al. 2006b). The total energy E has been obtained from three independent calculations: $E = \frac{1}{4} E^{\text{MM},X+Y} + \frac{3}{4} E^{\text{QM},X} - E^{\text{MM},X}$, because of the limitations of the B3LYP hybrid density functional to properly balance exchange and correlation contributions to the energies of the states. $E^{\text{MM},X+Y}$ is the energy of the complete system computed at the molecular mechanics level of theory, while $E^{\text{QM},X}$ and $E^{\text{MM},X}$ correspond to the energy of the reduced-system computed at the QM and MM levels of theory, respectively. Electrostatic interactions between regions X and Y are included in the calculation of both $E^{\text{QM},X}$ and $E^{\text{MM},X}$ at the quantum mechanical and molecular mechanics levels, respectively. Thus, the electrostatic interactions computed at the MM level in $E^{\text{MM},X}$ and $E^{\text{MM},\text{full}}$ cancel and the resulting DFT QM/MM evaluation of the total energy involves a quantum mechanical description of the polarization of the reduced system, due to transition metals, giving an error of at least 5 kcal mol⁻¹ in several instances (Siegbahn 2006). This weakness might be critical in investigations of the mechanisms of water splitting, which are strongly dependent on metal–O₂ binding. introduced by correcting the atomic charges of amino-acid residues in close contact with the QM layer, according to the self-consistent polarization protocol MoD-QM/MM (Gascon et al. 2006; Sproviero et al. 2006b, 2007, 2008b).

Other recently reported benchmark studies have examined the accuracy of the DFT/B3LYP method in calculations of O–H bond dissociation enthalpies in a variety of redox-active mononuclear and polynuclear manganese complexes (Lundberg and Siegbahn 2005a). In general, the functional $E^{\text{QM},X}$ and $E^{\text{MM},X}$ at the quantum mechanical and molecular mechanics levels, respectively. Thus, the electrostatic interactions computed at the MM level in $E^{\text{MM},X}$ and $E^{\text{MM},\text{full}}$ cancel and the resulting DFT QM/MM evaluation of the total energy involves a quantum mechanical description of the polarization of the reduced system, due to transition metals, giving an error of at least 5 kcal mol⁻¹ in several instances (Siegbahn 2006). This weakness might be critical in investigations of the mechanisms of water splitting, which are strongly dependent on metal–O₂ binding.

QM/MM modeling

QM/MM modeling of the OEC of PSII has been based on Structural models of the OEC the ONIOM (our own N-layered integrated molecular orbital plus molecular mechanics) method (Dapprich et al. 1999; Vreven and Morokuma 2000) with electronic models of the OEC in PSII in the dark-stable State, Recent DFT-QM/MM studies have introduced structural models of the OEC in PSII in the dark-stable State,

including the complete ligation of the metal cluster MnCa by amino-acid residues, water, hydroxide, and chloride (see Fig. 2, top) (Sproviero et al. 2006b). These QM/MM models were developed by structural refinement of the XRD model of PSII from the cyanobacterium *Thermosynechococcus elongatus* (Ferreira et al. 2004, assuming a minimum displacement of the ligating residues from their crystallographic positions after completing the coordination spheres of the metals by hydration. These QM/MM structural models were constructed under the assumption that the crystallographic positions of the amino-acid residues in the XRD models were essentially correct, within the experimental resolution, with electronic density maps only broadened by structural disorder and radiation damage induced by photoreduction of Mn ions. Fully relaxed QM/MM configurations were obtained by energy minimization and were found to preserve the architecture of the Mn₃CaO₄ cuboidal core with a dangling Mn ligated to corner μ_4 -oxide ion, suggested by the XRD model as previously proposed by EPR studies (Britt et al. 2004; Peloquin et al. 2000). The nature of the amino-acid residues directly ligated to the MnCa cluster were also consistent with the ligands proposed by the XRD structure of Ferreira et al. (2004), although with significant differences with regards to the actual ligation scheme. The resulting molecular structures are expected to provide biologically relevant insight into the OEC structure since room-temperature thermal fluctuations have negligible effects on the structure, protonation state, or charge localization effects of PSII, as indicated by XAS studies carried out at 20 K and room-temperature (Haumann et al. 2005b).

Water ligation

In contrast to the XRD structures, the QM/MM models have included manganese and calcium ions completely ligated consistently with standard coordination chemistry assumptions, supported by biochemical and spectroscopic data. This required hydration of the XRD models and addition of several small molecules as metal ligands to the OEC in order to satisfy the usual coordination numbers of high-valent Mn (5 or 6 ligands) and Ca ions (typically 6 coordinate) (Sproviero et al. 2006b). It was assumed that most (if not all) of these small ligands were either water bound or water-derived species like hydroxide or oxo ligands. Hydration involved an iterative procedure of "soaking" the XRD model in water and allowing the hydrated structure to relax in a process of energy minimization. This procedure led to the ligation of about six water and water-derived ligands to the metals of the OEC. Two of these waters, attached respectively to Ca and to Mn(4), were identified as possible substrate water molecules (see below), in agreement with

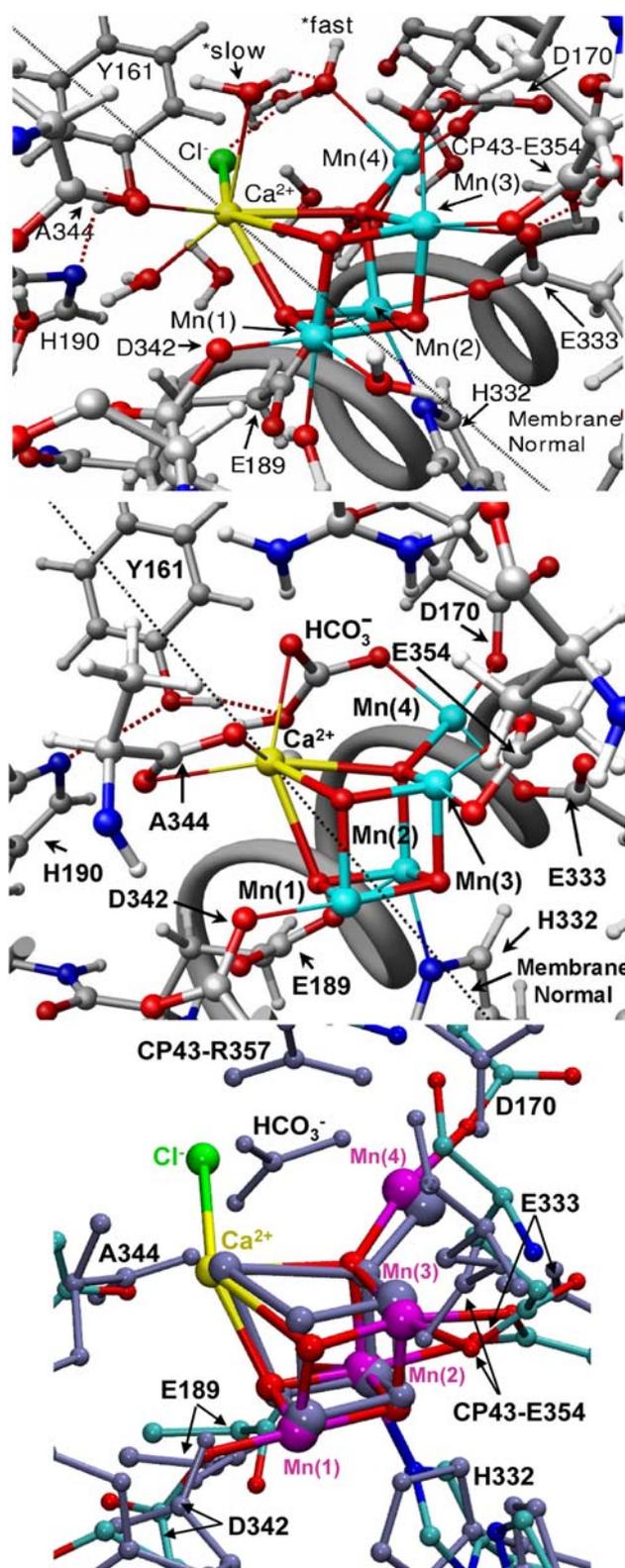


Fig. 2 The OEC and its surrounding molecular environment as described by the QM/MM model (Sproviero et al. 2006b top), the XRD structure 1S5L (Ferreira et al. 2004 middle), and the superposition of the pentanuclear MnCa clusters of the two models (bottom)

