Water-oxidizing complex in Photosystem II: Its structure and relation to manganese-oxide based catalysts

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Review

Water-oxidizing complex in Photosystem II: Its structure and relation to manganese-oxide based catalysts

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ABSTRACT

Cyanobacteria, green algae, and higher plants provide the major part of molecular O₂ of Earth atmosphere via water oxidation of oxygenic photosynthesis. The water-oxidizing complex is a manganese-calcium oxide-based cluster embedded in Photosystem II that oxidizes water with high turnover frequency. The atomic structure and analysis of the Mn-Ca cluster are important in understanding the mechanism of water oxidation and for the design of efficient artificial water-oxidizing catalysts. With this short review, we aim to introduce the basic features of the biological water oxidation to the new-comers in the field. Taking into account the recent structural studies, including a high-resolution, radiation-damage-free structure of the water-oxidizing complex, and structures of intermediate S-states revealed by femtosecond X-ray free electron lasers, we discuss the structure and functions of the biologically active site and its implications for the development of inorganic catalysts for solar fuels production.

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Contents

1. Introduction	2
2. Biological water oxidation	4
3. Structure of the water-oxidizing complex	4
4. Metal ions in the biological water-oxidizing complex	6
4.1. Mn1	7

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4.2.	Mn2	7
4.3.	Mn3	7
4.4.	Mn4	7
4.5.	Ca	7
5.	The second coordination sphere around the Mn-Ca cluster	7
5.1.	Cl ⁻	8
5.2.	Tyrosine 161 (Y _Z)	9
5.3.	Channels	9
6.	Mechanism of biological water oxidation	9
7.	Implications for the synthetic MnO _x catalysts	12
8.	Conclusions	12
	Acknowledgments	13
	Conflict of interest	13
	References	13

1. Introduction

Oxygenic photosynthesis provides the source of foods (energy) indispensable for sustaining life in its current form on the Earth. This process is virtually an energy-conversion process by which the light energy from the Sun is converted into chemical energy stored in the form of sugars. The organic compounds are synthesized by reducing atmospheric CO₂ with the reducing equivalents (electron and protons) obtained by splitting water molecule. As a by-product, molecular oxygen is released in the atmosphere. The whole process of photosynthesis is common to cyanobacteria, algae, and terrestrial plants, and is conventionally divided into four sets of reactions [1,2]:

- (1) light absorption and energy transfer
- (2) primary electron transfer
- (3) energy stabilization by secondary processes
- (4) synthesis and export of products

The steps of (1)–(3) take place in two large transmembrane protein-cofactor complexes: Photosystem I (PSI) and Photosystem II (PSII) embedded in the thylakoid membrane of the photosynthetic organisms (Fig. 1b).

The redox-active cofactors bound in these two photosystems form an electron transport pathway known as Z-Scheme [1,2], where water molecules oxidized by PSII serve as an electron source [3,4]. PSII is a large protein-cofactor complex, also known as the water-plastoquinone oxidoreductase. The reactions in PSII start with a photon absorption (wavelengths 400–680 nm) by the antenna pigments of PSII followed by excitation of the primary electron donor (chlorophyll *a* molecule(s)) in the heart of the reaction center of PSII. Within a few ps an electron from the excited chlorophylls is transferred to the primary electron acceptor, a nearby protein-bound pheophytin molecule (Pheo, see Fig. 1c). For long time it was accepted that a special pair of chlorophyll molecules, P₆₈₀, bound to D1 and D2 proteins with nearly parallel porphyrin rings spaced by only several angström distance, serves as a primary electron donor. Recent studies show that the real situation is more complicated and an additional pair of chlorophyll *a* molecules, also bound to D1 and D2 proteins (so-called accessory pigments), is likely to be involved [5]. For example, a multimer model was suggested where there is no ‘special pair’ and the excitation energy may be dynamically localized basically on any combination of neighbouring chlorins [6]. In other words, there are indications that the primary charge separation may happen between one of the accessory chlorophylls and the primary electron acceptor (Pheo), a reaction followed by electron transfer from the special pair P₆₈₀ to the accessory Chl [6]. The electron-hole created on P₆₈₀/P₆₈₀⁺, is compensated by a fast electron transfer (<1 μs) from a nearby Tyr residue, Tyr161 from the D1 protein, often

denoted also as Y_Z [1,2]. The oxidized Y_Z subsequently oxidizes the water-oxidizing center, the Mn₄CaO₅ cluster firmly bound to PSII. In the acceptor side, the electron is transferred from the reduced pheophytin to the primary quinone acceptor, Q_A, then to the exchangeable Q_B molecule, part of a pool of plastoquinone acceptors (PQ pool) dissolved in the lipid bilayer of the thylakoid membrane. The electrons from the PQ pool are subsequently transferred through several electron carriers to PSI and used to reduce NADP (see Fig. 1). Thus, the water-splitting reaction in PSII can be viewed as a starting point of the whole photosynthetic electron-transfer chain (in terms of the electron source, but not in terms of time sequence). This reaction is also the source of molecular oxygen in the atmosphere on our planet.

The basic structural features of PSII are similar in all oxygenic photoautotrophs, and in this short review, we will focus on the PSII in cyanobacteria. PSII is a dimeric membrane-protein supercomplex (with dimensions of ~10.5 (nm) × 20.5 (nm) × 11.0 (nm) for cyanobacterial PSII dimers) [7,8] consisting of 20 protein subunits and over 70 cofactors for each of the monomers, resulting in a total molecular mass of 700 kDa for the dimer [3,4]. In each monomer, two polypeptides, D1 and D2, form the reaction center and bind all of the cofactors involved in the electron transfer, including the special chlorophyll pair P₆₈₀, four additional chlorophyll *a* molecules (a pair of accessory chlorophyll molecules and a pair of so-called periphery chlorophyll molecules at a relatively larger distance), Mn₄CaO₅ cluster, two pheophytins, two plastoquinones (one firmly bound quinone, Q_A, and one exchangeable with the plastoquinone pool, Q_B). Also, 35 chlorophyll molecules and some β-carotene molecules, two heme Fe atoms, one non-heme Fe atom, and more than 1400 water molecules [3,7,8] are found in each PSII monomer whose structure is analyzed at a resolution of 1.9 Å (Fig. 1c).

