

The mechanism of photosynthetic water splitting†

James P. McEvoy, Jose A. Gascon, Victor S. Batista and Gary W. Brudvig*

Department of Chemistry, Yale University, PO Box 208107, New Haven, CT, 06520-8107, USA. E-mail: gary.brudvig@yale.edu

Received 12th May 2005, Accepted 7th September 2005
First published as an Advance Article on the web 4th October 2005

Oxygenic photosynthesis, which provides the biosphere with most of its chemical energy, uses water as its source of electrons. Water is photochemically oxidized by the protein complex photosystem II (PSII), which is found, along with other proteins of the photosynthetic light reactions, in the thylakoid membranes of cyanobacteria and of green plant chloroplasts. Water splitting is catalyzed by the oxygen-evolving complex (OEC) of PSII, producing dioxygen gas, protons and electrons. O₂ is released into the atmosphere, sustaining all aerobic life on earth; product protons are released into the thylakoid lumen, augmenting a proton concentration gradient across the membrane; and photoenergized electrons pass to the rest of the electron-transfer pathway. The OEC contains four manganese ions, one

calcium ion and (almost certainly) a chloride ion, but its precise structure and catalytic mechanism remain unclear. In this paper, we develop a chemically complete structure of the OEC and its environment by using molecular mechanics calculations to extend and slightly adjust the recently-obtained X-ray crystallographic model [K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber and S. Iwata, *Science*, 2004, 303, 1831–1838]. We discuss our mechanistic hypothesis [J. P. McEvoy and G. W. Brudvig, *Phys. Chem. Chem. Phys.*, 2004, 6, 4754–4763] with reference to this structure and to some important recent experimental results.

Introduction

Water is photochemically oxidized to dioxygen in photosystem II (PSII) by the oxygen-evolving complex (OEC), a

† Dedicated to Professor James Barber on the occasion of his 65th birthday.

James P. McEvoy grew up in Yorkshire, England, and read Chemistry at Merton College, Oxford University, obtaining his MChem degree in 1998. He remained at Oxford (at Linacre College) for his doctoral work, developing variable-temperature protein film voltammetry under the supervision of Fraser A. Armstrong. Having earned his DPhil in 2002, he spent a year working with John S. Foord, also at Oxford, investigating the use of diamond electrodes in protein electrochemistry. In 2003, he joined Gary W. Brudvig at Yale University as a postdoctoral associate and has since worked on the mechanism of oxygen evolution in photosystem II, developing his interests in bioinorganic redox chemistry.

Jose A. Gascon received a BSc degree in Physics from the Institute of Mathematics, Astronomy and Physics (FAMAF) at the National University of Cordoba in Argentina. In 1998, he moved to Louisiana State University to pursue a PhD in Chemistry studying theoretical aspects of electronic and structural properties of rare gas cation clusters. In 2002, he began a postdoctoral fellowship under the supervision of Victor S. Batista at Yale University. His current research involves QM/MM studies of bio-macromolecules, including the study of photochemical reactions in rhodopsin and structural and electronic properties of the oxygen-evolving complex in photosystem II.

Victor S. Batista received his Lic. Ciencias Químicas degree from Universidad de Buenos Aires, Argentina (1989), and the Sugata Ray Award (1995) and a PhD degree in Theoretical Chemistry (1996) from Boston University. After completing postdoctoral programs with William H. Miller at the University of California, Berkeley (1997–1999) and Paul Brumer at the University of Toronto (2000), he joined the Yale faculty in 2001, where he has received the ACS PRF-G6 Award (2002), the Research Corporation Innovation Award (2002), the NSF Career Award (2004), the Sloan Fellowship (2005–2006) and the Camille Dreyfus Teacher-Scholar Award (2005).

Gary W. Brudvig received his bachelor's degree in Chemistry from the University of Minnesota in 1976 and went on to gain his PhD in Chemistry from the California Institute of Technology in 1980 where he investigated the metal centers in cytochrome c oxidase under the direction of Sunney I. Chan. Following two years as a Miller Fellow at the University of California, Berkeley studying with Kenneth Sauer, he joined the faculty at Yale University, where he is now Professor and Chair of Chemistry, and where he applies both biophysical and bioinorganic techniques to the study of photosystem II.



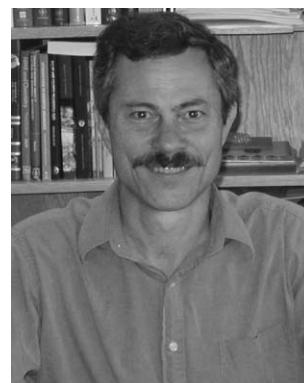
James P. McEvoy



Jose A. Gascon

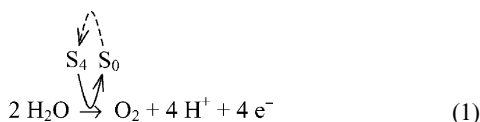


Victor S. Batista



Gary W. Brudvig

redox-catalytic center on the luminal side of PSII which includes four manganese ions, a calcium ion and (almost certainly) a chloride ion. Seminal experiments conducted independently by Kok and Joliet established that the OEC operates in a four-step photo-catalytic cycle, existing successively in five oxidation states ("S-states").¹ The OEC is oxidized in one-electron steps from S_0 (the most reduced state) to S_4 (the most oxidized) by a repeatedly photo-oxidized chlorophyll center, P_{680} , which converts collected light energy into redox potential energy. The OEC thus accumulates four equivalents of oxidizing power through the cycle and uses it to split oxidatively two molecules of water. An O_2 molecule is released very soon after the OEC attains the S_4 state, and the complex is itself returned to the S_0 state. Eqn (1) describes the overall electrochemical half-reaction, which in PSII is associated with the eventual two-electron reductions of two molecules of plastoquinone.



After the discovery that the OEC contained four manganese ions,^{2,3} it was assumed that this oxidizing power resides principally, if not entirely, in these transition metal centers, and subsequent experiments have confirmed this. The precise manner in which the complex gains and wields oxidizing power is still, however, unknown. Spectroscopic techniques which have been used to report on the OEC cluster and its surroundings include varieties of electron paramagnetic resonance (EPR) spectroscopy,⁴⁻⁶ X-ray absorption spectroscopy (XAS),⁷⁻⁹ optical spectroscopies^{10,11} and Fourier-transform infra-red (FTIR) spectroscopy.¹²⁻¹⁴ These and other spectroscopies have been combined with biochemical modifications of the protein, such as depletion or substitution of the OEC's component metal ions, addition or removal of basic species (such as halides or ammonia) that are able to interact with the OEC, and site-directed mutations of nearby amino acid residues. Classical enzyme kinetic studies are impossible, because water is one of the enzyme's substrates and its concentration is effectively invariable, but precise time-resolved measurements of proton and dioxygen production have been performed.¹⁵⁻¹⁷ Hillier and Wydrzynski have rapidly introduced $H_2^{18}O$ to the OEC and used time-resolved mass spectrometry to observe the subsequent appearance of ^{18}O in the product dioxygen molecules.^{17,18} This technique has provided important insights into the OEC mechanism (see below). Most recently a number of X-ray diffraction structures of PSII have been published, which provide structural information hard to obtain by other methods.¹⁹⁻²²

Thermodynamics of water oxidation

The reduction potential of half-reaction eqn (1) has a pH-dependence of about -60 mV per pH unit.²³ At pH 5.0, which is roughly the lowest luminal pH at which the OEC is thought to operate,²⁴ the standard reduction potential is $+0.93$ V. The OEC cluster must therefore provide a maximum of around $+0.93 \times 4 = +3.72$ eV ($+359$ kJ mol⁻¹) of oxidizing free energy to catalyze the four-electron oxidation of water to oxygen. Researchers have made efforts to establish whether this energy is delivered all at once, in the $S_4 \rightarrow S_0$ transition, or whether it is delivered in a series of steps over the course of the S-state cycle. Krishtalik's analysis of this problem²⁵ showed that any mechanism involving four one-electron oxidations of water (for example, ref. 26) is heavily disfavored by the difficulty of abstracting a hydrogen radical from water to form OH^\bullet in the first step. Both the concerted four-electron reaction and the pathway involving two successive two-electron reactions are more feasible energetically. Crucially, moreover, Krishtalik recognized the need to reduce the overall positive free energy of the oxidation by strongly binding product protons to a nearby base. He pointed out that the pK_a of

this proton-accepting species must be coupled to the oxidation state of the OEC, because otherwise it will simply obtain a proton from the bulk solution and lose its ability to abstract protons from the oxidized OEC-water complex. Krishtalik reasoned that the species must therefore be activated as a base just when it is needed, by an abrupt alteration of its pK_a .