earlier proposals (Haumann and Junge 1999; Hoganson and Babcock 1997; McEvoy and Brudvig 2004; Messinger 2004; Schlodder and Witt 1999), but in contrast to other models suggesting substrate water coordination as oxo-bridges among Mn ions (Brudvig and Crabtree 1986; Nugent et al. 2001; Pecoraro et al. 1994; Robblee et al. 2001; Yachandra et al. 1996). The two molecules accounted for W^{fast} the electronic density in the 1S5L XRD structure that was initially assigned to bicarbonate (Ferreira et al. 2004).

There is some important experimental information concerning water binding at and around the OEC. Pulsed EPR experiments using D_2O -solvated PSII reveal the presence of several exchangeable deuterons near the OEC in the S_0 , S_1 , and S_2 states, which is broadly consonant with the QM/MM model, although the best fit to the EPR data involves rather fewer waters than are seen in the QM/MM structure (Britt et al. 2004). Reducing the number of waters also common in contact with the cluster in the QM/MM model remains to be explored and would involve: (1) some or all of the bridging, η^2 mode (as in the crystallographic model of Loll et al. 2005); and/or (2) the incorporation of non-water-derived small molecule ligands like bicarbonate (Dasgupta et al. 2004; McEvoy and Brudvig 2004; Sproviero et al. 2006b), of which there is little evidence.

Time-resolved mass spectrometry experiments, analyzing the production of ^{18}O -containing-dioxygen by the $H_2^{18}O$ -solvated OEC, have made important contributions to the question of substrate water binding at the OEC (Hendry and Wydrzynski 2002, 2003; Hillier et al. 1998; Hillier and Wydrzynski 2000, 2001, 2004; Messinger et al. 1995). These observations have been recently addressed by calculations of transition state energy barriers for water exchange in structural models of the OEC in the S_1 and S_2 states while progressively detaching substrate water molecules from Ca^{2+} and the dangling Mn(4) (Sproviero et al. 2008d). The resulting structural rearrangements provided insight on the water exchange mechanisms and the relative binding strengths, considering that elongation of the metal–oxygen bond is likely the primary step in water exchange and rate-determining (Lundberg et al. 2003; Rotzinger 1997; Rotzinger 2005). These calculations complemented earlier studies of water exchange in transition metal complexes (Cady et al. 2006; Helm and Merbach 1999; Houston et al. 2006; Rotzinger 1997; Rotzinger 2005; Tagore et al. 2006, 2007), including theoretical studies of manganese complexes, based on Hartree–Fock and complete active-space self-consistent field theories (Lundberg et al. 2003; Rotzinger 1997, 2005; Tsutsui et al. 1999) as well as DFT studies of water exchange in other transition metal complexes (Deeth and Elding 1996; Hartmann et al. 1997, 1999; Lundberg et al. 2003; Vallet et al. 2001).

It was found that the QM/MM models are consistent with two substrate waters in different environments within the OEC and so exchanging at different rates, with one of the substrate waters bound to calcium (Hendry and Wydrzynski 2003). Stretching the $Ca^{2+}O^{low}$ bond was energetically more demanding than stretching the Mn(4) O^{fast} bond due to charge delocalization in the oxomanganese complex, where charge transfer between manganese ions and ligand/oxo-bridges can affect the net ionic charges

of metal centers. Charge delocalization partially neutralizes the net ionic charges of the Mn centers, leaving a smaller positive charge on Mn(4) ($q = +1.35$) than on Ca^{2+} ($q = +1.77$). These results are thus consistent with O^{low} attached to Ca^{2+} , even when such a metal center has a smaller oxidation number than Mn(4) (Hendry and Wydrzynski 2003). The underlying charge delocalization is common to synthetic oxomanganese complexes in contact with the cluster in the QM/MM model remains to be explored and would involve: (1) some or all of the between partial ionic charges and formal oxidation numbers (Sproviero et al. 2006a, b, 2007).

In agreement with experiments (Hillier and Wydrzynski 2004), the QM/MM models also predict that the exchange rate of the slowly exchangeable water molecule increases upon $S_1 \rightarrow S_2$ oxidation (Sproviero et al. 2008d). Important mechanistic evidence for this observation also comes from metal-substitution studies at the calcium binding site (Vrettos et al. 2001b). QM/MM calculations indicated that charge transfer interactions between Ca^{2+} and D1-A344 decreased the partial ionic charge of calcium ($q = -0.21$), weakening the $Ca^{2+}O^{low}$ bond. Therefore, the exchange rate of W^{low} was predicted to increase upon $S_1 \rightarrow S_2$ oxidation of the QM/MM model, due to the corresponding changes in partial ionic charges modulated by charge transfer interactions.

Chloride QM/MM structures included Cl in the model since it is known to be required for transitions beyond the S_2 state (Wincencjusz et al. 1997). The binding position was found by replacing each water molecule by Cl and selecting the lowest energy configuration (McEvoy et al. 2005b; Sproviero et al. 2006b). Chloride was found to ligate loosely to the metal cluster, between calcium and Mn, in accordance with pulsed EPR data obtained from the acetate-inhibited OEC. EPR experiments indicate that acetate (known to bind competitively with chloride) (Kure et al. 1999; Sinclair 1984) binds at 3.1 Å from Y_Z (Force et al. 1997), in agreement with *in silico* substitution of calcium-bound chloride by acetate (McEvoy et al. 2005b). Therefore, the QM/MM structures predict that chloride is not directly bound to a Mn center but rather loosely bound to

the metal cluster by electrostatic interactions, at approximately 5 Å from the nearest Mn.

Protein ligands

The QM/MM models predict that the proteinaceous ligation of the OEC involves several amino-acid residues already thought to be ligands to the metal cluster on the basis of XRD (Biesiadka et al. 2004; Ferreira et al. 2004; Loll et al. 2005), site-directed mutagenesis, and spectroscopic studies (Boerner et al. 1992; Chu et al. 1994, 1995, 2004; Clausen et al. 2004; Debus 2001; Debus et al. 2005; Diner 2001; Kramer et al. 1994; Nixon and Diner 1992; Nixon et al. 1992; Roffey et al. 1994; Strickler et al. 2005, 2006). The proposed QM/MM coordination is similar to the proposal by Ferreira et al. (2004), except that D1-Glu333 in the QM/MM models bridges Mn(2) and Mn(3) in a η² mode, instead of monodentally binding Mn(4). In addition, CP43-Glu354 is protonated in the QM/MM models and binds in a monodentate fashion to Mn(3), instead of binding as a bidentate, chelating fashion as suggested by the crystal structure. These changes were found to be consistent not only with the overall electronic density maps associated with XRD models but also with EXAFS spectra and mechanistic data, as discussed in the following sections. A detailed description of metal-metal distances and oxidation states is presented in Table 1 and 2.