The active site for water oxidation is a CaMn₄O₅ cluster maintained by its surrounding protein environment. It is often called water-oxidizing complex (WOC) or oxygen-evolving complex (OEC) and utilizes earth-abundant, environmentally-friendly metal ions Ca and Mn to oxidize water with a low overpotential and a high turnover number [3–11]. The WOC has a high turnover frequency of 500 s⁻¹ [11]. The structure of this ‘super catalyst’ is evolutionary preserved and virtually identical from cyanobacteria to various algae and higher plants, and dates back to 2.4 billion years ago [2]. Due to the extensive di-μ-oxo bridging between the metal atoms in the structure it can be considered as a small nano-sized Mn-Ca oxide (with dimensions of ~0.5 (nm) × 0.25 (nm) × 0.25 (nm)) (Fig. 1c–e) [3,8,11]. Thus, the water-splitting part of the PSII may serve as a model for artificial photosynthetic systems, where water oxidation is used to provide cheap electrons from water according to Eq. (1). These electrons can be used to reduce protons (Eq. (2)) and produce hydrogen in the overall water splitting reaction (Eq. (3)) [12–14]:

In total:

- (1) When catalyzed by synthetic, artificial catalysts and powered by electrical energy from energy sources such as wind or sun light, the water electrolysis (Eq. (3)) is a possible method for storage of clean, renewable energies in the form of hydrogen [13,15–17].
- (2) Alternatively, the electrons from water can also be used to reduce CO_2 into methanol or other carbonaceous fuels. In general, water is the most attractive source of electrons and protons and thus water
- (3)

oxidation is likely to be a central process for any renewable fuel generation. Understanding the structure and function of the biological Mn_4CaO_5 catalytic center is often believed to be one of the key steps in producing efficient catalysts for water oxidation.

2. Biological water oxidation

To split water to produce a molecule of dioxygen, four electrons must be removed from two molecules of water. In 1969 Pierre Joliot observed an oscillating pattern in the flash-induced oxygen evolution of dark-adapted *Chlorella* cell suspensions [18]. The maximum yield of oxygen evolution was observed after the 3rd flash, and then after every fourth flash (i.e. after the 7th flash, the 11th flash, etc.). Thus, oxygen evolution occurs with a periodicity of four, which was modeled by Bessel Kok in his famous S-state cycle (Kok cycle, Scheme 1) [19]. In the original model of Kok [19] an electron was removed from the catalyst during each S-state transition from S_0 to S_4 state, which eventually leads to accumulation of four positive charges in the S_4 state. To explain the flash-induced oxygen evolution pattern, the S_1 state must be dark-stable and the $S_4 \rightarrow S_0$ transition should be light-independent. All other S-state transitions, $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4$, are induced by the photochemical oxidation of P_{680} , the reaction center chlorophyll of PSII. It took much longer time to discover that the water-oxidation catalyst is an Mn_4CaO_5 cluster where the metal centers are strongly interconnected by bridging oxygen atoms (see the discussion below). In this system of electronically coupled manganese ions, the accumulation of positive charges will hinder the removal of the next electron by a dramatic increase of the redox potential of the complex. Thus, excess accumulation of the positive charges has to be avoided by the release of protons and electrons in a strictly alternating way, as first suggested by Dau and co-workers [20,21]. Therefore, the extended S-state cycle assumes that four oxidizing equivalents, but not four charges, are accumulated sequentially at the CaMn_4O_5 cluster and an oxygen molecule is evolved in the final step (Scheme 1) [3,11,22]. The strict alternative removal of protons and electrons suggests the formation of additional intermediate states, and some of them were also experimentally confirmed [20,23–30].

The extended S-state cycle predicts nine different intermediate steps in the water-oxidation cycle [18,21,22,24,31,32]. Some of these intermediates were trapped experimentally in time-resolved X-ray and UV-Vis spectroscopy, photothermal beam deflection experiments or delayed chlorophyll fluorescence measurements [24,27,29,30,33–38]. The pK_a values for the proton removal during the individual S-state transitions were also determined experimentally by delayed fluorescence measurements, EPR and FTIR measurements [25,39–41]. The proposed sequence of events is in line with some of the most prominent computational models of the reaction cycle of photosynthetic water oxidation [27,42–50].

Although it is clear that the proton and electron removal steps from the WOC are related (so-called proton-coupled electron transfer, PCET) [51–53], the exact mechanism of the individual electron and proton removal steps is still unknown. Besides the sequential (consecutive) mechanism (ET/PT or PT/ET), also the role of concerted transfer of an electron and a proton (CEPT) is discussed in the literature. For example, Multiple Site-Electron Proton Transfer (MS-EPT) pathway involving the His190 residue (crucial for the $\text{Tyr}_Z \rightarrow P_{680}^+$ electron transfer) is suggested by Babcock and co-workers [54] where simultaneous transfer of an electron (to P_{680}^+) and proton (to His190) might take place, thus avoiding the formation of high-energy intermediate Tyr_ZOH^+ [55,56]. Whether or not the CEPT is part of the biological water oxidation mechanism, the concerted electron and proton removal is of interest when developing artificial catalysts for water oxidation [56,57].

3. Structure of the water-oxidizing complex

The role of Mn in oxygenic photosynthesis was first proposed by A. Pirson in 1937 [58]. Extensive studies in the subsequent decades showed that the WOC contained four Mn, one Ca and also one Cl^- ion, and various models were proposed for the structure of the Mn-Ca cluster [59–71]. Among these models, a dimer-of-dimers model consists of two Mn ‘dimers’ (each being a di- μ -oxo-bridged Mn_2 unit) has received extensive attention, and some hypotheses regarding the mechanism of water-splitting were proposed based on this model [59]. On the other hand, Britt et al.