Babcock and co-workers were the first to suggest a definite identity for this redox-coupled basic species.²⁷ They had earlier identified a tyrosine residue (Y_Z) as the electron-transfer intermediary between P_{680}^{++} and the OEC, reducing the former and oxidizing the latter.^{28,29} It was found that rapid proton-release occurs upon the oxidation of Y_Z ³⁰ and that Y_Z is closely associated with the residue D1-His190.^{31,32} Babcock went on to suggest that the oxidized Y_Z^\bullet radical simultaneously oxidizes and deprotonates the hydrated OEC, acting as a hydrogen atom abstractor, and that it dispatches the abstracted protons to the protein surface *via* D1-His190.³³ Tyrosine has a pK_a of *ca.* -2 in its oxidized, radical form, a value which is dramatically increased to *ca.* $+10$ in its reduced form. It was pointed out that the residue's change in acidity with oxidation state means that the side-chain is very likely to exist in the deprotonated, neutral state in its oxidized radical form, and in the protonated, neutral state in its reduced form. By simultaneously abstracting an electron and a proton from the OEC, Y_Z would both satisfy the thermodynamic criteria identified by Krishtalik and maintain charge-neutrality throughout the reaction, presumably decreasing its activation energy.

Our proposal for the mechanism of water splitting in the OEC

The role of such proton-coupled electron transfer (PCET) in the S-state cycle continues to be a much debated topic, and one which motivates our research. We have been particularly interested in the related role of the single calcium ion in the OEC. It has become apparent that this calcium is very close to the manganese ions, and is required for water splitting.³⁴⁻³⁸ We and others have sought to identify its role by experiments in which the metal is either removed from the OEC or replaced by other metal ions. There is good evidence that calcium ligates one of the two substrate water molecules in the OEC.³⁶

On the basis of considerable experimental and theoretical evidence, our previously published mechanistic proposal^{39,40} incorporates the following suggestions: (1) that the two substrate water molecules of the OEC are coordinated respectively to Ca^{2+} and to one of the Mn ions; (2) that the substrate-binding Mn ion is highly oxidized during the course of the S-state cycle, arriving at the formal $+5$ oxidation state in the S_4 state; (3) that, beyond the S_2 state, the Mn-bound substrate water is twice deprotonated as the metal is oxidized in processes of concerted PCET; and, finally, (4) that the resulting $Mn^V=O$ species in the S_4 state is nucleophilically attacked by the calcium-bound substrate water, producing the O-O bond of the product dioxygen molecule. In this perspective, we discuss how our proposal stands up in the light of some recent experimental results on the subject. We make use of our new molecular mechanics computational structure of the OEC⁴¹ to refine our proposals. This model is based on the recent 3.5 Å-resolution X-ray diffraction structure of the OEC²² and completes the coordination of the metal sites in a chemically realistic fashion, mostly with the addition of water molecules and a single chloride ion. A network of hydrogen bonded species is seen around the proposed catalytically-active face of the OEC cluster, providing insight into the movement of protons and water in the site.

Computational methods

A reduced model of the protein cavity surrounding the OEC cluster was constructed according to the recently reported X-ray structure of photosystem II.²² The model included all residues whose α -carbons are within 15 Å of any OEC cluster atom.

It is likely that the crystal structure represents the lower S-states more closely than the higher S-states, because of radiation-induced reduction of the cluster,⁴² so we have computationally modeled the OEC in the S₀ state. We note that it is also possible that X-ray induced reduction causes some structural rearrangement of the OEC; we address the sensitivity of our mechanistic model to changes in the structural model later in the paper. Electrostatic charges on the OEC cluster ions were assigned according to their formal charges as previously hypothesized in the S₀ state^{39,40} and further discussed below: Mn(1) = +4, Mn(2) = +4, Mn(3) = +3, Mn(4) = +2 (Ca²⁺ = +2, bridging oxides = -2).

Manganese in its higher oxidation states is usually six-coordinate (octahedral), although Mn^{III} may sometimes be five-coordinate (square-based pyramidal).⁴³ Calcium is typically six- to eight-coordinate.⁴⁴ These considerations imply that the ligating amino acid residues revealed in the crystal structure are not sufficient to satisfy the valence requirements of any of the metal ions. We satisfied these requirements by the addition of exogenous species (mostly water molecules) which are not expected to be revealed in a crystal structure of this resolution. Manganese water ligands were added deliberately to complete an octahedral ligand set, whereas calcium water ligands were added by general hydration of the structure (see below). In both cases, we assumed a *minimum displacement of the ligating amino acid residues* from their crystallographic positions. Computational models were also constructed according to the principle of *minimum incorporation of ligand water molecules*. In other words, we constructed models in which various suitable residues near to the OEC were recruited as ligands to the manganese ions, in a variety of chelating modes. This meant that fewer water molecules were needed to complete the Mn coordination spheres. However, these alternative models were dismissed because they required significant displacements of the ligating amino acids from their crystallographic positions.

The geometry of the resulting model was optimized using the Amber force field. The positions of the OEC cluster ions were fixed according to their crystallographic coordinates, and it was assumed that protein-cluster interactions are dominated by electrostatic terms. The following residues were allowed to move during geometry optimization: D1-Asp61, D1-Tyr161, D1-Gln165, D1-Asp170, D1-Glu189, D1-His332, D1-Glu333, D1-His337, D1-Asp342, D1-Ala344, CP43-Glu354 and CP43-Arg357. To prevent unrealistically large deviations from its crystallographic location, CP43-Arg357 was harmonically constrained with a force constant of 400 kJ mol⁻¹. The heavy atoms of all other residues in the model were kept fixed at their crystallographic locations. Optimization of the model resulted in a 0.64 Å root mean square (rms) displacement of the listed residues from their crystallographic coordinates.

The resulting molecular mechanics model of the ligated OEC cluster was compared to a quantum mechanical, *ab initio* model system optimized at the B3LYP/lacvp level of theory, in which ligating carboxylate groups were modeled as formates and D1-His332 as imidazole. The resulting minimized *ab initio* model (not shown) is consistent with the molecular mechanics model. Details and results of these calculations will be published separately.

Additional hydration of the OEC environment

Water molecules, in addition to those bound to Mn ions, were added to the computational model by embedding the molecular structure in a box of water molecules at thermal equilibrium. Waters within 15 Å of the cluster's center of mass were added if they did not sterically conflict with the protein or with coordinating water molecules. The completed structure was then relaxed by geometry optimization. Because such a relaxation creates room for more water molecules, the procedure was repeated until the number of waters converged to a constant

value. Hydration added 85 more water molecules to the model. Two of these completed the coordination of calcium, and the rest engaged in hydrogen-bonding interactions with the protein and/or with each other.

Addition of chloride

There is experimental evidence indicating that one Cl⁻ ion is bound to the OEC⁴⁵ near to D1-Tyr161⁴⁶ and participates in the mechanism of oxygen evolution, perhaps by affecting proton movement in the later S-states.⁴⁷ In order to determine the most likely position of Cl⁻, we analyzed the relative stabilities of molecular structures obtained by replacing each one of the non-Mn-bound water molecules by Cl⁻. After geometry optimization, the most stable structure was selected. The resulting model became electrostatically neutral around the OEC cluster after the addition of Cl⁻, and contained 2106 atoms.

Structural model of the OEC and its surroundings

Fig. 1 shows our molecular mechanics computational structure model of the OEC and its immediate surroundings. The coordinations of the OEC protein ligands remain mostly unchanged from the X-ray structure upon which our model is based.²² The bicarbonate ion which appears in the crystallographic model has not been included because we consider its presence at this location adventitious, although we do not discount the possibility of bicarbonate's involvement elsewhere in the OEC.^{39,48} D1-Asp342 binds monodentally to Mn(1) and CP43-Glu354 chelates bidentally to Mn(3). Metal hexacoordination is achieved in each case by the addition of two waters to Mn(1) and one hydroxide ion to Mn(3). Three water molecules are added to complete the coordination of Mn(4), ligated monodentally by D1-Asp170 and D1-Glu333. To complete the coordination of Mn(2), D1-Glu333 was moved slightly from the crystal structure to form an η² carboxylate bridge between Mn(4) and Mn(2). The resulting rms displacement of the residue is 0.56 Å, with a 1.6 Å displacement of its carboxylate oxygens from their original X-ray positions. The carboxylate terminus of the D1 subunit, D1-Ala344, was also moved (rms displacement of 0.83 Å) to coordinate bidentally the calcium ion. This residue has been implicated in OEC ligation⁴⁹ although recently obtained FTIR S₁/S₂ difference spectra have suggested that it binds to manganese rather than to calcium.^{13,50-52} It may be that the FTIR frequencies of the carboxylate terminus are more sensitive to the overall charge of the cluster (hence its shift in the S₁ → S₂ transition^{13,52}) than to whether it is ligated to calcium or strontium.⁵¹ Until this question is resolved, perhaps by quantum mechanical calculations of the relevant vibrational frequencies, we proceed along the lines indicated by the crystallographic data. Our mechanistic proposal does not depend upon the ligation of the terminal carboxylate to the OEC.

A total of seven ligating water molecules are included as metal ligands (two on Ca²⁺, three on Mn(4) and two on Mn(1)) as well as one hydroxide ion on Mn(3). This extensive hydration of the cluster is in line (though not in perfect agreement) with pulsed EPR experiments which reveal the presence of several exchangeable deuterons near the Mn cluster in the S₀, S₁ and S₂ states.⁵³ We identify two of the ligand waters (marked with asterisks in Fig. 1), one bound to Ca²⁺ and the other to Mn(4), as probable substrate water molecules. Their respective oxygen atoms are 2.6 Å apart in this model, and may be brought yet closer together in the S₄ state (following deprotonation of the Mn-bound substrate water) to achieve O-O bond formation in the S₄ → S₀ transition.