Oxidation states

Two redox isomers were found to have comparable stability in the S₁ resting state, including model (a) shown in Fig. 2 (top), where the dangling manganese Mn(4) is pentacoordinated and the Mn oxidation states are Mn(1) = IV, Mn(2) = IV, Mn(3) = III, Mn(4) = III, also referred as Mn(IV, IV, III, III); and model (b) (not shown

in Fig. 2), where the dangling manganese is hexacoordinated with an additional water and the oxidation states are Mn₄(IV, III, III, IV). These results are consistent with EPR (Peloquin and Britt 2001; Peloquin et al. 2000) and X-ray spectroscopy (Dau et al. 2001; Yachandra et al. 1993) as well as recent XANES (Haumann et al. 2005b) and ⁵⁵Mn ENDOR (Kulik et al. 2005a, b, c) experiments, but disagree with low-valent Mn(III, III, III, III) proposals (Kuzek and Pace 2001; Zheng and Dismukes 1996). The two models are neutral and predict anti-ferromagnetic coupling between Mn(1) and Mn(2), between Mn(2) and Mn(3), and between Mn(3) and Mn(4), but frustrated spin-coupling between Mn(1) and Mn(3) in the cuboidal structure. Both models include complete coordination of the high-valent Mn centers, with the preferential spin state III or IV of Mn(4) determined by the number of ligands (five or six, respectively). The coordination of H332 to the Mn cluster stabilizes the oxidation state IV for Mn(2) when Mn(4) is pentacoordinated, and the oxidation state III (with a Jahn-Teller elongation along the Mn-H332 axis) when the coordination sphere of Mn(4) is complete.

XRD data and EXAFS spectra The XRD data has yielded molecular structural models of PSII resolving nearly all cofactors and most of the amino-acid residues in the protein complex structure at 3.0–3.5 Å resolution (Ferreira et al. 2004; Loll et al. 2005). The precise positions of the Mn ions, substrate water molecules, and proteinaceous ligands, however, remain uncertain since the coordinate error in the density maps is usually as high as 1 Å (Kamiya and Shestakov 2003) and the resolution of bridging ligands is typically out of reach (Dau et al. 2003, 2004). Nevertheless, the XRD models have assigned ligands from the amino acid side chains which form the cavity of the Mn cluster and the assignment of these amino

Table 1 Interionic distances and bond angles relative to the membrane normal in the DFT QM/MM structural models of the OEC of PSII in the S₀, S₁(a), S₁(a) with P-EXAFS optimization, S₂, S₃, and S₄ states (Sproviero et al. 2007), including comparisons to the 1S5L XRD model (Ferreira et al. 2004)

Bond vector	S ₀		S ₁ (a)		S ₁ (a) Opt. P-EXAFS		S ₂		S ₃		S ₄		X-ray	
	Length [Å]	Angle	Length [Å]	Angle	Length [Å]	Angle	Length [Å]	Angle	Length [Å]	Angle	Length [Å]	Angle	Length [Å]	Angle
Mn(1)–Mn(2)	2.65	59	2.76	57	2.72	51	2.78	58	2.69	57	2.69	54	2.65	59
Mn(1)–Mn(3)	2.92	76	2.76	85	2.71	75	2.77	81	2.81	73	2.82	74	2.67	79
Mn(2)–Mn(3)	2.96	78	2.82	63	2.78	68	2.86	65	2.82	77	2.58	72	2.72	71
Mn(2)–Mn(4)	3.79	54	3.34	54	3.23	58	3.29	59	3.84	58	3.55	61	3.25	58
Mn(3)–Mn(4)	3.04	21	3.72	29	3.33	34	3.55	35	2.81	21	2.81	27	3.26	38
Ca–Mn(2)	3.59	63	3.31	53	3.39	62	3.78	57	3.63	63	3.61	71	3.40	59
Ca–Mn(3)	3.51	50	3.95	35	3.43	40	4.00	36	3.74	53	3.58	57	3.38	39

Table 2 Mulliken spin population analysis and ESP atomic charges in the DFT QM/MM models of the OEC of PSII in S₀, S₁, S₂, S₃, and S₄ states (Sproviero et al. 2007)

Ion center	S ₀			S ₁			S ₂			S ₃			S ₄		
	Spin pop.	Ox. #	ESP charge	Spin Pop.	Ox. #	ESP charge	Spin pop.	Ox. #	ESP charge	Spin pop.	Ox. #	ESP charge	Spin pop.	Ox. #	ESP charge
Mn(1)	-2.88	+4	+1.30	-2.80	+4	+1.11	-2.79	+4	+1.14	-2.87	+4	+1.38	-2.85	+4	+1.32
Mn(2)	+3.83	+3	+1.20	+2.75	+4	+1.08	+2.92	+4	+1.02	+3.15	+4	+1.16	+3.19	+4	+1.72
Mn(3)	-3.87	+3	+1.27	-3.82	+3	+1.26	-2.74	+4	+1.59	-2.97	+4	+1.62	-2.84	+4	+1.72
Mn(4)	+3.80	+3	+1.15	+3.80	+3	+1.35	+3.79	+3	+1.49	+2.98	+4	+1.13	+3.10	+4	+0.97
O(5)	+0.00	-2	-0.75	+0.05	-2	-0.60	+0.09	-2	-0.53	+0.03	-2	-0.68	+0.03	-2	-0.76
O(6)	+0.05	-2	-0.92	+0.02	-2	-0.80	+0.02	-2	-0.81	+0.03	-2	-0.84	+0.01	-2	-0.99
O(7)	+0.00	-2	-0.74	+0.02	-2	-0.67	-0.03	-2	-0.78	+0.10	-2	-0.72	+0.07	-2	-0.72
O(8)	-0.03	-2	-0.95	-0.07	-2	-0.98	-0.09	-2	-0.86	-0.04	-2	-1.11	-0.05	-2	-1.49
Ca	-0.00	+2	+1.60	-0.01	+2	+1.77	-0.00	+2	+1.56	-0.00	+2	+1.65	-0.00	+2	+1.66
Cl	-0.04	-1	-0.54	-0.00	-1	-0.71	+0.00	-1	-0.67	-0.00	-1	-0.68	-0.27	-1	-0.48

acids by Ferreira et al. (2004) has been confirmed by Loll intensities in momentum $\hat{O}k$ -space. The Fourier transform et al. (2005), with only very slight modifications which of these oscillations determine the metal-metal distances, were within the errors of the electron density maps. Furthermore, the coordination of Mn ions, and the coordination bond lengths with ligands, including oxo-bridges, water/hydroxo species and amino-acid residues directly ligated to Mn ions have been tentatively proposed consistently with the overall electronic density maps and the Mn-Mn distances determined (see Figs 3, 5, and 8).

mined by XAS studies (Dau et al. 2001; Robblee et al.

2001).