Fig. 1. a: Important redox cofactors operating in the apparatus for natural photosynthesis, including type I and II reaction centers (simplified Z-scheme). The electron transfer steps are indicated with black arrows. P_{680} : reaction center pigment of PSII (chlorophyll *a*) that has an absorption peak at 680 nm; P_{700} : reaction center pigment of PSI (analogous to chlorophyll *a*) that has an absorption peak at 700 nm; P_{680}^* and P_{700}^* : the excited states of P_{680} and P_{700} ; Mn: water oxidation catalyst (Mn_4CaO_5 oxide-based cluster); Tyr: redox-active tyrosine D1-Tyr161 (Tyr_Z), part of the D1 protein of PSII; Pheo: pheophytin, the primary electron acceptor of PSII; Q_A : primary plastoquinone electron acceptor; Q_B : secondary plastoquinone electron acceptor; PQ: pool of plastoquinone molecules, exchangeable with Q_B ; FeS: Rieske iron-sulfur protein; Cyt f: cytochrome *f*; PC: plastocyanin; A_0 : primary electron acceptor of PSI (chlorophyll *a* molecule); A_1 : phylloquinone; F_X , F_A , F_B : three separate iron-sulfur centers; FD: ferredoxin; FNR: ferredoxin-nicotinamide adenine dinucleotide phosphate (NADP) reductase. The process illustrated by the Z-scheme is driven by the absorption of photons at PSII and PSI, resulting in the formation of the excited states P_{680}^* and P_{700}^* , respectively. P_{680}^* provides an electron to reduce pheophytin, a step that is followed by a step-wise electron transfer from pheophytin to P_{700}^* (the oxidizing species after the electron transfer from P_{700}^*). Following this initial electron transfer, P_{680}^* can oxidize Tyr_Z and subsequently the Mn_4CaO_5 water-oxidizing cluster. Four photons are needed to be absorbed by PSII to drive the oxidation of two molecules of water, the liberation of one molecule of oxygen and the supply of 4 electrons to the Z Scheme. Light absorption in PSI creates P_{700}^* , which provides an electron to reduce A_0 to FNR, and finally reduces NADP^+ , the major cofactor needed to reduce CO_2 in the Calvin-Benson Cycle. The image and caption are reprinted with permission from Ref. [12]. Copyright (2012) by Macmillan Publishing Group. b: Representation of the membrane-protein complexes involved in the photosynthetic light-energy conversion reactions, located in the thylakoid membranes of various oxygenic photosynthetic organisms. c: Structure of one monomer of Photosystem II multi-subunit protein-cofactor complex. The D1 protein subunit is shown in blue, D2 subunit is shown in green and the rest of the protein subunits are shown in grey color. The antennae chlorophyll molecules are shown with thin green lines and the porphyrin rings of the primary electron donor, P_{680} , are shown with thicker green lines (Mg in the porphyrin rings shown in purple). The other cofactors involved in the electron transfer are: Mn_4CaO_5 cluster (Mn shown as magenta spheres, Ca is green and O in red); redox-active Tyr residue of D1 protein (shown as red lines); the primary electron acceptor, the pheophytin porphyrin rings (orange lines); the primary plastoquinone acceptor, Q_A (one-electron acceptor, firmly bound to D2 subunit) and the secondary plastoquinone acceptor, Q_B (two electrons and two protons acceptor, bound to D1 subunit; fully reduced $Q_B\text{H}_2$, PQ, is released from the Q_B binding pocket and transfers the electrons to the next protein-cofactor complex in the photosynthetic electron-transfer chain, the cytochrome b_6/f complex). Quinone rings of both Q_A and Q_B are shown as purple lines, the hydrophobic chains are omitted. The electron transfer path within PSII is indicated with red arrows. The protons released by the oxidation of water molecules in the Mn_4CaO_5 cluster are transported towards inner, luminal, part of the thylakoid membrane; the protons for Q_B reduction are taken from the outer, stromal, part of the membrane. In this way, the electron transfer within PSII contributes to the formation of the transmembrane proton gradient, utilized for ATP synthesis. The β -carotene molecules also found in PSII are shown with thin yellow lines. Also, about 1400 water molecules per monomer are resolved within the PSII complex (now shown in the scheme). The approximate dimensions of PSII embedded in the membrane are also indicated. Image using data from Ref. 7. d: The first and second coordination environment of the Mn_4CaO_5 cluster, which in a very simplified view can be considered as a small nano-sized Mn-Ca oxide with dimensions of $\sim 0.5 \times 0.25 \times 0.25$ nm. Black lines indicate covalent bonds, and broken lines indicate hydrogen-bonds between the Mn_4CaO_5 cluster and its ligands. e: The radiation-damage-free structure of the Mn_4CaO_5 cluster in PSII from *Thermosynechococcus vulcanus* in the S_1 state at a resolution of 1.95 Å determined with the use of femtosecond X-ray pulses of the SPring-8 free-electron laser.

Scheme 1. Extended reaction cycle of photosynthetic water oxidation (outer cycle) compared to the classical Kok model (inner cycle). The extended cycle includes not only the electron transfer from Mn₄CaO₅ complex to Y₂, but also the proton removal from the complex or its ligand environment. The subscripts indicate the number of oxidation equivalents accumulated at the Mn-complex and the superscripts indicate the charge relative to the dark-stable S₁ state (+, positive; n, neutral). The indicated pK values were determined. The image and caption are reprinted with permission from ref. [27] Copyright (2011) by Springer.

found that the 'dimer-of-dimers' model is not consistent with the data obtained from electron paramagnetic resonance (EPR) studies [71]. Instead, they proposed a trimer-monomer model, where three Mn ions are connected by di-μ-oxo bridges so that they form a strongly exchange-coupled Mn₃ core, and the fourth Mn ion (the 'dangler' Mn) is connected to the trinuclear core with a mono-μ-oxo bridge [71].