Chloride is found in our model as a ligand to calcium, and is located 3.4 Å from the phenol oxygen of Y_Z. This is in good agreement with pulsed EPR data obtained from the OEC in which chloride has been replaced by an acetate ion, and the distance measured between the acetate methyl group and tyrosine Z.^{41,46}

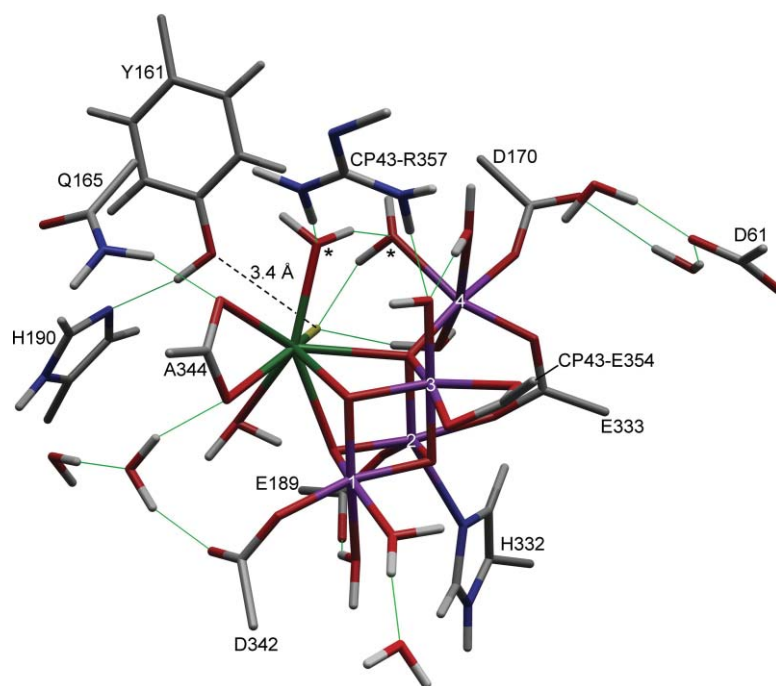


Fig. 1 Computational structural model of the OEC in the S_0 state, and its immediate environment. Atom colors are as follows: manganese, purple; calcium, green; oxygen, red; chlorine, yellow; carbon, gray; nitrogen, blue; hydrogen, white. The two water molecules marked with asterisks are those suggested to be the substrate water molecules. Hydrogen bonds are shown as thin green lines. All residues belong to the D1 polypeptide unless otherwise labeled. Manganese atom numbering follows that in ref. 22.

Mechanistic implications of the computational structural model

We present in Fig. 2 a molecular mechanistic model based on our structural model. We have previously noted that the crystallographic structure of the OEC obtained by Ferreira *et al.*²² is consistent with our mechanistic ideas³⁹ if we suppose that the two substrate water molecules are respectively bound to Mn(4) and to calcium, in place of the crystallographically-modeled bicarbonate ion. Furthermore, the crystal structure raised the possibility that the CP43–Arg357 residue plays the role of the redox-coupled catalytic base in the latter half of the S-state cycle.³⁹ Following Babcock and co-workers, we had previously assigned this role to Y_Z , but the crystal structure argues against this by showing the residue to be on the other side of the OEC cluster from the apparent proton-exit pathway.^{22,39} Refinements to the crystal structure, however, may yet show that Y_Z is more closely associated with this channel. In our computational model, the Y_Z phenol group hydrogen-bonds to the imidazole ϵ -N of the D1–His190 side-chain. This hydrogen-bonding partnership has been predicted on the basis of mutational and spectroscopic studies.^{32,54–56} On the basis of the existing data, we hypothesize that this pairing exists to allow fast loss of the tyrosine phenol proton to D1–His190 upon tyrosine oxidation, and fast re-binding of the same proton upon reduction of the tyrosine by the OEC. This “proton-rocking” mechanism is widely believed to occur at Y_D , the analogous tyrosine residue in the D2 subunit.⁵⁷ Its operation at Y_Z would imply that Nature has employed Y_Z purely on the basis of its redox potential (*ca.* 1.0 V in solution at pH 5, ref. 58) and is indifferent to the residue’s acid/base characteristics, except inasmuch as they control its reduction potential. However, we propose that there exists a vital electrostatic interaction between the Y_Z –D1–His190 pair and the OEC, responsible in our model for modulating the protonation state of CP43–Arg357 (see ref. 39 and Fig. 2). This electrostatic influence of a charge retained in Y_Z ’s vicinity is consistent with proton-release and charge-transfer kinetic data^{59–61} and with EPR spectroscopic experiments examining the cryogenically trapped $S_n Y_Z^*$ intermediates of S-state transitions.⁶²

The detailed structure that we have computationally obtained here suggests some further refinements to our mechanistic model by indicating hydrogen-bonding interactions amongst amino acid residues and water molecules surrounding the OEC (see Fig. 1 and Fig. 2). There are several hydrogen bonds revealed around the “active face” of the OEC, near the substrate waters. These involve the substrate waters themselves, the calcium-bound chloride, the CP43–Arg357 sidechain, D1–Gln165, and two non-substrate manganese-ligating waters bound respectively to Mn(3) and Mn(4). We anticipate that a similar hydrogen-bonding arrangement is important in the second half of the S-state cycle, both in expediting deprotonation of the Mn-bound substrate water molecule, and in orienting the Ca-bound substrate water molecule correctly for the O–O bond-forming reaction in the $S_4 \rightarrow S_0$ transition. Our model indicates that D1–Gln165 and CP43–Arg357 are potentially important in orienting the Ca-bound water properly for its nucleophilic attack upon the Mn^Y(4)=O oxo oxygen in this transition. Although in our S_0 model the two substrate waters are hydrogen-bonded with each other, it is important that this does not persist later in the cycle. By the S_4 state, the hydrogens of the Ca-bound water must be held away from the Mn^Y(4)=O oxo oxygen, allowing the oxygen atoms of the two species to approach one another closely. This function, it would seem, may only be performed by nearby hydrogen-bond acceptor species, such as the carboxy oxygen of the D1–Gln165 amide sidechain.

The other function of the OEC hydrogen-bonding network beyond the S_2 state is to expedite the deprotonation of the Mn-bound substrate water. In our mechanism, this water must be oriented so that it readily gives up its protons to the temporarily-neutral CP43–Arg357 guanidine sidechain, which acts as a base in the reaction. (We suggest that this residue is previously deprotonated due to its proximity to two positively charged species: the OEC cluster itself, and the D1–His190 residue.³⁹ Arginine acts similarly as a general acid/base catalyst in the fumarate reductases.^{63,64}) Our computational model suggests that the calcium-bound chloride plays a prominent role in this alignment by hydrogen-bonding to the Mn(4)-bound substrate water molecule prior to its deprotonation by arginine.