Isotropic EXAFS spectra

The best structural data of the OEC comes from high-

resolution EXAFS measurements (Dau et al. 2001, 2003;

2004; Haumann et al. 2005b; Yachandra et al. 1987; Yano

et al. 2005b; 2006), although the structural models are still

underdetermined. Therefore, the QM/MM models have

been carefully analyzed as compared to both XRD models

and the most recent high-resolution EXAFS data. The

two redox isomers obtained at the DFT-QM/MM level of

comparisons of calculated and experimental EXAFS data

show that there is very good agreement in the

description of the peaks associated with multiple

scattering from the N and O Mn-ligand centers at 1.8 Å

emitted by the Mn ions upon X-ray absorption. Calcula-

tions have been carried out according to the Real Space

backscattering due to the dangling Mn and Ca

FEFF8 (version 8.2) (Ankudinov et al. 2002; Bouldin et al.

2001). The method is based on the theory of the oscillatory

spectra is the slightly different structure of the peak at

reduced distance 1.6 Å, where model (b) has a more pro-

nounced shoulder due to the slightly shorter 2.1 Å

coordination bond length between Mn(2) and the carbox-

ylate oxygens of E333, while the corresponding bond

length in model (a) is 2.2 Å

or EXAFS data, has been obtained with the module

FEFF83, explicitly considering atoms within 10 Å from

any metal in the OEC. The quantum mechanical interfer-

ence of outgoing photoelectrons generated by X-ray

absorption, with the scattered waves from atoms sur-

rounding the Mn ions, generates oscillations of EXAFS

Figure 3 shows the comparison of calculated and experi-

mental EXAFS spectra in k -space (left) as well as the

corresponding spectra in reduced distances (right). The

experimental spectra in k -space were kindly provided by

Prof. Holger Dau. The calculated spectra correspond to the

two redox isomers obtained at the DFT-QM/MM level of

theory and the X-ray model structure 1S5L. The compari-

son shows that there is very good agreement in the

description of the peaks associated with multiple

scattering from the N and O Mn-ligand centers at 1.8 Å

emitted by the Mn ions upon X-ray absorption. Calcula-

tions have been carried out according to the Real Space

backscattering due to the dangling Mn and Ca

FEFF8 (version 8.2) (Ankudinov et al. 2002; Bouldin et al.

2001). The method is based on the theory of the oscillatory

spectra is the slightly different structure of the peak at

reduced distance 1.6 Å, where model (b) has a more pro-

nounced shoulder due to the slightly shorter 2.1 Å

coordination bond length between Mn(2) and the carbox-

ylate oxygens of E333, while the corresponding bond

length in model (a) is 2.2 Å

The quantitative analysis of interatomic bond lengths

and bond orientation angles relative to the membrane

normal in QM/MM models has allowed for rigorous

comparisons with high-resolution EXAFS spectra and

X-ray diffraction models (see Table 2) (Sproviero et al.

2006b; 2007, 2008b). The interatomic distances compatible

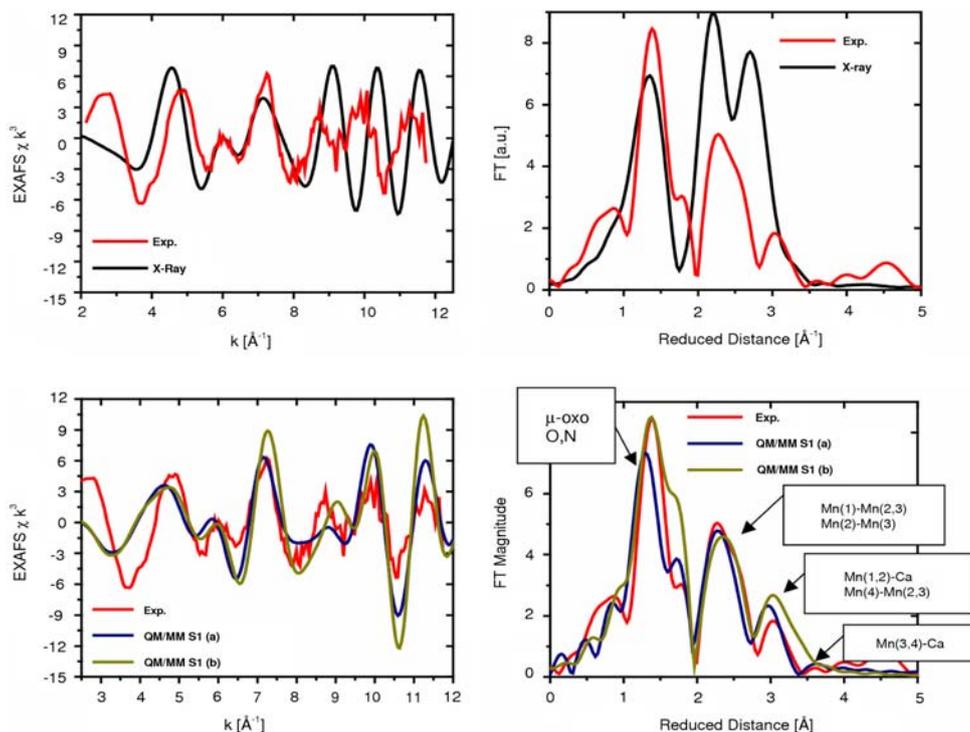


Fig. 3 Comparison between experimental (Haumann et al. 2005b, 2006b), including model (a) where the dangling manganese is pentacoordinated and the oxidation states are Mn(1) = IV, Mn(2) = IV, Mn(3) = III, Mn(4) = III; and (b) where the dangling manganese is hexacoordinated with an additional water and the oxidation states are Mn(1) = IV, Mn(2) = III, Mn(3) = III, Mn(4) = IV. Copyright 2006 American Chemical Society

with EXAFS measurements are: $2 \times 2.7 \text{ \AA}$ Mn–Mn distances, $1 \times 2.8 \text{ \AA}$ Mn–Mn distance, $1 \times 3.3 \text{ \AA}$ Mn–Mn distance, and $2 \times 3.4 \text{ \AA}$ Mn–Ca distances. Interatomic distances $> 3.4 \text{ \AA}$ are difficult to measure by EXAFS, in fact the most reliable measured distances are those below 3 \AA . Model (a) yields the following distances: $2 \times 2.76 \text{ \AA}$ Mn–Mn distances, $1 \times 2.82 \text{ \AA}$ Mn–Mn distance, $1 \times 3.34 \text{ \AA}$ Mn–Mn distance, and $1 \times 3.31 \text{ \AA}$ Mn–Ca distance. It is, therefore, concluded that the agreement between QM/MM models and EXAFS data is very good, with the most significant discrepancy being that the two shortest Mn–Mn distances are slightly too long. In contrast, the calculated EXAFS spectrum, based on the X-ray model 1S5L structure shows much worse agreement with EXAFS data. This is mainly due to the slightly different geometry of the metal cluster and the incomplete coordination of the metal centers.

associated with X-ray diffraction data, it is difficult to judge whether the oxomanganese cores in the QM/MM model and in the three X-ray diffraction structures are truly identical or whether there are any significant differences. The QM/MM model is also consistent with the original analysis of ^{55}Mn -ENDOR measurements on the S state (Britt et al. 2000). The significance of these results with respect to the S state is determined by the fact that the S and S states have almost identical geometry, as discussed later in this article. The ^{55}Mn -ENDOR measurements disfavored the dimer of dimers model over models with a trinuclear-Mn core and a fourth Mn set off from the core by a longer Mn–Mn internuclear distance, or a dangler model. Recent work on polarized EXAFS data, however, favored the dimer of dimers model over the cuboidal Mn_4Ca complex with a dangler Mn (Yano et al. 2006).