To reveal the structure of the catalytic core for water oxidation, it is essential to solve the crystal structure of PSII. Horst Witt and Wolfram Saenger first reported the structure of PSII isolated from a cyanobacterium *Thermosynechococcus elongatus* at a relatively low resolution of 3.8 Å [72]. This structure assigned the major protein subunits within PSII and most of the electron transfer cofactors; however, its resolution was not enough to reveal the geometric organization of the Mn₄CaO₅-cluster. Subsequently, Kamiya and Shen reported the structural analysis of *Thermosynechococcus vulcanus* (*T. vulcanus*) PSII [73], which assigned some additional protein subunits not resolved previously. The first evidence for a cubane structure of the Mn₄Ca cluster was reported by James Barber and So Iwata in 2004 [74], who analyzed the structure of PSII at 3.5 Å resolution and showed a Mn₃Ca-cubane with a 4th Mn atom attached at a more distant position. In spite of the progress in solving the structure of PSII, the detailed organization of the WOC was still not clear owing to the limited resolution achieved, which was not enough to separate the individual atoms within the metal cluster. In particular, the bridging oxygen atoms were not identified from the electron density maps due to the

much weaker densities of oxygen atoms than those of the nearby Mn ions, and practically no ligating water molecules to the metal cluster were visible. There were also ambiguities regarding the patterns and distances of amino acid ligands coordinated to the metal cluster that is highly sensitive to radiation damage. In 2011, Jian-Ren Shen and Nobuo Kamiya reported the high-resolution structure of PSII crystals from the thermophilic cyanobacterium *T. vulcanus* at 1.9 Å (Fig. 1c–e) [7]. This atomic-resolution data enabled them to unambiguously assign the positions of each of the metal ions, oxo-bridged oxygen atoms, amino acid, and water ligands, as well as some water molecules that form extended hydrogen-bonding networks around the cluster [7,75].

To avoid the possible radiation damage in the structural analysis, Shen and his colleagues further employed femtosecond X-ray free electron lasers (XFEL) to analyze the high-resolution, radiation damage-free structure of PSII in the dark-stable S₁-state [10]. The results showed that the Mn₄CaO₅ cluster adopts a distorted-chair structure, with Mn₃CaO₄ organized in a cubic core and a 4th Mn attached to the core via two oxo-bridges. The distortion in the shape of the cluster suggests its structural instability, or flexibility [3,4,8–11,76], an important structural feature that may allow the cluster to undergo structural rearrangements during the S-state cycle, which is a prerequisite for its catalytic activity [3]. Recent investigations of PSII with XFEL using an elegant technique of serial femtosecond X-ray crystallography allowed identification of the structural changes in the Mn₄CaO₅ cluster during the S-state transitions (see below) [76,77].

In the structure of the WOC, there are also four terminal water ligands (W1–W4) and seven amino acid ligands (Fig. 1d, e) [7,10]. Among these four water molecules, two (W1, W2) are coordinated to the dangling Mn (Mn4) and the other two (W3, W4) are coordinated to Ca. Thus, the chemical formula of the cluster is more precisely described as $\text{Mn}_4\text{CaO}_5(\text{H}_2\text{O})_4$. Among the seven amino acid ligands, six are carboxylate groups and one is an imidazole group [3,8,10,75]; these groups are stable under oxidizing conditions. Among the carboxylate groups, one is monodentately coordinated, while all of the other carboxylate ligands are bidentately coordinated [7,10]. Shen et al. also analyzed the structure of PSII with Ca replaced by Sr biosynthetically from *T. vulcanus* at a resolution of 2.1 Å [79]. The structure showed that the Ca was indeed replaced by Sr, however, the position of Sr was moved toward the outside of the Mn_3SrO_4 cubane, causing an elongation of the bond distances between Sr and its surrounding atoms due to the larger ionic radius of Sr compared to Ca. This may be the reason for the lowered water-splitting activity of the cluster compared to the native, Ca-containing cluster. They found also an apparent elongation of the Sr–W3 bond, whereas the Sr–W4 distance was not much changed. This result suggests a weaker binding and a mobile nature of W3, which could imply either direct or indirect involvement of this water molecule in the O=O bond formation [79,80].

Dismukes et al. analyzed the nature of the bridging oxygen atoms based on the crystal structure [11], and they emphasized that tetrahedral geometry imposes an sp^3 hybridization of the oxygen orbitals for the bridging O atom, with no π bonding to the Mn ion, yielding longer and weaker Mn–O bonds. This bonding is less directional and more flexible than an $\text{sp}^2 + \text{p}_z$ (multiple) bonding as required for $\text{Mn}_3(\mu_3\text{-O})$ in planar geometry [11]. Dismukes et al. suggested that prevention of planarization is important for the water-oxidation activity and Ca(II) helps to prevent such planarization and provides additional flexibility to the cubane structure which is needed for the O–O bond formation [11]. In addition to its structural role, it was suggested that Ca is involved in the regulation of the redox-properties of the Mn ions, as it was shown that coordination of Ca ions to the bridging oxygen atoms in structurally closely related Mn(Ca) complexes could tune the redox potentials of the Mn ions [80,81].

Among the $\mu\text{-O}$ atoms, the position of O5 is unique and interesting from the mechanistic point of view. O5 connects Mn1 in the cubane with Mn4 located outside of the cubane with unusually long bonds of 2.2–2.7 Å as compared to the typical Mn–O bond length of 1.8–2.1 Å found for other oxo-bridged Mn complexes. O5 is also connected to Ca and Mn3 within the cubane. However, as analyzed by Dismukes, the arrangement of the O5 coordination sphere can be considered as an incomplete octahedron missing two coordination sites *trans* to the vectors Mn3O5 and Ca–O5, indicating that the O5 orbitals are unhybridized and comprise valence $\text{s}+3\text{p}$ atomic orbitals [11]. The space *trans* to Mn3–O5 and Ca–O5 has no closely-positioned atoms. The closest group to Mn3–O5 is a methyl group 3.68 Å away (D1–Val185), and in the closest position to CaO5 is a Cl^- that is 6.89 Å away. Quantum mechanical (QM)/molecular mechanical (MM) calculations based on the crystal structure suggested that O5 is a hydroxide ion (OH^-), rather than an oxo ligand (O^{2-}) [82]. However, models, where O5 is deprotonated are also discussed in the literature [83]. Starting from the unique position of O5 in the S_1 -state, theoretical calculations have suggested that O5 may adopt two alternative positions in the S_2 state, namely, one with the O5 close to Mn4 (open-cubane, right-side open structure) and the other one with the O5 close to Mn1 (close-cubane, left-side open) [81,84]. These two structures have been suggested to correspond to two interconvertible structures represented by two EPR signals, one is a multiline signal, and the other one is the $g = 4.1$ signal, observable in the S_2 -state at low temperature [84,85].