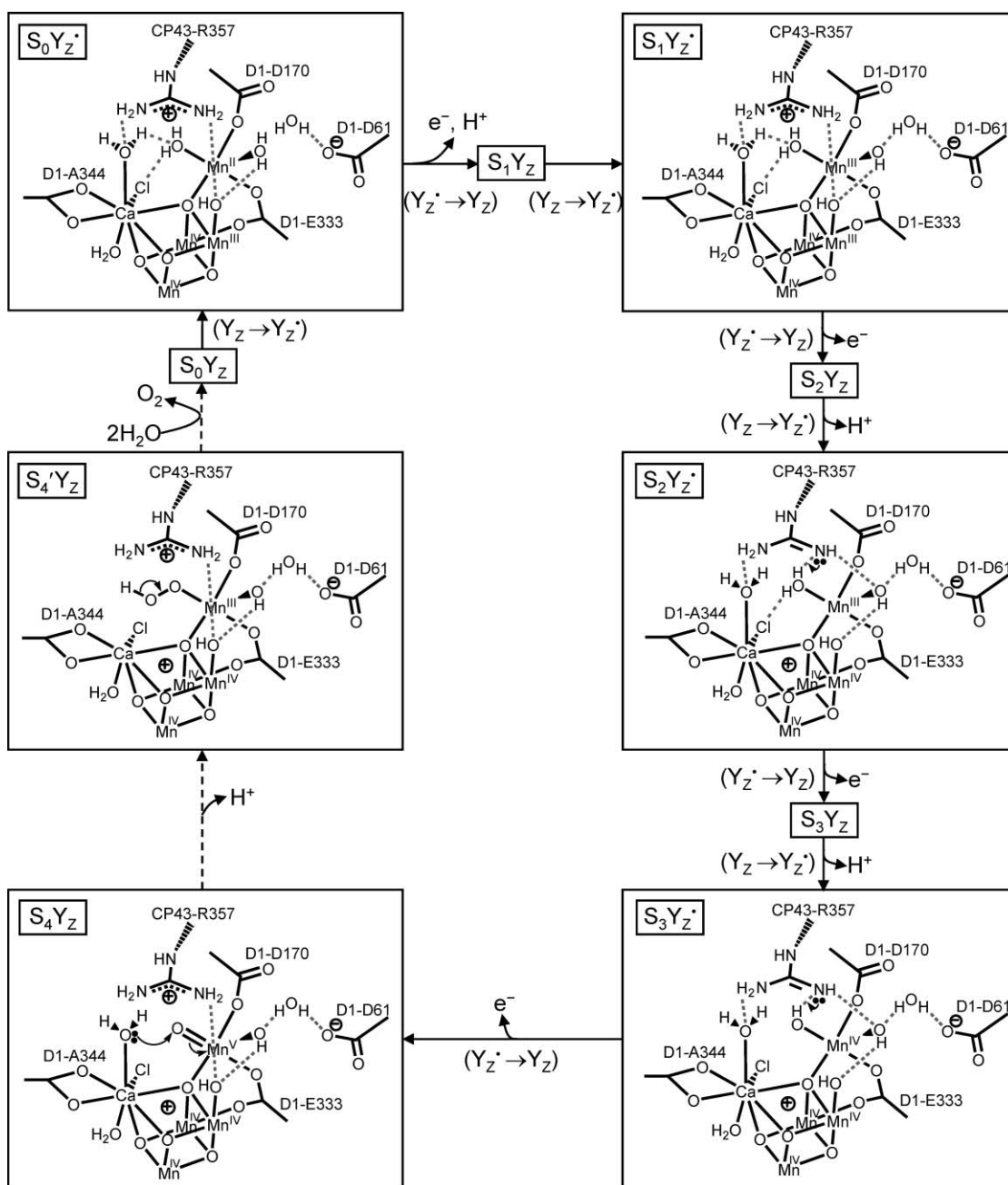


Fig. 2 Proposed mechanism of water splitting by the OEC, incorporating the computational structural model shown in Fig. 1. Hydrogen bonds are shown as dashed lines. The oxidation state changes of D1-Tyr161 (YZ) are shown next to the solid reaction arrows, which denote light-driven steps in the S-state cycle; dashed reaction arrows indicate spontaneous steps.

We suggest that chloride's role in the OEC is to provide a well-defined and localized hydrogen-bond acceptor for the Mn-bound substrate water, able reliably to cede its place to the CP43-Arg357 guanidine sp² nitrogen immediately after this residue's deprotonation (see Fig. 2). It is vital that the neutral arginine is thus brought immediately into contact with the Mn-bound substrate water, because otherwise this powerful guanidine base will acquire a proton from some other species and will be prevented from performing its catalytic function. If chloride (or another hydrogen-bond acceptor such as bromide or nitrate) is not bound to calcium in this location, the Mn-bound substrate water molecule may be free to form promiscuous hydrogen-bonding attachments with other species, preventing it from associating with the deprotonated arginine side-chain as soon as the latter is formed. This proposal is in agreement with the discovery by Yocum and co-workers that chloride is only required for the OEC redox transitions beyond the S₂ state.⁶⁵

We also consider the manner in which arginine might give up these protons to the proton-exit pathway in the second half of the catalytic cycle, specifically in the S₂Y_Z → S₂Y_Z[•] and S₃Y_Z → S₃Y_Z[•] tyrosine oxidations. These protons are expected to move *via* a Grotthus-type mechanism to the protein surface by way of a proton-exit channel comprising charged amino acid residues and water molecules organized in a dynamic, hydrogen-bonded assembly. Our S₀ model contains D1-Asp61, which appears from the crystal structure to be the first residue of this pathway,²² along with two hydrogen-bonded water molecules located between D1-Asp61 and the Mn(4) ligand sphere. Either or both of the Mn(4) ligands, D1-D170 and the non-substrate water uppermost in Fig. 1, plausibly link the hydrogen-bonding network around the OEC with that of the proton-exit pathway in later S-states. The latter option is indicated in Fig. 2. Judging by our structural model, it seems possible that some small movement of the arginine occurs immediately after its deprotonation to bring it towards the substrate water during the

$S_2 \rightarrow S_3$ transition, a movement that would be associated with a re-organization of the hydrogen-bonding network (see Fig. 2). This is in accord with experimental evidence suggesting that the OEC structure changes somewhat during this transition.⁶⁶

Although it is illuminating to consider the details of the OEC catalytic mechanism in relation to its structure, further developments in spectroscopy and crystallography will inevitably modify this structural model to some extent. The main features of our mechanistic proposal—namely, the combined roles of calcium and a highly-oxidized manganese ion, along with organized proton transfer from the Mn-bound substrate water—are not precisely dependent upon all of the atomic positions presented here. However, the usefulness of a mechanistic model lies in its testability, and some details of our proposal, such as the involvement of CP43–Arg357 in proton transfer, do depend precisely upon the structure, and may be directly disproved by further experiment.

The oxidation states of the Mn ions in the OEC

It is important to consider the oxidation states of the four manganese ions in the various S-states of the OEC. The X-ray crystal structure probably represents an average of various (low) S-states and therefore is not immediately helpful in answering the question. On the basis of considerable spectroscopic and biochemical data,^{9,67–72} the S_1 and S_2 states are generally considered to comprise the III, III, IV, IV and III, IV, IV, IV manganese oxidation states, although there is some dissent.^{73,74} The $S_2 \rightarrow S_3$ transition is controversial: some groups have interpreted EXAFS data to mean that it involves the oxidation of a manganese ion,⁷⁰ while others have maintained that it does not.⁷⁵ We believe that the balance of evidence favors a manganese-centered oxidation in the $S_2 \rightarrow S_3$ transition, which, accepting the consensus oxidation states of S_2 , implies a IV, IV, IV, IV set in S_3 . The S_0 state is thought to represent either the II, III, IV, IV or III, III, III, IV manganese oxidation states. The S_0 EXAFS and multiline EPR signal have been interpreted by some researchers^{76–79} as indicating the presence of Mn^{II} and, although this assignment has been questioned (most recently in ref. 80), it is the II, III, IV, IV assignment that we have used for S_0 .

The $S_3 \rightarrow [S_4] \rightarrow S_0$ transition is the most important and mysterious step in the catalytic cycle. We (and others) have proposed that Mn(4) is oxidized in the $S_3 \rightarrow S_4$ transition to an unstable Mn^V oxo species, which rapidly reacts with a calcium-bound water to release water and reform the S_0 state.^{40,81} This aspect of our mechanism has met with some criticism⁴⁸ on the grounds that the coordinative environment of Mn(4) implied by the crystal structure is not sufficiently electron-donating to stabilize the Mn^V oxidation state. In contrast, the non-porphyrin ligands that the groups of Collins^{82–85} and O'Halloran⁸⁶ have used synthetically to stabilize $Mn^V=O$ are highly-donating macrocyclic Schiff bases.

We agree that the Mn(4) ligand environment in our structure (3 neutral waters, 2 monoanionic carboxylate oxygens, one dianionic μ_4 oxide) is not, as it stands, likely to stabilize a +5 oxidation state. However, our proposal is that, as the S-state cycle progresses, one of the Mn(4) water ligands is deprotonated to hydroxide (we suggest in the $S_0 \rightarrow S_1$ transition, see Fig. 2) and the substrate water itself is doubly deprotonated (in the $S_2 \rightarrow S_3$ and the $S_3 \rightarrow S_4$ transitions) to give Mn(4) the following ligands in the S_4 state: 1 neutral water, 1 monoanionic hydroxide, 2 monoanionic carboxylate oxygens, one dianionic μ_4 oxide and one divalent oxo atom (oxidation state = –2). This ligand assembly is more electron-donating than the S_0 assembly and correspondingly better able to stabilize the Mn^V oxidation state. Furthermore, there is evidence from EXAFS spectroscopy that the $S_2 \rightarrow S_3$ transition produces a new 2.7 Å Mn–Mn distance, possibly representing the creation of a μ -oxo bridge between a pair of Mn ions.^{66,68} Our structural model of the S_0 state, and the crystal structure upon which it is based, suggest that any such

bridge would appear between Mn(4) and Mn(3). In the $S_2 \rightarrow S_3$ transition we propose that Mn(4) is oxidized from Mn^{III} to Mn^{IV} . The former is sometimes penta-coordinate, whereas Mn^{IV} is not.⁴³ This means that Mn(4) might be penta-coordinate in the S_2 (and S_1) states, gaining a sixth ligand in the form of a μ -oxo ion in the $S_2 \rightarrow S_3$ step. This would add yet another electron-donating dianionic ligand to Mn(4)'s coordination sphere in the S_3 state. Alternatively, the new 2.7 Å Mn–Mn distance seen in the S_3 state might be the result of the shortening of a longer Mn–Mn distance within the cuboidal cluster. Quantum mechanical calculations involving the higher S-states will be useful in assigning the individual oxidation states of the Mn ions and in assessing the plausibility of these structural rearrangements.