In addition to the observed agreement between calculated and experimental isotropic EXAFS spectra, the configuration of the cuboidal Mn_4Ca complex with a dangler Mn in the QM/MM hybrid model shares common structural features with the X-ray diffraction model. In fact, due to the limited resolution of the electronic density maps, EXAFS amplitudes that provide information on the

orientations of the Mn–Mn and Mn–Ca vectors. These models, as compared to the studies reduced an original set of 11 possible empirical corresponding spectra based on models I, IIa, and III of EXAFS models (Derose et al. 1994) of the Mn₄Ca cluster, Yano et al. (2006). These studies are particularly important including the “dimer of dimers” model extensively discussed in the past (Yachandra et al. 1996), to one model for the reconciliation of XRD and EXAFS models since the DFT-QM/MM model provides isotropic EXAFS spectra in much better agreement with experimental data than the XRD models (see Figs 3, 5), and yet it is fully consistent with the protein ligands proposed by the two independently developed X-ray diffraction structures (Ferreira et al. 2004; Loll et al. 2005).

Figure 5 shows the comparison of polarized EXAFS spectra for model IIa, proposed by Yano et al. (2006) and the corresponding polarized EXAFS spectra based on the 1S5L XRD model (top) and the DFT-QM/MM model (middle) in the S state. These results show that the polarized-EXAFS spectra of model IIa agree much better with the polarized-EXAFS spectra of the DFT-QM/MM model than with the corresponding spectra of the 1S5L XRD model. In addition, Fig 5 (bottom) shows that quantitative agreement with the polarized-EXAFS spectra of model IIa can be obtained by slightly re-orienting the orientation of some metal–O vectors in the QM/MM model, without significantly affecting the metal–metal or metal–ligand distances. Figure 6 shows a superposition of the inorganic Mn₄Ca core in the re-orientated QM/MM (R-QM/MM) model (Sproviero et al. 2008c) and 1S5L XRD (Ferreira et al. 2004) models. Furthermore, Table 1 shows the metal–metal and metal–ligand bond lengths and orientations, relative to the membrane normal, in the QM/MM and R-QM/MM models. These results show that the QM/MM cuboidal Mn₄Ca cluster with a dangler Mn model and ligated consistently with XRD models can provide polarized EXAFS spectra that are fully consistent with experimental data.

One possible explanation (favored by the Berkeley group) for the lack of consistency between the polarized EXAFS models and the XRD model is that XRD data might be fundamentally flawed because of radiation-induced damage, although the cuboidal model of the OEC with a dangler Mn is at least partially consistent with Mn-ENDOR measurements as well as QM/MM studies. Another possibility is that more complete structural models might be able to reconcile the polarized-EXAFS and XRD data, as recently investigated with DFT-QM/MM models (Sproviero et al. 2008c).

Theoretical studies (Sproviero et al. 2008c) have analyzed the polarized-EXAFS spectra based on the DFT-QM/MM examining the vibrational properties of amino-acid residues in close contact with the OEC by using FTIR spectroscopy (Strickler et al. 2006; Strickler et al. 2005). These FTIR studies indicated that the vibrational frequencies of carboxylate groups associated with amino-acid residues D1-D170, D1-D342, and D1-E189 are not shifted as the OEC is oxidized from the S₀ to the S₃ states (Strickler et al. 2005, 2006). The experimental evidence is given by the S-state difference FTIR spectra (i.e., S₃ – S₀ spectra) obtained both from the wild-type PSII and from a site-directed mutant in which the amino-acid residue under scrutiny has been replaced by a different residue. If significant differences were observed between the wild-type

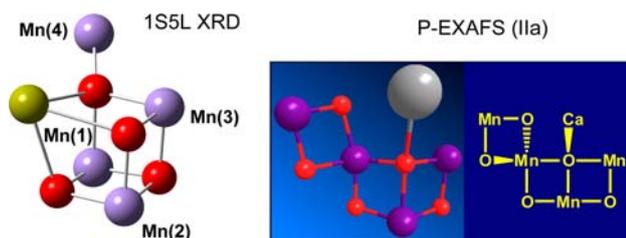


Fig. 4 Structure of the Mn₄Ca inorganic core of the OEC of PSII suggested by the X-ray diffraction structure 1S5L (Ferreira et al. 2004) and model IIa of (Yano et al. 2006). Purple: Mn; Red: O; Gray/yellow: Ca

Fig. 5 Fourier transforms of polarized Mn EXAFS spectra from single crystals of PSII in the S₂ state as described by model IIIa from Yano et al. (2006) (red), 1S5L XRD model (Ferreira et al. 2004) (black upper panel) and the QM/MM models (blue), including the unreduced QM/MM model (blue, middle panel) (Sproviero et al. 2006b) and the reduced-QM/MM (blue, bottom panel) along the axis normal to the membrane (Z) and the two orthogonal axis in the plane of the membrane (X and Y), as defined in Fig. 3 of Yano et al. (2006)

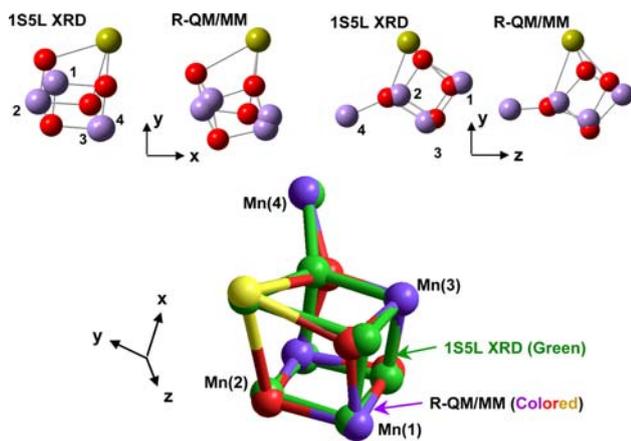
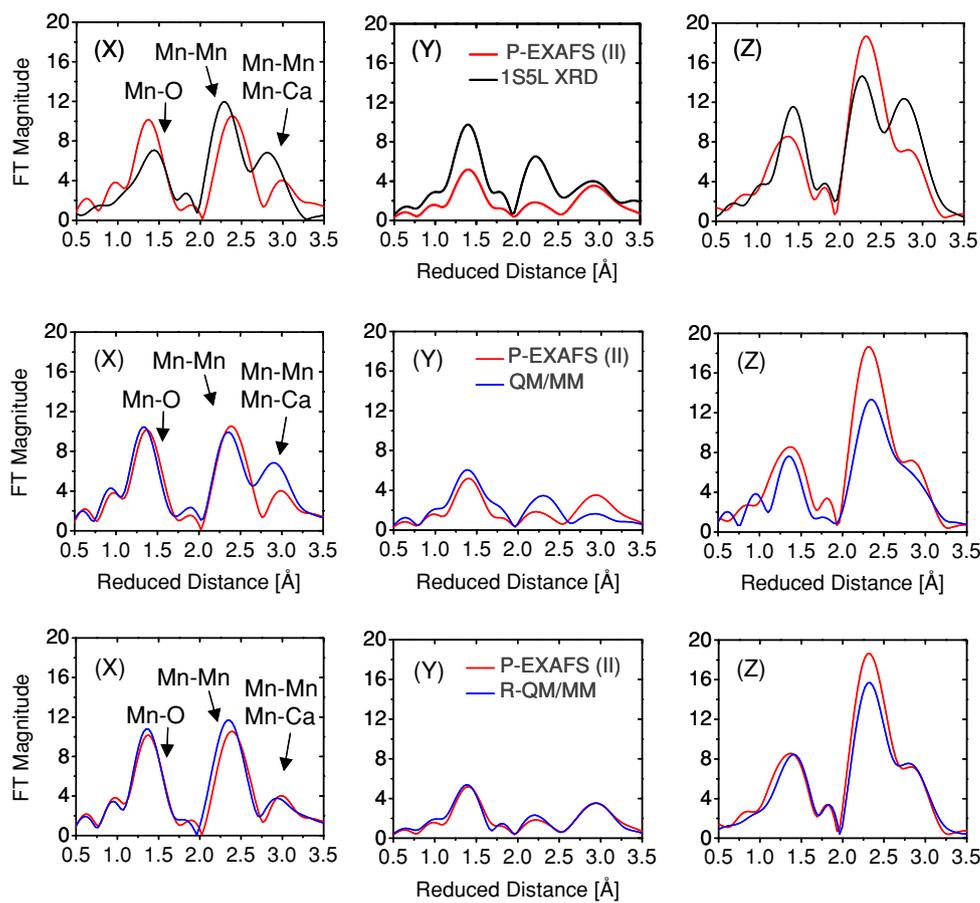