The unique position of O5 and its unusually long distances to Mn1 and Mn4 suggested the possibility of O5 as one of the substrate O atoms in the O–O bond formation. This means that the area involving O5 in the Mn_4CaO_5 -cluster forms the reaction site for the O–O bond formation [3,9]. This hypothesis is in agreement with the extensive theoretical calculations about the possible mechanism of water oxidation performed by Per Siegbahn [44]. However, the model is based on the S_1 -state structure only, and there are several possibilities even if O5 provides indeed one of the substrate O atoms [3,9]. Thus, analyses of the intermediate S-state structures are necessary to fully understand the mechanism of the O–O bond formation. This has been made possible by the use of the ultra-short, extremely strong X-ray pulses of XFEL that became available recently [76,77].

As mentioned above, Shen and co-workers have utilized the XFEL pulses to solve the radiation damage-free structure of PSII at the S_1 -state at a high resolution using large PSII crystals at a cryogenic temperature [10]. In doing so, they used a “serial femtosecond fixed-target rotational crystallography (SF-ROX) method” [86] in which the large crystals are fixed at low temperature while irradiated with each XFEL pulse, followed by a small rotation and movement of the crystal to provide a fresh point for the next XFEL pulse irradiation. This approach allowed to collect high-resolution data from large PSII crystals. However, using this approach it is not possible to solve the intermediate S-state structure since the crystals used are too large for the penetration of the exciting light and the intensity of the light flashes that could be used to illuminate the crystals is not sufficient to reliably generate the intermediate S-states. Also, since the XFEL pulses are so strong that every pulse will destroy the irradiation point of the crystals, each diffraction image has to be collected from a fresh area on the crystals, whereas the intermediate S-states cannot be generated separately on one single crystal. That means that new crystal has to be used for every XFEL pulse irradiation; thus, a huge number of crystals would be needed to collect a full dataset. Alternatively, a new method has been developed to collect the diffraction data with the femtosecond XFEL pulses, which is called “serial femtosecond crystallography (SFX)” [87]. In this method, micro-crystals are ejected into a liquid jet flow through and XFEL pulses are synchronized with the flow of the liquid jet to irradiate the micro-crystals and yield the diffraction image. As the small PSII crystals will be destroyed during irradiation, there is no possibility that two XFEL pulses will hit the same crystal. The obtained diffraction images are random in terms of the orientation of the crystals, and new software has been developed to process such kind of image data [33,88,89]. Early studies aimed to solve the structures of the S-state intermediates yielded low-resolution data [90–92], which were not enough to discern the small structural changes occurring during the S-state transitions, but the recent data were collected with high resolution (2.04–2.08 Å for the S-state intermediates) [76,77] represent a major breakthrough in the understanding of the structural changes of Mn_4CaO_5 cluster during the S-state cycle.

4. Metal ions in the biological water-oxidizing complex

In this section, we discuss details of the coordination chemistry of Mn and Ca ions [94], and the groups near the metal ions (Fig. 1d, e). The coordination number for all of the 4 Mn ions in the $\text{Mn}_4\text{-CaO}_5$ cluster is 6 in the S_1 state (with Mn1 being a special case), which is usual for Mn(III) or Mn(IV) ions. In the case of Mn1 atom, the Mn1–O5 distance is longer (~ 2.70 Å) than the other Mn–O/N bonds (mean of ~ 1.95 Å), so that Mn1 in effect can be seen as 5-coordinated, forming open cubane structure of the Mn_4CaO_5 cluster [3,10].

In literature there are two main hypotheses about the oxidation state of Mn ions in the S_1 state, based on different reaction mechanisms: (i) high-valent scheme, where two of the Mn ions in S_1 state are in oxidation state III, and two are in oxidation state IV [3,10]; and (ii) low-valent scheme, where four Mn atoms are in III, or one II, one III and two IV in S_1 state [95,96].

Although a lot of debates took place concerning this over the last decade, the experimental evidence from conventional and advanced EPR and EXAFS studies favor the high-valent scheme [97] (see [98] for a review). This is supported by a large number of experimental data [30,99,100], as well as the recent XFEL high-resolution structure [10], and today is largely accepted to be valid. The oxidation states of the individual Mn ions in the dark-adapted S_1 state are discussed below.

4.1. Mn1

Two μ_3 -O atoms were observed as hard ligands for this Mn that connect it to Ca, Mn2 and Mn3 (longer 2.7 Å distance between Mn1 and O5 forms the open coordination site on the cubane structure). One monodentate carboxylate (D1-E189), one bridging carboxylate (D1-D341, bridging between Mn1 and Mn2), and one imidazole group (from D1-H332) were also found in the first coordination sphere (Fig. 1d); these ligands could stabilize the oxidation number of III or IV for this Mn1 ion. In the S_1 state, the oxidation state for the Mn1 ion is believed to be III based on the presence of a Jahn-Teller axis suggested from the high-resolution, damage-free crystal structure [10]. In the recent XFEL study of the Berkeley group this ion is identified as 5-coordinated due to the 2.7 Å distance to O5 [76]. In general, Mn(II), Mn(III), and Mn(IV) are hard Lewis acids, and usually, as we observe in the Mn-Ca cluster, they prefer oxygen donor ligands, such as O^{2-} , OH^- and COO^- . Only one imidazole group from histidine-332 is found in the Mn-Ca cluster, and this ligand is coordinated to Mn1, in *trans* position to the open coordination site (the longer Mn1-O5 distance).