To summarize, although our structural model (and the crystal structure upon which it is based) does not provide Mn(4) with a very strongly electron-donating ligand set, this observation is compatible with our proposal that the center is highly oxidized in the S-state cycle, culminating in a formal Mn^V oxidation state in S_4 . It is not necessary that Mn(4) should be provided with extremely electron-donating ligands, such as have been used synthetically to obtain stable $Mn^V=O$ compounds (see above), because the $Mn^V=O$ moiety in the OEC must be unstable to reductive water attack in our proposed $S_4 \rightarrow S_0$ transition. The ligands used by Collins and co-workers, in contrast, give rise to water-stability⁸³ and low $Mn^{V/IV}$ reduction potentials, indeed as low as –560 mV in acetonitrile.⁸² Nature must ensure that the successive oxidations of the manganese center proceed at approximately equal redox potentials of just under +1 V.⁵⁸ We suggest that this is achieved by altering the ligand environment during the cycle, providing the metal with a more electron-donating ligand set later in its higher oxidation states. μ -Oxo and water ligands are good ligands in this context because their electron-donating properties vary according to their protonation state.⁸⁷

It might be that this charge-compensating effect of proton-coupled electron transfer is responsible for the recent observation that the FTIR stretching frequencies of D1–Asp170 are not altered in any S-state transition up to S_3 .⁸⁸ Each oxidation of Mn(4) is, we propose, accompanied by the deprotonation of one of its water ligands. Perhaps the vibrations of the ligating D1–Asp170 residue are unaffected by these oxidations because they are not accompanied by changes in net charge at the manganese center. We also note that electron delocalization within the OEC cluster is expected to reduce the accumulated positive charge at any single oxidized manganese center.

The binding of substrate water

The timing and location of substrate water binding in the OEC have been long-standing points of controversy. Early mechanistic models generally proposed either that the water molecules bound in the $S_4 \rightarrow S_0$ transition (see, for example, ref. 89) or in the $S_2 \rightarrow S_3$ transition (see ref. 90). Compelling evidence for water binding early in the S-state cycle has since come from pulsed EPR experiments⁵³ (which do not distinguish between substrate and non-substrate waters) and from the time-resolved mass-spectrometric measurements of ¹⁸O-labeled oxygen evolution by the OEC (a method which specifically targets substrate waters).⁹¹ These latter experiments, performed on thylakoid membranes at physiological pHs, have yielded four particularly important results:¹⁷ (1) that one substrate water molecule is bound to the OEC in all observable S-states (S_0 – S_3), and that the other is bound by at least the S_2 state; (2) that one substrate water molecule exchanges with bulk water at a faster rate than the other, and that these differently-bound waters do not exchange with one another; (3) that the rates of water exchange with bulk solution differ according to the S-state of the OEC, in the order S_0 (10 s^{-1}) > $S_2 = S_3$ (2 s^{-1}) > S_1 (0.02 s^{-1}) for the slow-exchanging water, and S_1 ($>120\text{ s}^{-1}$) > S_2 (120 s^{-1}) > S_3 (40 s^{-1}) for the fast-exchanging

water; and (4) that replacing calcium with strontium in the OEC increases the rate of exchange of the slow-exchanging water, but leaves that of the fast-exchanger unchanged. This last result strongly indicates that calcium binds the slow-exchanging water, and therefore that the fast-exchanging water binds to manganese (Mn(4) in our scheme).

To analyze these data, it would be helpful if there were manganese and calcium ligand water exchange rates (k_{ex}) available from a range of well-characterized model compounds at physiological pHs. Unfortunately, the only direct data available for the two metals are from the simple hexaqua $[\text{Mn}^{\text{II}}(\text{H}_2\text{O})_6]^{2+}$ ion, whose $k_{\text{ex}} = 2.1 \times 10^7 \text{ s}^{-1}$ at $\text{pH} \approx 1$.⁹² The Mn^{II} solution value is not very useful as a comparison with the fast-exchanging OEC water because: (1) the OEC fast-exchange rate is not resolved in the S_0 state, the only one in which we propose a +2 oxidation state for Mn(4); and (2) the hexaqua k_{ex} was measured at a much lower pH than was the OEC k_{ex} . There has not been a great deal of work done on the pH-dependence of solution k_{ex} values, but where it has been examined, as in the case of $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$, the pH effect has been found to be predictably pronounced.^{93,94} The pH-dependence of k_{ex} in the OEC is even less studied (only in the S_3 state¹⁷ to our knowledge) and is expected to be more complicated than that of bare aqua ions, with contributions from the protein as well as from the metal itself.

The lowest S-state for which the k_{ex} of the fast-exchanging OEC substrate water has been reliably measured is S_2 , where we propose that Mn(4) is formally in the +3 oxidation state. The solvated $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$ ion is unstable and its k_{ex} has not been measured. However, it has been observed that both iron and ruthenium hexaqua ions exchange ligand waters *ca.* 10^4 times slower when the metal is in the +3 oxidation state than when it is in the +2 oxidation state. A similar effect in the case of manganese would give a k_{ex} of *ca.* 10^3 s^{-1} for $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$ at $\text{pH} \approx 1$ (following similar reasoning, Hillier *et al.*⁹⁵ arrive at 10^{-3} – 10^{-1} s^{-1} , which appears to be an error). However, the real k_{ex} value for $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$ is probably much higher than 10^3 s^{-1} because Mn^{III} is a d^4 ion, and its ${}^5\text{E}_g$ high-spin ground-state term is subject to considerable Jahn–Teller stabilization. This is predicted to be achieved by lengthening its Mn–O bonds along one axis, and $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$ is predicted to be particularly labile as a result. Cr^{2+} is isoelectronic with Mn^{3+} , and $[\text{Cr}(\text{H}_2\text{O})_6]^{2+}$ has a much higher k_{ex} than either $[\text{V}(\text{H}_2\text{O})_6]^{2+}$ or $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$, exchanging water about 10^4 times faster than predicted on the basis of its charge density and undistorted ligand-field stabilization energy.⁹⁴ This means that the k_{ex} of $[\text{Mn}(\text{H}_2\text{O})_6]^{3+}$ might be as great as 10^7 s^{-1} (the same as that of $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$) and thus many orders of magnitude greater than that measured for the fast-exchanging water in the S_2 state of the OEC.

The $\text{S}_2 \rightarrow \text{S}_3$ photo-oxidation decreases the Mn-assigned fast exchange rate approximately three-fold. While following the trend predicted by simple electrostatic considerations, this decrease (as noted by Hendry and Wydrzynski)⁹¹ seems to be too small to account for our proposal in this step, which involves an oxidation of the substrate-binding Mn(4) from Mn^{III} to Mn^{IV} . Furthermore, we also propose that the substrate water is deprotonated in this step, presumably strengthening the Mn–O bond. Changes in the OEC structure between the S_2 and S_3 states, and particularly the reorganization of the hydrogen-bonding network around the OEC (see above, and Fig. 2), may be responsible for this flattening of the k_{ex} response to changing oxidation state. The increase in bond strength in going from $\text{Mn}^{\text{III}}\text{–OH}_2$ to $\text{Mn}^{\text{IV}}\text{–OH}$ may be partially offset by improving access of the site to bulk water *via* a reorganized hydrogen-bonding network. Hillier and Wydrzynski have recently found evidence for a hydrogen-bonding interaction of the fast-exchanging substrate water, in the form of a 2° H/D isotope effect for the exchange of this water in the S_3 state.¹⁷ The authors take this to mean that the water is hydrogen-bonded to a base, and that this interaction affects the water exchange. This agrees well with our proposal that the mechanistically vital

deprotonation of the Mn-bound substrate water in the S_3 state is achieved through the mediation of a hydrogen-bonding network involving the chloride ion and the CP43–Arg357 guanidine side chain (see Fig. 2).

No direct measurement of the rate of water exchange in $[\text{Ca}(\text{H}_2\text{O})_6]^{2+}$ exists, but it is predicted certainly to be greater than that of $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$, whose $k_{\text{ex}} = 6.7 \times 10^5 \text{ s}^{-1}$ at $\text{pH} \approx 1$.⁹⁶ Acoustic methods have been used to estimate $k_{\text{ex}} \geq 10^8 \text{ s}^{-1}$ for the calcium hexaqua ion at $\text{pH} \approx 9$.⁹⁷ These rates are much greater than the slow substrate water exchange rate observed in thylakoid membranes, which is identified with the calcium-binding substrate water. At its fastest, in the S_0 state, this k_{ex} is *ca.* 10 s^{-1} at $\text{pH} \approx 7$, about 10^8 times slower than the $[\text{Ca}(\text{H}_2\text{O})_6]^{2+}$ aqua ion value at $\text{pH} \approx 9$. This comparison implies that the Ca^{2+} environment in the OEC is very different indeed from its naked hexaqua environment. Furthermore, calcium-bound water seems to exchange with bulk slower than the fast-exchanging (Mn-bound) water by a factor of >6000 in S_1 , *ca.* 60 in S_2 and *ca.* 20 in S_3 . We suggest that two factors are important. The first is that charge density in the OEC does not simply reflect the changes in the formal oxidation states of the metal ions, because electrons are delocalized throughout the structure. The strength of the metal–water bond reflects the real electron density at the metal center, not the formal oxidation state. We are currently conducting *ab initio* quantum mechanical calculations on the OEC cluster to determine the positive charge densities at each of the metal sites in the various S-states. Preliminary results indicate that the $\text{Ca}^{2+}\text{–OH}_2$ bond is stronger than the $\text{Mn}(4)\text{–OH}_2$ bond even when Mn(4) is formally in the +3 oxidation state. Secondly, the rate of water exchange does not simply reflect the strength of the water–metal bond, but also the rate at which water is able to enter and leave the site. It is highly likely that the crystallographically revealed proton exit pathway (beginning at D1–Asp61) is full of water, and also serves as the water-entrance channel to the OEC. Mn(4) lies at one end of this channel, and our structural model (Fig. 1) shows that it is in close contact with two of the hydrogen-bonded waters which fill the channel. The manganese-bound substrate water therefore has better access to bulk water than does the calcium-bound substrate water. We expect the Mn(4)-bound substrate water to be particularly well-linked by hydrogen bonds to the channel beyond the S_2 state, because it is in the $\text{S}_2 \rightarrow \text{S}_3$ and $\text{S}_3 \rightarrow \text{S}_4$ transitions, we propose, that this water is rapidly stripped of protons. In contrast, and in accordance with our scheme shown in Fig. 2, the slow-exchanging (calcium-associated) water appears to have no hydrogen-bonding partner in the S_3 state that promotes the water exchange reaction.