Fig. 6 Comparison of the Mn₄Ca metal cluster of PSII, as described by the 1S5L XRD (Ferreira et al. 2004) and R-QM/MM (Sproviero et al. 2006b) models of the OEC

Charge delocalization, protonation state transitions, and changes in structural rearrangements are all factors that must be considered to elucidate the origin of vibrational frequency shifts (Sproviero et al. 2008a). However, the simplest possible interpretation has been that none of these amino-acid residues are ligated to the Mn ions oxidized during the S₂ transitions, including D1-Asp342 (Strickler et al. 2007), D1-Glu189 (Strickler et al. 2006) and D1-Asp170 (Debus et al. 2005), in marked disagreement with QM/MM and X-ray diffraction models. Furthermore, the only residue which is identified by FTIR spectroscopy as ligating a redox-active Mn ion (and definitely not Ca) is the D1 carboxyl terminus, D1-Ala344 (Chu et al. 2004; Strickler et al. 2005), which in the QM/MM model provides the only protein ligand of calcium. The sensitivity of D1-Ala344 to the S₂ → S₁ and S₂ → S₀ transitions has also been documented with FTIR spectroscopy by Ono and co-workers (Kimura et al. 2005b). The interpretation of these results has been that D1-A344 is ligated to the Mn ion oxidized during the S₂ transition (Chu et al. 2004), in disagreement with QM/MM and X-ray diffraction models. Unfortunately, more rigorous interpretations of these studies on the influence that the oxidation of Mn centers

and mutant spectra then the mutated residue would exhibit vibrational frequencies which are S-state-dependent as expected for a ligand of a redox-active ion. However, significant differences were observed in S-state difference spectra obtained from wild-type PSII and those from PSII experiments have been hindered by the lack of systematic with the mutated residues.

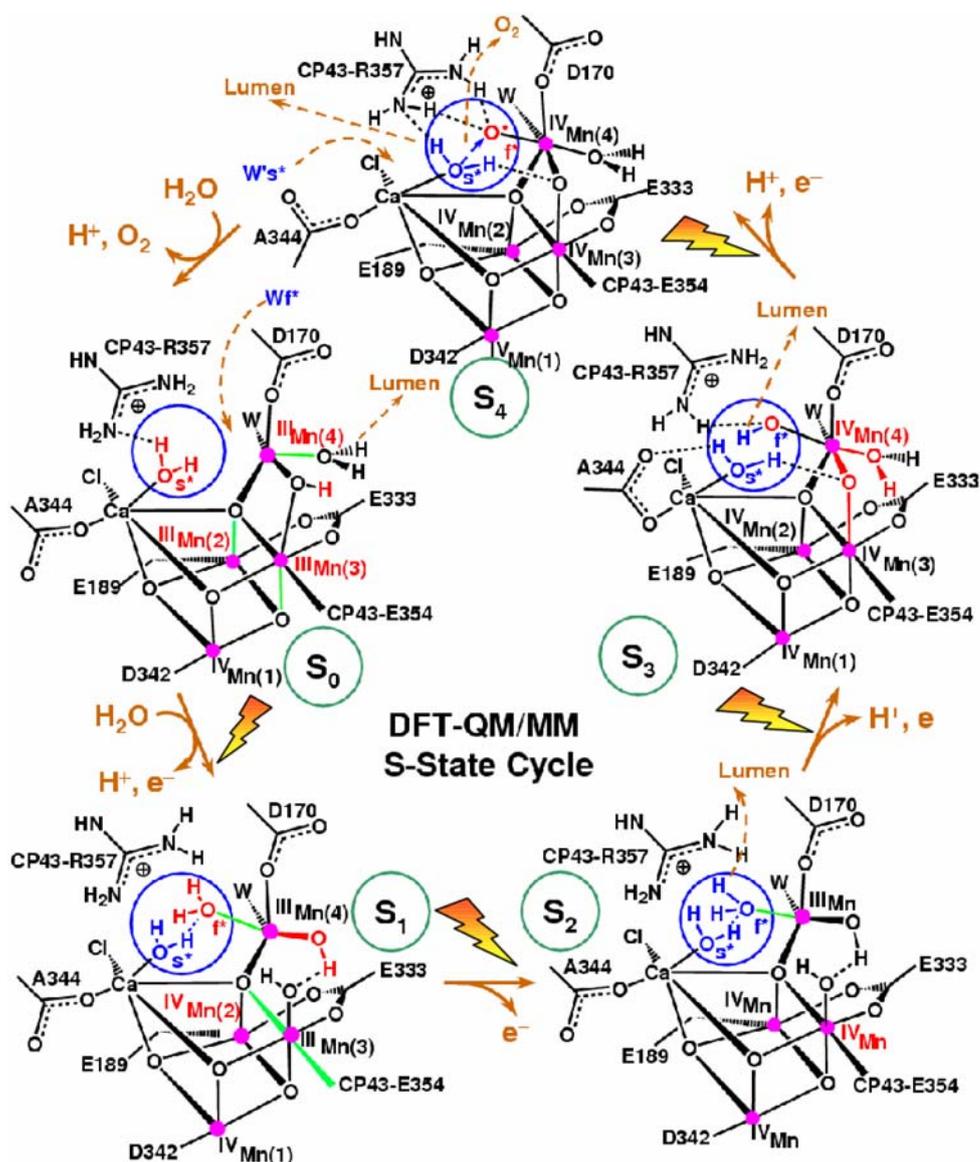
has on the vibrational frequencies of carboxylate ligands in (Sproviero et al. 2008). These studies predict that the high-valent oxomanganese complexes. These contradictions between the FTIR spectroscopy and QM/MM stretch is significant upon $Mn^{III} \rightarrow Mn^{IV}$ oxidation only descriptions the proteinaceous ligation scheme are also when the carboxylate group is coordinated along the Jahn-Teller axis of a Mn^{III} . Otherwise, vibrations of carboxylate ligands can often be quite insensitive to Mn oxidation. It is important to note, however, that carboxylate groups are usually strong ligands and, therefore, seldom ligate along the elongated Jahn-Teller axis of Mn^{III} . In fact, the QM/MM models suggest that neither D1-D170, D1-D342, nor FTIR data and the XRD and QM/MM models. One possible explanation (favored by Debus and coworkers) is that when the OEC is in the S_2 states. Therefore, these the XRD data are flawed because of radiation damage results are consistent with negligible changes in the resulting in the gross derangement of nearby amino-acids vibrational frequencies of carboxylate ligands even when Another possibility is that it is hard to decisively assign they are directly ligated to Mn centers that undergo oxidation state transitions as suggested by the DFT-QM/MM produced by the mutation of only one residue, to either models. The only amino-acid residue ligated along the direct ligation effects or to indirect structural perturbations. Jahn-Teller axis of a Mn^{III} ion is CP43-E354 for which This difficulty has been recently highlighted by the mark-there should be an observable vibrational frequency shift as edly different interpretations of similar FTIR data by two the OEC evolves from the S_0 to the S_2 state. This prediction groups investigating the structural role of D1-Glu189 has been recently confirmed by experiments (Strickler et al. (Kimura et al. 2005a Strickler et al. 2006). Another possibility (favored by QM/MM studies) is that the apparent disagreement between FTIR and X-ray diffraction models is due to the intrinsic difficulties associated with the QM/MM mechanistic model of water splitting interpretation of the FTIR frequency shifts as resulting from the electronic and structural rearrangements in the QM/MM computational modeling work has been focused complex biomolecular environment, including changes mainly on the development of structures of the OEC of hydrogen bonding, protonation states of the ligands, and PSII that are consistent with a wide range of experiments. formation of oxo-bridges (Gascon et al. 2007). The resulting structural models have distinct mechanistic implications (Sproviero et al. 2006b, c, d). Most notably, modes of carboxylate ligands to manganese ions might be the QM/MM model of the OEC in the S_2 state includes two insensitive to changes in the formal oxidation states of the terminal ligand water molecules that plausibly represent ions, because of electron delocalization within the cluster the two substrate water molecules responsible for oxygen (Gascon et al. 2007). At the same time, the smearing out of evolution by catalytic water splitting, one bound to Ca and electrostatic charge might induce vibrational frequency shifts of carboxylate groups not directly ligated to redox-atoms 2.7 Å apart. Furthermore, the QM/MM structural active manganese ions due to charge rearrangements models allowed for the investigation of structural changes associated with S_2 -state transitions. In support of this induced by oxidation/reduction of the OEC and the effect argument, recent calculations have shown that the observed such electronic changes on the underlying coordination/ shift in carboxylate vibrational frequency of the terminal protonation state of the ligands along the catalytic cycle of D1-Ala344 residue upon $S_1 \rightarrow S_2$ transition is compatible water oxidation.