4.2. Mn2

The six ligands in the coordination sphere of this ion are three μ_3 -O (bridging to Mn1, Mn3 and Ca) and three bridging COO^- ligands, bridging to Mn1 (D1-D341), Mn3 (CP43-E354) and Ca (D1-A344) (Fig. 1d). As in the case of Mn1, these ligands could stabilize both the oxidation state of III or IV. Based on the Mn-O distances, the new XFEL study indicated that the oxidation state for this Mn ion is IV [10].

4.3. Mn3

The six ligands around this ion are three μ_3 -O (bridging to Mn1, Mn2 and Ca), one μ_2 -O (bridging to Mn4), and two bridging COO^- ligands, connecting it to Mn2 (CP43-E354) and Mn4 (D1-E333). Four hard $\mu_{2/3}$ -O ligands will stabilize Mn(IV), which agrees with the new XFEL study [10].

4.4. Mn4

One μ_3 -O (O5 atom, connected to Ca and Mn3; there is a long 2.7 Å distance between O5 and Mn1, forming open coordination site in the cubane), one μ_2 -O (bridging to Mn3), two bridging COO^- (D1-D170 to Ca and D1-E333 to Mn3) and two H_2O ligands are coordinated to this Mn ion (Fig. 1d) [7,10]. One of the two water molecules is a candidate for the substrate for dioxygen formation [3,11]. These ligands likely stabilize Mn(III). However, deprotonation of the water ligands could stabilize higher oxidation state for Mn, such as Mn(IV) (Eq. (4)):

Most likely, in S_1 state Mn4 is in oxidation state III. In higher S -states, when it is oxidized to IV state, deprotonation of one of the terminal water molecules is likely to occur.

The new XFEL study also suggested that the oxidation state for Mn4 is III in the S_1 state [10]. However, the formal oxidation state may be insufficient for a complete description of the Mn ions in the Mn-Ca oxide-based cluster, since electrons may be delocalized by direct ligands or other groups around the site. The delocalization of the charge between the metal atom and the ligand molecules is suggested to be important for the understanding of the mechanism of water oxidation [98].

4.5. Ca

Ca has a structural role in some biological systems, and also has a unique role as a component of the WOC in the PSII. No other cation can replace Ca in the WOC and retain the functional activity except strontium(II), which can functionally substitute Ca in the water-oxidizing complex but with lower catalytic activity [30,45,78,101–104]. The coordination number of the Ca ion varies from 6 to 10 in different compounds. According to the crystal structure of Umena et al. [7] in the WOC, Ca is surrounded by seven ligands: three μ_3 -O (connecting it to Mn1, Mn2 and Mn3 and Mn4), as well as two bridging COO^- groups (D1-A344 to Mn2 and D1-D170 to Mn4), and two H_2O molecules (W3, W4) (Fig. 1d). According to the XFEL structure of Kern et al. [76], Ca is 8-coordinated, as it is also ligated to D1-E189. One of the water molecules bound to Ca may serve as the substrate for water oxidation [7,10,78]. Among the two water molecules, W4 is directly connected to the redox active tyrosine residue, Y_Z , by hydrogen bonds and W3 is H-bonded to Y_Z through another water molecule (Fig. 2a) [7,10]. W3 is further H-bonded to W1 and W2, which together with the neighboring amino acid residues and with other water molecules form an H-bonding network around the cluster [3,10,105,106]. Thus, at least one important role of the Ca ion is to maintain and regulate the H-bonding network, connecting the water molecules and Y_Z ; this also implies that Ca is important in maintaining the position and orientation of the Y_Z moiety. Recently, Barry's group reported spectroscopic evidence that calcium ion interacts with Y_Z in its radical form and singlet states. Multiple conformational states of the Y_Z radical/singlet are also identified [107].

5. The second coordination sphere around the Mn-Ca cluster

Nature has used side chains of a few more amino acid residues to stabilize the Mn-Ca cluster by filling in its second coordination sphere. For example, the nitrogen atom of D1-His337 is hydrogen-bonded to O3, one of the μ -O atoms (Fig. 1d). CP43-Arg357 is hydrogen-bonded to both O2 and O4 of the Mn-Ca cluster, the carboxylate oxygen atom of the D1-Asp170, and to that of the D1-Ala344 residue (Fig. 1d). In this way, CP43-Arg357 may stabilize the structure of the WOC by compensating the negative charges induced by the oxo bridges and carboxylate ligands [7,10]. It was suggested that CP43-Arg357 is the primary base that is deprotonated during the $S_3 \rightarrow S_0$ transition [32,11].

Near CP43-Arg357 a putative proton exit pathway, which begins with D1-Asp61, is placed. Mutation in this amino acid results in strong retardation of the electron transfer from the Mn_4CaO_5 cluster of Y_Z and severe delay of the O_2 release step [35,108].

Another important amino acid, D1-Val185, is located close to O5 and occupies a location contacting the water molecules between Y_Z and D1-Asp61. Mutations in this amino acid also retard dramatically the O_2 evolution, most likely due to the disturbance of

Fig. 2. a: Hydrogen-bond connections between the Mn_4CaO_5 -cluster and Y_Z . b: Redox coactors in PSII based on the crystal structure⁷ with distances between them are indicated in Å.

the hydrogen bonding network around the Mn_4CaO_5 cluster and the environment of the nearby chloride co-factor of PSII [109].

5.1. Cl^-

Cl^- depletion suppresses O_2 evolution [110–113] and the concentration of this anion is regulated effectively under a variety of conditions in chloroplasts [9,113]. Two chloride ions in the structure of the WOC were identified [7,10,114,115]. Both Cl^- ions are surrounded by two water molecules and two amino acid residues. One of the roles proposed earlier for Cl^- was its involvement in the ligation of the Mn ions [97,112,116]. However, the results of the high-resolution structural analysis do not support this hypothesis [7,10,115]. Other roles suggested for the Cl^- ions include regulation of the redox potentials of the cluster [117], hydrogen-bonding [118], and activation of the substrate water [45,46]. Based

on the crystal structure, the two Cl^- ions appeared to be required for stabilizing the Mn-Ca cluster, since both ions are coordinated to the main chain nitrogen atom of a Glu residue, whose carboxylate group is coordinated to the Mn-Ca cluster [7,10,115]. It can be imagined that loss of the Cl^- ions would lead to the destabilization of the Glu residues, which in turn would destabilize the Mn-Ca cluster. The Cl^- -1 binding site is also located in the entrance of an H-bonding network involving D1-Asp61, and theoretical calculations showed that in the absence of this Cl^- , a salt bridge between D2-Lys317 and D1-Asp61 is induced that would hinder the transfer of protons to the lumen [119]. Also, in PSII active centers are inhibited by competitive binding of the herbicide terbutryn to the Q_B binding site in the D1 protein, instead of a single Cl^- binding site, two binding sites with partial occupancy separated by the side chain of D2-Lys317 were found. Possible movement of the Cl^- between these two positions has been suggested and

the results were discussed in the context of proton transfer to the lumen [120].