There is a pressing need for ligand exchange rates to be measured in high-valent manganese model complexes which resemble the OEC cluster. Unpublished results in the Brudvig laboratory indicate that $\mu\text{-O}$ exchange in $\text{Mn}^{\text{III}}/\text{Mn}^{\text{IV}}$ and $\text{Mn}^{\text{IV}}/\text{Mn}^{\text{IV}}$ dimers occurs several orders of magnitude slower than the rate of substrate water exchange observed in the OEC, whereas the terminal waters of these model complexes exchange on a timescale comparable with that seen in the biological system.⁹⁸ Further investigations into the rates and mechanisms of high-valent manganese ligand exchange reactions will be invaluable in the continued elucidation of the OEC mechanism.

Acknowledgements

G. W. B. acknowledges National Institutes of Health grant GM32715 for the support of this work. V. S. B. acknowledges a generous allocation of supercomputer time from the National Energy Research Scientific Computing Center and financial support from an NSF Career Award, a Research Innovation Award from Research Corporation, a Petroleum Research Fund Award (type G) from the American Chemical Society, a junior faculty award from the F. Warren Hellman Family, and start-up package funds from the Provost's office at Yale University.

References

- 1 P. Joliot and B. Kok, Oxygen evolution in photosynthesis, in *Bioenergetics of Photosynthesis*, ed. Govindjee, Academic Press, New York, 1975, pp. 387–412.
- 2 C. F. Yocum, C. T. Yerkes, R. E. Blankenship, R. R. Sharp and G. T. Babcock, Stoichiometry, inhibitor sensitivity, and organization of manganese associated with photosynthetic oxygen evolution, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 7507–7511.
- 3 G. M. Cheniae and I. F. Martin, Sites of function of manganese within photosystem II—roles in O₂ evolution and system II, *Biochim. Biophys. Acta*, 1970, **197**, 219–239.
- 4 G. W. Brudvig, Electron paramagnetic resonance spectroscopy, *Methods Enzymol.*, 1995, **246**, 536–554.
- 5 R. D. Britt, J. M. Peloquin and K. A. Campbell, Pulsed and parallel-polarization EPR characterization of the photosystem II oxygen-evolving complex, *Annu. Rev. Biophys. Biomol. Struct.*, 2000, **29**, 463–495.
- 6 A. Boussac, S. Un, O. Horner and A. W. Rutherford, High-spin states ($S > 5/2$) of the photosystem II manganese complex, *Biochemistry*, 1998, **37**, 4001–4007.
- 7 H. Dau, P. Liebisch and M. Haumann, X-Ray absorption spectroscopy to analyze nuclear geometry and electronic structure of biological metal centers-potential and questions examined with special focus on the tetra-nuclear manganese complex of oxygenic photosynthesis, *Anal. Bioanal. Chem.*, 2003, **376**, 562–583.
- 8 V. K. Yachandra, Structure of the manganese complex in photosystem II: insights from X-ray spectroscopy, *Philos. Trans. R. Soc. London, Ser. B*, 2002, **357**, 1347–1357.
- 9 J. E. Penner-Hahn, Structural characterization of the Mn site in the photosynthetic oxygen-evolving complex, *Struct. Bonding*, 1998, **90**, 1–36.
- 10 B. Ke, *Photosynthesis: Photobiochemistry and Photobiophysics*, ed. Govindjee, Kluwer Academic Publishers, Dordrecht, 2001.
- 11 J. Clausen and W. Junge, Detection of an intermediate of photosynthetic water oxidation, *Nature*, 2004, **430**, 480–483.
- 12 C. Berthomieu, R. Hienerwadel, A. Boussac, J. Breton and B. A. Diner, Hydrogen bonding of redox-active tyrosine Z of photosystem II probed by FTIR difference spectroscopy, *Biochemistry*, 1998, **37**, 10547–10554.
- 13 H. Chu, W. Hillier and R. J. Debus, Evidence that the C-terminus of the D1 polypeptide of photosystem II is ligated to the manganese ion that undergoes oxidation during the S₁ to S₂ transition: an isotope-edited FTIR study, *Biochemistry*, 2004, **43**, 3152–3166.
- 14 T. Noguchi and M. Sugiura, Structure of an active water molecule in the water-oxidizing complex of photosystem II as studied by FTIR spectroscopy, *Biochemistry*, 2000, **39**, 10943–10949.
- 15 J. Clausen, R. J. Debus and W. Junge, Time-resolved oxygen production by PSII: chasing chemical intermediates, *Biochim. Biophys. Acta*, 2004, **1655**, 184–194.
- 16 M. Haumann and W. Junge, Proton and charge indicators in oxygen evolution, in *Oxygenic Photosynthesis: The Light Reactions*, ed. D. R. Ort and C. F. Yocum, Kluwer Academic Publishers, Dordrecht, 1996, pp. 165–192.
- 17 W. Hillier and T. Wydrzynski, Substrate water interactions within the photosystem II oxygen evolving complex, *Phys. Chem. Chem. Phys.*, 2004, **6**, 4882–4889.
- 18 W. Hillier and T. Wydrzynski, The affinities for the two substrate water binding sites in the O₂ evolving complex of photosystem II vary independently during S-state turnover, *Biochemistry*, 2000, **39**, 4399–4405.
- 19 A. Zouni, H. T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger and P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, *Nature*, 2001, **409**, 739–743.
- 20 N. Kamiya and J. R. Shen, Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 98–103.
- 21 J. Biesiadka, B. Loll, J. Kern, K. D. Irrgang and A. Zouni, Crystal structure of cyanobacterial photosystem II at 3.2 Å resolution: a closer look at the Mn-cluster, *Phys. Chem. Chem. Phys.*, 2004, **6**, 4733–4736.
- 22 K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber and S. Iwata, Architecture of the photosynthetic oxygen-evolving center, *Science*, 2004, **303**, 1831–1838.
- 23 M. Pourbaix, *Atlas of Electrochemical Equilibria*, Pergamon Press, Oxford, 1966.
- 24 D. M. Kramer, C. A. Sacksteder and J. A. Cruz, How acidic is the lumen?, *Photosynth. Res.*, 1999, **60**, 151–163.
- 25 L. I. Krissalik, Energetics of multielectron reactions-photosynthetic oxygen evolution, *Biochim. Biophys. Acta*, 1986, **849**, 162–171.
- 26 G. Renger, Model for molecular mechanism of photosynthetic oxygen evolution, *FEBS Lett.*, 1977, **81**, 223–228.
- 27 C. W. Hoganson, N. Lydakis-Simantiris, X. S. Tang, C. Tommos, K. Warncke, G. T. Babcock, B. A. Diner, J. McCracken and S. Styring, A hydrogen-atom abstraction model for the function of Y_Z in photosynthetic oxygen evolution, *Photosynth. Res.*, 1995, **46**, 177–184.
- 28 J. G. Metz, P. J. Nixon, M. Rogner, G. W. Brudvig and B. A. Diner, Directed alteration of the D1 polypeptide of photosystem II: Evidence that tyrosine-161 is the redox component, Z, connecting the oxygen-evolving complex to the primary electron donor, P680, *Biochemistry*, 1989, **28**, 6960–6969.
- 29 R. J. Debus, B. A. Barry, I. Sithole, G. T. Babcock and L. McIntosh, Directed mutagenesis indicates that the donor to P₆₈₀⁺ in photosystem II is tyrosine-161 of the D1 polypeptide, *Biochemistry*, 1988, **27**, 9071–9074.
- 30 M. Haumann and W. Junge, Extent and rate of proton release by photosynthetic water oxidation in thylakoids-electrostatic relaxation versus chemical production, *Biochemistry*, 1994, **33**, 864–872.
- 31 F. Mamedov, R. T. Sayre and S. Styring, Involvement of histidine 190 on the D1 protein in electron/proton transfer reactions on the donor side of photosystem II, *Biochemistry*, 1998, **37**, 14245–14256.
- 32 A. M. A. Hays, I. R. Vassiliev, J. H. Golbeck and R. J. Debus, Role of D1–His190 in proton-coupled electron transfer reactions in photosystem II: a chemical complementation study, *Biochemistry*, 1998, **37**, 11352–11365.
- 33 C. W. Hoganson and G. T. Babcock, A metalloradical mechanism for the generation of oxygen from water in photosynthesis, *Science*, 1997, **277**, 1953–1956.
- 34 J. S. Vrettos, D. A. Stone and G. W. Brudvig, Quantifying the ion selectivity of the Ca²⁺ site in photosystem II: Evidence for direct involvement of Ca²⁺ in O₂ formation, *Biochemistry*, 2001, **40**, 7937–7945.
- 35 C. Lee and G. W. Brudvig, Investigation of the functional role of Ca²⁺ in the oxygen-evolving complex of photosystem II: A pH-dependence study of the substitution of Ca²⁺ by Sr²⁺, *J. Chin. Chem. Soc. (Taipei)*, 2004, **51**, 1221–1228.
- 36 G. Hendry and T. Wydrzynski, ¹⁸O isotope exchange measurements reveal that calcium is involved in the binding of one substrate-water molecule to the oxygen-evolving complex in photosystem II, *Biochemistry*, 2003, **42**, 6209–6217.
- 37 R. M. Cinco, J. H. Robblee, J. Messinger, C. Fernandez, K. L. M. Holman, K. Sauer and V. K. Yachandra, Orientation of calcium in the Mn₄Ca cluster of the oxygen-evolving complex determined using polarized strontium EXAFS of photosystem II membranes, *Biochemistry*, 2004, **43**, 13271–13282.
- 38 S. H. Kim, W. Gregor, J. M. Peloquin, M. Brynda and R. D. Britt, Investigation of the calcium-binding site of the oxygen evolving complex of photosystem II using ⁸⁷Sr ESEEM spectroscopy, *J. Am. Chem. Soc.*, 2004, **126**, 7228–7237.
- 39 J. P. McEvoy and G. W. Brudvig, Structure-based mechanism of photosynthetic water oxidation, *Phys. Chem. Chem. Phys.*, 2004, **6**, 4754–4763.
- 40 J. S. Vrettos, J. Limburg and G. W. Brudvig, Mechanism of photosynthetic water oxidation: combining biophysical studies of photosystem II with inorganic model chemistry, *Biochim. Biophys. Acta*, 2001, **1503**, 229–245.
- 41 J. P. McEvoy, J. A. Gascon, E. M. Sproviero, V. S. Batista and G. W. Brudvig, Computational structural model of the oxygen evolving complex in photosystem II: Complete ligation by protein, water and chloride, in *Photosynthesis: Fundamental Aspects to Global Perspectives*, ed. D. Bruce and A. van der Est, Allen Press, Lawrence, Kansas, 2005, vol. 1, pp. 278–280.
- 42 H. Dau, P. Liebisch and M. Haumann, The structure of the manganese complex of photosystem II in its dark-stable S₁ state-EXAFS results in relation to recent crystallographic data, *Phys. Chem. Chem. Phys.*, 2004, **6**, 4781–4792.
- 43 F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, Wiley-Interscience, New York, 1999.
- 44 A. K. Katz, J. P. Glusker, S. A. Beebe and C. W. Bock, Calcium ion coordination: A comparison with that of beryllium, magnesium, and zinc, *J. Am. Chem. Soc.*, 1996, **118**, 5752–5763.
- 45 K. Lindberg and L. E. Andreasson, A one-site, two-state model for the binding of anions in photosystem II, *Biochemistry*, 1996, **35**, 14259–14267.
- 46 D. A. Force, D. W. Randall and R. D. Britt, Proximity of acetate, manganese, and exchangeable deuterons to tyrosine Y_Z in acetate-inhibited photosystem II membranes: Implications for the direct involvement of Y_Z in water-splitting, *Biochemistry*, 1997, **36**, 12062–12070.