with ligation of D1-Ala344 to calcium, rather than to the manganese ion which undergoes a change in its formal oxidation state (Gascon et al. 2007). It has been recently suggested, however, that charge delocalization and 2, showing that Mn(2), Mn(3), and Mn(4) accumulate unlikely to account for all of the numerous changes in oxidizing equivalents while Mn(1) remains redox inactive FTIR spectra which are observed to accompany S_2 -state transitions (Strickler et al. 2007). accumulated as a terminal oxyl radical MnO^\bullet of the

Preliminary computational studies of oxomanganese dangling manganese (an oxidized form of a substrate water complexes have also addressed the effect of oxidation state molecule, deprotonated and ligated to Mn) and is essential transitions on the vibrational frequencies of carboxylate for O-O bond formation. Dioxygen evolution, during the ligands directly attached to redox active Mn ions $S_4 \rightarrow S_0$ transition, involves a nucleophilic attack of the

Fig. 7. The quantitative analysis of structural and spin-electronic state rearrangements is presented in Tables and 2, showing that Mn(2), Mn(3), and Mn(4) accumulate throughout the cycle. The fourth oxidizing equivalent is accumulated as a terminal oxyl radical MnO^\bullet of the

Fig. 7 Catalytic cycle of water splitting suggested by DFT QM/MM models of the OEC of PSII (Sproviero et al 2007). Dashed arrows in brown 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 (also shown in blue). Coordination bonds elongated by Jahn–Teller distortions are marked in green. The oxidation states of Mn ions are indicated as Roman superscripts, while the orientation of the metal cluster corresponds to Fig. 2, where Mn(1), Mn(2), Mn(3), and Mn(4) are indicated. Copyright 2008 American Chemical Society



calcium-bound water molecule on the electrophilic oxyl radical $\text{Mn}^{\text{IV}}\text{O}^\ominus$. This reaction is similar to the earlier proposals by Pecoraro et al. (1998) and Brudvig (Vrettos et al. 2001a), with the difference that in the QM/MM model the nucleophilic water attacks an oxyl radical rather than an oxo- Mn^{V} species. A nucleophilic attack of a calcium-bound activated water molecule onto an electrophilic oxyl radical molecule in the second coordination shell of Ca^{2+} was also analyzed, at the DFT level, in model systems long before the crystal structure of PSII was known (Siegbahn and Crabtree 1999), although more recently (Siegbahn 1996; Siegbahn 2002), or a manganese-bridging oxo group (Robblee et al. 2001). The overall reaction also disagrees with other proposals where manganese-bridging oxo-ligands react with one another during the O–O bond forming step (Brudvig and Crabtree 1986; Yachandra et al.

1996), where oxyl radicals react with μ -oxo bridges instead of reacting with terminal Ca^{2+} -bound water molecules (Hillier and Messinger 2005; Messinger 2004; Siegbahn 2006a; Siegbahn and Lundberg 2005), or where basic μ -oxo ligands deprotonate manganese-bound terminal water molecules (Dau et al. 2001).

The QM/MM mechanism is consistent with significant structural rearrangements in the metal cluster during the $S_0 \rightarrow S_1$ and $S_2 \rightarrow S_3$ transitions, as evidenced by the EXAFS spectroscopy (Robblee et al. 2002). During the $S_0 \rightarrow S_1$ transition, the protonated μ -oxo bridge between Mn(4) and Mn(3) is opened due to changes in oxidation and protonation states that stretch the Mn(4)–O distance leaving the OH group ligated to Mn(3). Strengthening the coordination between Mn(3) and its axial ligand changes the Jahn–Teller distortion (elongated coordination bond from axial to equatorial, shortening the Mn(1)–Mn(3) distance. As shown in Table 4, these results are consistent with the EXAFS observation of a shortening of a Mn–Mn distance by approximately 0.15 Å (Robblee et al. 2002), with a single short (2.7 Å) Mn–Mn distance per Mn tetramer in the S_2 state and two short (2.7 Å) Mn–Mn distances in the S_3 state, with a third distance close to 2.8 Å. The interpretation of EXAFS data, however, has been based on deprotonation of one OH-bridge (Haumann et al. 2005b; Robblee et al. 2002) rather than on opening of a protonated μ -oxo bridge between Mn(4) and Mn(3).