5.2. Tyrosine 161 (Y_Z)

Upon absorption of photons by the RC of PSII, primary charge separation occurs, resulting in the formation of oxidized P_{680} (P_{680}^+) and reduced pheophytin ($Pheo^-$) [121]. P_{680}^+ is subsequently reduced by electron transfer from a tyrosine (Tyrosine 161 of the D1 subunit) residue (Y_Z , Fig. 2) to form a tyrosine radical ($Y_Z^{\cdot+}$) [122]. Electrons for the reduction of $Y_Z^{\cdot+}$ are donated from the Mn_4CaO_5 cluster. As described above, the Y_Z residue forms a strong hydrogen bond, involving one water molecule (W4) coordinated to Ca, and is located in the direct neighborhood of Ca, rather than Mn ions. Another strong hydrogen bond is observed between Y_Z and the ϵ -nitrogen atom of a histidine residue (D1-His190), which causes rapid movement of protons and electrons in this local system [3,7,8,122,123]. A hydrogen-bonding network involving this Tyr-His pair was observed in the crystal structure and was suggested to function as a proton extractor [3,7,8,10,82]. Recently, Noguchi's group using Fourier transform infrared spectroscopy and theoretical calculations suggested that the water molecules participating in H-bonding network connecting the Tyr-His pair to the surface of the PSII complex play an essential role in the water-oxidation mechanism, specifically in a concerted process of proton transfer and water insertion during the $S_2 \rightarrow S_3$ transition [106,124,125].

5.3. Channels

Since the nano-sized Mn-Ca cluster is embedded within the PSII protein matrix and is shielded by a large protein barrier from the bulk solution, it is important for it to have channels connecting the catalyst with the outside solution to ensure the efficient access of substrate water molecules to the catalytic site, and the exit of products protons and oxygen. Indeed, such channels have been found in the crystal structures [3,7,10,82,106,126–128], and as mentioned above, multiple H-bonding networks have been found starting from the catalyst and ending in the surface of the protein complex [3,7,10,127,128].

6. Mechanism of biological water oxidation

Despite all the detailed information about the structure of PSII protein complex, important steps in the 4-electron/4-proton chemistry of water oxidation remain still unclear. We will focus our discussion on the now accepted high-valent scheme (one Mn(IV) and three Mn(III) ions in S_0 state) and discuss the possible mechanism of water-oxidation reaction. This scheme predicts that on each S-state transition from S_0 to S_3 state one Mn(III) ion gets oxidized to Mn(IV), which leads to the important conclusion that before the S_4 state is formed, there is no partial oxidation of a substrate water molecule [22]. During the $S_3 \rightarrow S_4$ transition both Mn-centered and ligand-centered (substrate) oxidation have been discussed in the literature [129].

A significant step towards the elucidation of the mechanism of biological water oxidation was the proposal of the extended S-state cycle of water oxidation, which besides the Mn-cluster oxidation by Y_Z also includes a strict alternation of electron transfer steps with proton removal reactions (Scheme 1). In the strongly electronically coupled Mn ions in the Mn_4CaO_5 cluster, proton removal steps are needed to prevent the oxidation-induced increase of the redox potential of the cluster [20,24,25].

According to this now largely accepted framework of events, the O—O bond formation occurs upon the $S_4 \rightarrow S_0$ transition

(Scheme 1), although the binding of the two H_2O molecules likely occurs earlier at different S states. In H_2O^{16}/H_2O^{18} exchange experiments water exchange rates were determined, revealing two substrate water molecules characterized with different exchange rates [130–133]. Experimental evidence [59,113,134,135], as well as quantum-chemical calculations [26,44–50], suggest that one of the substrate water molecules binds in the S_2 state, causing major rearrangements in the Mn_4CaO_5 cluster. Theoretical models suggested water binding to the cluster in the $S_2 \rightarrow S_3$ transition by a carousel or pivot rearrangement of water ligands around Mn4 [48,136]. The models propose high-spin closed cubane structure (see below) and involve binding of water molecule between Mn4 and O5. The present XFEL structural data also indicate binding of one water molecule during the $S_2 \rightarrow S_3$ state transition [76,78,137] but there are no indications supporting the pivot or carousel mechanism [76]. These hypotheses were also ruled out by the recent model of Per Siegbahn [138]. The other substrate water may bind in the S_4 state, presumably after O_2 release and might be incorporated into the oxo-bridge framework of the Mn_4CaO_5 complex (e.g., replacing O5) upon the formation of the S_0 state. There are experimental evidence that one water molecule (most likely the slowly exchanged water) is presumably bound to Ca and at least one substrate water is partially protonated up to S_3 state [139–141].

Different mechanisms of O—O bond formation in biological water oxidation have been discussed in the literature. The two main hypotheses are (i) nucleophilic attack between water molecule bound to Ca (or Mn4) and adjacent water substrate oxygen atom (terminal or bridging ligand most likely of Mn4) [3,49,142–145] and (ii) oxo/oxyl radical coupling mechanism between the bridging O5 and an oxyl radical [3,50,54,77,137,146,147].