- 47 K. Olesen and L. E. Andreasson, The function of the chloride ion in photosynthetic oxygen evolution, *Biochemistry*, 2003, **42**, 2025–2035.
- 48 J. Dasgupta, R. T. van Willigen and G. C. Dismukes, Consequences of structural and biophysical studies for the molecular mechanism of photosynthetic oxygen evolution: functional roles for calcium and bicarbonate, *Phys. Chem. Chem. Phys.*, 2004, **6**, 4793–4802.
- 49 P. J. Nixon, J. T. Trost and B. A. Diner, Role of the carboxy terminus of polypeptide D1 in the assembly of a functional water-oxidizing manganese cluster in photosystem II of the cyanobacterium *Synechocystis* Sp Pcc 6803-assembly requires a free carboxyl group at C-terminal position 344, *Biochemistry*, 1992, **31**, 10859–10871.
- 50 Y. Kimura, K. Hasegawa and T. Ono, Characteristic changes of the S_2/S_1 difference FTIR spectrum induced by Ca^{2+} depletion and metal cation substitution in the photosynthetic oxygen-evolving complex, *Biochemistry*, 2002, **41**, 5844–5853.
- 51 M. A. Strickler, L. M. Walker, W. Hillier and R. J. Debus, Evidence from biosynthetically incorporated strontium and FTIR difference spectroscopy that the C-terminus of the D1 polypeptide of photosystem II does not ligate calcium, *Biochemistry*, 2005, **44**, 8571–8577.
- 52 Y. Kimura, N. Mizusawa, T. Yamanari, A. Ishii and T. Ono, Structural changes of D1 C-terminal alpha-carboxylate during S-state cycling in photosynthetic oxygen evolution, *J. Biol. Chem.*, 2005, **280**, 2078–2083.
- 53 R. D. Britt, K. A. Campbell, J. M. Peloquin, M. L. Gilchrist, C. P. Aznar, M. M. Dicus, J. Robblee and J. Messinger, Recent pulsed EPR studies of the photosystem II oxygen-evolving complex: implications as to water oxidation mechanisms, *Biochim. Biophys. Acta*, 2004, **1655**, 158–171.
- 54 A. M. A. Hays, I. R. Vassiliev, J. H. Golbeck and R. J. Debus, Role of D1-His190 in the proton-coupled oxidation of tyrosine Y_z in manganese-depleted photosystem II, *Biochemistry*, 1999, **38**, 11851–11865.
- 55 R. A. Roffey, D. M. Kramer, Govindjee and R. T. Sayre, Lumenal side histidine mutations in the D1 protein of photosystem II affect donor side electron-transfer in *Chlamydomonas reinhardtii*, *Biochim. Biophys. Acta*, 1994, **1185**, 257–270.
- 56 B. A. Diner and P. J. Nixon, Evidence for D1-His190 as the proton acceptor implicated in the oxidation of redox-active tyrosine Y_z of PSII, in *Photosynthesis: Mechanisms and Effects*, ed. G. Garab, Kluwer Academic Publishers, Dordrecht, 1998, vol. 2, pp. 1177–1180.
- 57 X. S. Tang, D. A. Chisholm, G. C. Dismukes, G. W. Brudvig and B. A. Diner, Spectroscopic evidence from site-directed mutants of *Synechocystis* PCC6803 in favor of a close interaction between histidine 189 and redox-active tyrosine 160, both of polypeptide D2 of the photosystem II reaction center, *Biochemistry*, 1993, **32**, 13742–13748.
- 58 C. Tommos and G. T. Babcock, Proton and hydrogen currents in photosynthetic water oxidation, *Biochim. Biophys. Acta*, 2000, **1458**, 199–219.
- 59 F. Rappaport and J. Lavergne, Charge recombination and proton transfer in manganese-depleted photosystem II, *Biochemistry*, 1997, **36**, 15294–15302.
- 60 F. Rappaport, M. Blanchard-Desce and J. Lavergne, Kinetics of electron transfer and electrochromic change during the redox transitions of the photosynthetic oxygen-evolving complex, *Biochim. Biophys. Acta*, 1994, **1184**, 178–192.
- 61 J. Lavergne and W. Junge, Proton release during the redox cycle of the water oxidase, *Photosynth. Res.*, 1993, **38**, 279–296.
- 62 V. Petrouleas, D. Koulougliotis and N. Ioannidis, Trapping of metalloradical intermediates of the S-states at liquid helium temperatures. Overview of the phenomenology and mechanistic implications, *Biochemistry*, 2005, **44**, 6723–6728.
- 63 C. G. Mowat, R. Moyses, C. S. Miles, D. Leys, M. K. Doherty, P. Taylor, M. D. Walkinshaw, G. A. Reid and S. K. Chapman, Kinetic and crystallographic analysis of the key active site acid/base arginine in a soluble fumarate reductase, *Biochemistry*, 2001, **40**, 12292–12298.
- 64 M. K. Doherty, S. L. Pealing, C. S. Miles, R. Moyses, P. Taylor, M. D. Walkinshaw, G. A. Reid and S. K. Chapman, Identification of the active site acid/base catalyst in a bacterial fumarate reductase: A kinetic and crystallographic study, *Biochemistry*, 2000, **39**, 10695–10701.
- 65 H. Wincencjusz, H. J. van Gorkom and C. F. Yocum, The photosynthetic oxygen evolving complex requires chloride for its redox state $S_2 > S_3$ and $S_3 > S_0$ transitions but not for $S_0 > S_1$ or $S_1 > S_2$ transitions, *Biochemistry*, 1997, **36**, 3663–3670.
- 66 M. Haumann, C. Muller, P. Liebisch, L. Iuzzolino, J. Dittmer, M. Grabolle, T. Neisius, W. Meyer-Klaucke and H. Dau, Structural and oxidation state changes of the photosystem II manganese complex in four transitions of the water oxidation cycle ($S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and S_3 , $S_4 \rightarrow S_0$) characterized by X-ray absorption spectroscopy at 20 K and room temperature, *Biochemistry*, 2005, **44**, 1894–1908.
- 67 T. Kuntzleman and C. F. Yocum, Reduction-induced inhibition and Mn(II) release from the photosystem II oxygen-evolving complex by hydroquinone or NH_2OH are consistent with a Mn(III)/Mn(III)/Mn(IV)/Mn(IV) oxidation state for the dark-adapted enzyme, *Biochemistry*, 2005, **44**, 2129–2142.
- 68 H. Dau, L. Iuzzolino and J. Dittmer, The tetra-manganese complex of photosystem II during its redox cycle-X-ray absorption results and mechanistic implications, *Biochim. Biophys. Acta*, 2001, **1503**, 24–39.
- 69 V. K. Yachandra, V. J. DeRose, M. J. Latimer, I. Mukerji, K. Sauer and M. P. Klein, Where plants make oxygen - a structural model for the photosynthetic oxygen-evolving manganese cluster, *Science*, 1993, **260**, 675–679.
- 70 L. Iuzzolino, J. Dittmer, W. Dorner, W. Meyer-Klaucke and H. Dau, X-ray absorption spectroscopy on layered photosystem II membrane particles suggests manganese-centered oxidation of the oxygen-evolving complex for the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, and $S_2 \rightarrow S_3$ transitions of the water oxidation cycle, *Biochemistry*, 1998, **37**, 17112–17119.
- 71 T. A. Roelofs, W. C. Liang, M. J. Latimer, R. M. Cinco, A. Rompel, J. C. Andrews, K. Sauer, V. K. Yachandra and M. P. Klein, Oxidation states of the manganese cluster during the flash-induced S-state cycle of the photosynthetic oxygen-evolving complex, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 3335–3340.
- 72 J. Cole, V. K. Yachandra, R. D. Guiles, A. E. McDermott, R. D. Britt, S. L. Dexheimer, K. Sauer and M. P. Klein, Assignment of the $g = 4.1$ electron paramagnetic resonance signal to manganese in the S_2 state of the photosynthetic oxygen-evolving complex - an X-ray absorption-edge spectroscopy study, *Biochim. Biophys. Acta*, 1987, **890**, 395–398.
- 73 T. G. Carrell, A. M. Tyryshkin and G. C. Dismukes, An evaluation of structural models for the photosynthetic water-oxidizing complex derived from spectroscopic and X-ray diffraction signatures, *J. Biol. Inorg. Chem.*, 2002, **7**, 2–22.
- 74 D. Kuzek and R. J. Pace, Probing the Mn oxidation states in the OEC. Insights from spectroscopic, computational and kinetic data, *Biochim. Biophys. Acta*, 2001, **1503**, 123–137.
- 75 J. Messinger, J. H. Robblee, U. Bergmann, C. Fernandez, P. Glatzel, H. Visser, R. M. Cinco, K. L. McFarlane, E. Bellacchio, S. A. Pizarro, S. P. Cramer, K. Sauer, M. P. Klein and V. K. Yachandra, Absence of Mn-centered oxidation in the $S_2 \rightarrow S_3$ transition: implications for the mechanism of photosynthetic water oxidation, *J. Am. Chem. Soc.*, 2001, **123**, 7804–7820.
- 76 K. A. Ahrling, S. Peterson and S. Styring, An oscillating manganese electron paramagnetic resonance signal from the S_0 state of the oxygen evolving complex in photosystem II, *Biochemistry*, 1997, **36**, 13148–13152.
- 77 J. Messinger, J. H. Robblee, W. O. Yu, K. Sauer, V. K. Yachandra and M. P. Klein, The S_0 state of the oxygen-evolving complex in photosystem II is paramagnetic: detection of EPR multiline signal, *J. Am. Chem. Soc.*, 1997, **119**, 11349–11350.
- 78 J. Messinger, J. H. A. Nugent and M. C. W. Evans, Detection of an EPR multiline signal for the S_0 state in photosystem II, *Biochemistry*, 1997, **36**, 11055–11060.
- 79 R. D. Guiles, V. K. Yachandra, A. E. McDermott, J. L. Cole, S. L. Dexheimer, R. D. Britt, K. Sauer and M. P. Klein, The S_0 state of photosystem II induced by hydroxylamine-differences between the structure of the manganese complex in the S_0 and S_1 states determined by X-ray absorption spectroscopy, *Biochemistry*, 1990, **29**, 486–496.
- 80 L. V. Kulik, B. Epel, W. Lubitz and J. Messinger, Mn-55 pulse ENDOR at 34 GHz of the S_0 and S_2 states of the oxygen-evolving complex in photosystem II, *J. Am. Chem. Soc.*, 2005, **127**, 2392–2393.
- 81 V. L. Pecoraro, M. J. Baldwin, M. T. Caudle, W. Y. Hsieh and N. A. Law, A proposal for water oxidation in photosystem II, *Pure Appl. Chem.*, 1998, **70**, 925–929.
- 82 J. M. Workman, R. D. Powell, A. D. Procyk, T. J. Collins and D. F. Bocian, Vibrational and electrochemical properties of a series of stable manganese(v)-oxo complexes, *Inorg. Chem.*, 1992, **31**, 1548–1550.
- 83 T. J. Collins, R. D. Powell, C. Slebodnick and E. S. Uffelman, A water-stable manganese(v)-oxo complex-definitive assignment of a $\nu(Mn(v)O)$ infrared vibration, *J. Am. Chem. Soc.*, 1990, **112**, 899–901.
- 84 T. J. Collins and S. W. Gordon-Wylie, A manganese(v)-oxo complex, *J. Am. Chem. Soc.*, 1989, **111**, 4511–4513.
- 85 C. G. Miller, S. W. Gordon-Wylie, C. P. Horwitz, S. A. Strazisar, D. K. Peraino, G. R. Clark, S. T. Weintraub and T. J. Collins, A method for driving O-atom transfer: secondary ion binding to a tetraamide macrocyclic ligand, *J. Am. Chem. Soc.*, 1998, **120**, 11540–11541.