During the $S_2 \rightarrow S_3$ transition, the substrate water molecule bound to Mn(4) is deprotonated, consistently with electrochromism data (Haumann and Junge 1996; Junge et al. 2002; Lavergne and Junge 1993; Schlodder and Witt 1999; Witt 1996), and the oxidation state of Mn(4) is advanced from III to IV. These changes induce inter-ligand proton transfer from the OH ligand of Mn(3) to the hydrogen bonded OH ligand of Mn(4), strengthening the attractive interactions between Mn(4) and the OH⁻ ligand of Mn(3), forming a μ -oxo bridge between Mn(3) and Mn(4), and transforming the OH ligand of Mn(4) into a water ligand (the OH proton acceptor is regenerated by deprotonation of the water ligand in the $S_0 \rightarrow S_1$ transition). These rearrangements are consistent with a pH-dependent rate constant (Haumann et al. 1997), suggesting a transition kinetically steered by proton movements. The rearrangements are also consistent with the observation of conformational changes in the structure of the cluster upon formation of the S_3 state (Haumann et al. 2005b; Liang et al. 2000), as manifested in the EXAFS spectra (see Fig. 8) (Sproviero et al. 2008b). The proposed formation of the μ -oxo bridge, between Mn(3) and Mn(4) by proton transfer between OH ligands is similar to the transition proposed by Siegbahn (Siegbahn and Lundberg 2005) where, in contrast to the QM/MM model, the bridge is formed by double deprotonation of a

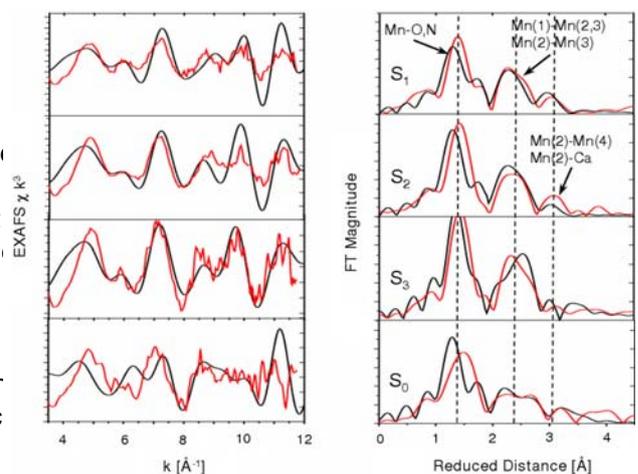


Fig. 8 Comparison between experimental (Sproviero et al. 2006d, red) and calculated (Haumann et al. 2005b, black) EXAFS spectra of OEC S-state intermediates of water splitting. *Left*: k -weighted EXAFS spectra. *Right*: Fourier-transformed spectra in r -space, showing three prominent peaks corresponding to scattering centers in the first (O, N), second (Mn in the core), and third (dangling Mn, Ca) coordination shells of Mn, respectively. Vertical dashed lines are included to facilitate the comparison of peaks for different S-states. Copyright 2006 American Chemical Society

water ligand attached to Mn(3) that releases a proton to the lumen and transfers the other proton to an OH ligand attached to the same Mn(3). In the QM/MM model, however, the substrate water molecule attached to the dangling Mn(4) is deprotonated, analogously to other proposals (Messinger 2004).

The proposed QM/MM molecular structures of catalytic intermediates have been partially validated through the comparison of experimental (Haumann et al. 2005b) and calculated EXAFS spectra as shown in Fig. 8 (Sproviero et al. 2008b). In addition to the comparison of the spectra in k -space, Fig. 8 (right panel) shows the Fourier transform EXAFS amplitudes characterizing the detailed structural rearrangements of the metal cluster along the catalytic cycle. These include changes in Mn–Mn and Mn–Ca distances as well as changes in the Mn–O ligand coordination bond lengths.

The origin of some of the deviations between calculated and experimental spectra might be due to contributions from other redox isomers of comparable energy, or a slightly different arrangement of ligands (Sproviero et al. 2006b). However, the overall comparison indicates that the DFT QM/MM models are qualitatively consistent with experimental data throughout the entire catalytic cycle. The first peak at reduced distance 1.41 Å (actual distance 1.8 Å) is determined by photoelectron scattering from N and O centers directly ligated to Mn ions. Scattering contributions from μ -oxo bridges and the ligated carboxylate group of E333 coordinated to Mn are responsible for a

shoulder at reduced distance 1.6 Å (actual distance 2.1 Å) close contact with this water (as it is in the QM/MM model), but its κ_a must also be affected by the electrostatics (actual distance 2.7 Å) corresponds to the characteristic static charge of both the OEC and the D1-H190 pair. This would make CP43-R357 the redox-coupled base reduced distance 3.0 Å and beyond corresponds to back-scattering from the dangling-Mn and Ca distances >3.3 Å. In agreement with experimental data (Haumann et al. 2005b), the simulated spectra of the OEC in the S state indicate that such a distribution of scattering amplitudes is consistent with a dangler cuboidal cluster (CP43-R342) to serine suppressed evolution (Hwang et al. 2007; Knoepfle et al. 1999). In addition, arginine is a well-known component of hydrogen bonding networks in proteins (Puustinen and Wikstrom 1999; Qian et al. 2004). A recent theoretical study has shown that the κ_a of CP43-R357 might indeed be particularly sensitive to an increase in the charge of the OEC (Ishikita et al. 2006). However, this remains to be examined in the QM/MM models. It is also possible that the κ_a of CP43-R357 is indirectly affected by the rearrangements of protonation states and hydrogen bonds of the ligands along the catalytic cycle. In particular, the OH ligand of Mn(3) experiences significant changes in protonation states along the cycle. It deprotonates by proton transfer to an OH ligand of Mn(4), during the S₂ → S₃ transition, transforming such a ligand into water and forming a μ -oxo bridge between Mn(3) and Mn(4). This event induces a rearrangement of hydrogen bonds: the hydrogen-bond between the two substrate water molecules is broken and two hydrogen bonds are formed, including a bond between W^{low} and the μ -oxo bridge and another one between W^{high} and CP43-R357. Upon further deprotonation of W^{high} in the S₃ → S₄ state, W^{low} forms a hydrogen bond with CP43-R357 in addition to its hydrogen bond with the μ -oxo bridge, establishing two deprotonation pathways. During the S₄ → S₀ transition, one proton transfers to the lumen via CP43-R357 while the other one translocates to the μ -oxo bridge and subsequently forms the OH⁻ ligand of Mn(3), upon opening of the bridge in the S₀ → S₁ transition.

In the S₃ state, the scattering peak at reduced distance 2.32 Å becomes narrower consistently with three Mn–Mn distances that become similar to each other. While this aspect is in agreement with the EXAFS data by Dau et al. (2003, 2004), it is still in disagreement with data from the Berkeley group indicating that one of the Mn–Mn distances is longer in the S₃ state (Sauer and Yachandra 2004; Yano et al. 2005b).

Proton exit channel

The QM/MM mechanistic model shown in Fig. 5 involves multiple changes in oxidation states. These transitions are coupled to proton transfer events balancing the overall charge of the cluster and rearranging the protonation states of water ligands. The QM/MM structural models also reveal a network of hydrogen bonds linking the proposed substrate water molecules with D1-Asp61 (see Fig. 5) the Prst residue of the proton exit pathway leading to the phenoxo oxygen of Y₂ is close to the chloride ligand (3.4 Å lumenal surface of PSII, suggested by XRD data (Ferreira et al. 2004). The sidechain of CP43-R357, along the more, the Y₂ phenol group is hydrogen bonded to the hydrogen-bonding network including both substrate water molecules and the calcium-bound chloride ion, might be bonding partnership is in line with mutational and spectroscopic studies (Hays et al. 1998, 1999; Roffey et al. 1994), bound substrate water in the second half of the S-state cycle (McEvoy and Brudvig 2004; Sproviero et al. 2006b). Considering the potential functional roles of CP43-R357 and D1-Y161, it is natural to expect that proton coupled

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