- 86 F. M. MacDonnell, N. L. P. Fackler, C. Stern and T. V. O'Halloran, Air oxidation of a 5-coordinate Mn(III) dimer to a high-valent oxomanganese(V) complex, *J. Am. Chem. Soc.*, 1994, **116**, 7431–7432.
- 87 M. J. Baldwin, A. Gelasco and V. L. Pecoraro, The effect of protonation on Mn(IV)($\mu(2)$ -O)₂ complexes, *Photosynth. Res.*, 1993, **38**, 303–308.
- 88 R. J. Debus, M. A. Strickler, L. M. Walker and W. Hillier, No evidence from FTIR difference spectroscopy that aspartate-170 of the D1 polypeptide ligates a manganese ion that undergoes oxidation during the S₀ to S₁, S₁ to S₂, or S₂ to S₃ transitions in photosystem II, *Biochemistry*, 2005, **44**, 1367–1374.
- 89 T. Kambara and Govindjee, Molecular mechanism of water oxidation in photosynthesis based on the functioning of manganese in two different environments, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 6119–6123.
- 90 G. W. Brudvig, H. H. Thorp and R. H. Crabtree, Probing the mechanism of water oxidation in photosystem II, *Acc. Chem. Res.*, 1991, **24**, 311–316.
- 91 G. Hendry and T. Wydrzynski, The two substrate-water molecules are already bound to the oxygen-evolving complex in the S₂ state of photosystem II, *Biochemistry*, 2002, **41**, 13328–13334.
- 92 Y. Ducommun, K. E. Newman and A. E. Merbach, High-pressure NMR kinetics. 11. High-pressure ¹⁷O NMR evidence for a gradual mechanistic changeover from I_a to I_d for water exchange on divalent octahedral metal ions going from manganese(II) to nickel(II), *Inorg. Chem.*, 1980, **19**, 3696–3703.
- 93 F. C. Xu, H. R. Krouse and T. W. Swaddle, Conjugate base pathway for water exchange on aqueous chromium(III)-variable-pressure and variable-temperature kinetic study, *Inorg. Chem.*, 1985, **24**, 267–270.
- 94 D. T. Richens, *The Chemistry of Aqua Ions*, John Wiley and Sons, Chichester, 1997.
- 95 W. Hillier, J. Messinger and T. Wydrzynski, Kinetic determination of the fast exchanging substrate water molecule in the S₃ state of photosystem II, *Biochemistry*, 1998, **37**, 16908–16914.
- 96 A. Bleuzen, P. A. Pittet, L. Helm and A. E. Merbach, Water exchange on magnesium(II) in aqueous solution: a variable temperature and pressure ¹⁷O NMR study, *Magn. Reson. Chem.*, 1997, **35**, 765–773.
- 97 G. Atkinson, M. M. Emara and R. Fernando, Ultrasonic absorption in aqueous solutions of calcium acetate and other bivalent metal acetates, *J. Phys. Chem.*, 1974, **78**, 1913–1917.
- 98 R. Tagore, H. Chen, R. H. Crabtree and G. W. Brudvig, 2005, manuscript submitted.