Direct evidence for the O—O bond formation step can come from crystallographic studies of PSII. Various experimental techniques indicated that there are no major structural changes in the Mn_4CaO_5 cluster during the $S_1 \rightarrow S_2$ transition while there are major structural rearrangements occur during $S_2 \rightarrow S_3$ transition [23,140]. These findings focused the efforts of the experimentalists towards solving of the crystal structure of the Mn_4CaO_5 cluster in its S_3 state, induced by providing of two consecutive saturating laser flashes to dark-adapted samples, predominantly in the S_1 state. The new experiments conducted by Shen's group on the S_3 state with the aid of femtosecond X-ray free electron lasers (XFEL) indicated the following changes as compared to S_1 state [77,137]:

- (i) Mn4 moves toward the outside of the cubane
- (ii) Ca moves away from Mn4
- (iii) A new oxygen atom O6 could be added near O5
- (iv) Glu189 of D1 moves away from the cubane
- (v) Movements of some of the water molecules in the second coordination sphere were detected
- (vi) some of the amino acids of D1 protein in close protein environment of Mn_4CaO_5 cluster (Asp61, Asp170, His332, and Ala344) exhibit slight structural changes

Based on these results, the research group proposed an oxygen evolution mechanism, which is depicted in Fig. 3.

In an earlier study of the Berkeley group with XFEL insertion of additional water or hydroxo ligand to the Mn_4CaO_5 cluster in the S_3 state was not found [93]. In a recent study however the authors combined serial femtosecond X-ray crystallography and X-ray emission spectroscopy and succeeded in solving the structures of all semi-stable intermediates (S-states) from the Kok cycle at high resolution (2.04–2.08 Å) [76]. The structure of Mn_4CaO_5 cluster in S_2 state remains fundamentally unchanged, with small shifts of Ca, Mn3 and Mn2 as well as D1-Asp170 and CP43-Glu354. The important finding is that according to these new data, in S_3 state

Fig. 3. A possible mechanism of O=O bond formation and proton transfer pathways from the WOC according to the model proposed in Suga et al. [86]. The dotted circle indicates the displacement of the water molecule (W665) next to the water (W567) H bonded to O4, which results in the approach of W567 to O4. Dashed arrows indicate possible pathways for proton transfer. The two pairs of oxygen atoms enclosed by dashed red lines represent the possible sites of O=O bond formation, with the one between O5 and O6 having a much shorter distance and therefore representing the more likely site of O=O bond formation. The image and caption are reprinted with permission from Ref. [86]. Copyright (2017) by Macmillan Publishing Group.

an insertion of O atom (oxo or hydroxo, labeled Ox) is identified near O5. The authors suggested that the Ox site occupied during $S_2 \rightarrow S_3$ transition is filled by W3 water molecule bound to Ca in S_1 and S_2 state. The following changes in S_3 state were also found:

- Mn1-Mn4 and Mn1-Mn3 distances are elongated by about 0.2 and 0.07 Å, respectively, relative to the S_2 structure
- The newly inserted Ox located about 1.8 Å from Mn1, occupies its sixth coordination site, resulting in a transition from 5-coordinated Mn1 to 6-coordinated Mn1 as proposed earlier based on EXAFS data [9,24,50]
- The newly inserted Ox besides Mn1 (1.8 Å distance) is also bound to Ca (2.50 Å). D1-E189 ligand (according to this model bridging Ca and Mn1 in S_1 and S_2 state) moves away from Ca, making space for Ox and thus preserving the coordination number of 8 for the Ca ion according to this model
- Additional changes in the positions of nearby residues (His332, Glu333, His337, Asp342, Ala344, Asp170 of D1, and Glu354, Arg357 of CP43) and substantial changes in the H-bonding network formed between the amino acids and water molecules surrounding the Mn_4CaO_5 cluster are observed as well.

It is not clear if Ox in the model of Kern et al. [76] and O6 in the model of Shen [77] represent the oxygen atom from the same newly inserted substrate water molecule. The interpretation of the results in terms of the reaction mechanism is different. According to Suga et al. [77] the newly inserted O6 is only 1.5 Å away from O5, but this distance is modified to 1.9 Å in a more recent study [137]. In the model of Kern et al. [76] the newly inserted Ox is at a distance 2.1 Å to O5 (location of Ox 0.9–1.0 Å away from O6), thus excluding the formation of peroxo-bond during the $S_2 \rightarrow S_3$ transition and suggests that the O-O bond may be formed between Ox and O5 during the $S_3 \rightarrow S_0$ transition (case 1 in Fig. 4). Alternatively, Ox can replace O5 during O_2 formation or release, with O-O bond formed in this scenario between O5 and one of the terminal water molecules bound to Mn4 (W2, case 2 in Fig. 4) or Ca (W3, case 3 in Fig. 4) [76]. Also, in the study of the Berkeley group, the structures of all S-states, and the structures of two transient states at 150 and 400 μs after the second laser flash were revealed to understand better the changes occurring during the $S_2 \rightarrow S_3$ transition. The structure of these two intermediates indicates that the elongation of the Mn1-Mn4 bond by 0.2 Å is already visible 150 μs after the second laser flash and it is followed by Ox binding to the Mn_4CaO_5 cluster visible in the structure 400 μs after the laser flash.

Fig. 4. Schematic structures of the S states in the Kok cycle of PSII and proposed reaction sequences for O-O bond formation in the S_4 state according to Kern et al. [76]. The likely position of Mn oxidation states (Mn(III) is depicted in orange, Mn(IV) in purple) as well as protonation and deprotonation reactions are indicated for each S state; the proposed steps in the $S_2 \rightarrow S_3$ transition, including O_x insertion, are indicated in the dashed box with blue dashed arrows signifying atom movements. Three likely options (1, 2 and 3) for the final $S_3 \rightarrow S_0$ transition are given in the bottom part, including possible order of 1) electron and proton release; 2) O-O bond formation and O_2 release, and 3) refilling of the empty substrate site. Images and captions: The image and caption are reprinted with permission from Ref. [76]. Copyright (2018) by Macmillan Publishing Group.

According to the extended S-cycle (Scheme 1) the $S_2 \rightarrow S_3$ transition involves deprotonation step followed by an electron transfer to Y_Z [19,30,148]. Kern et al. [76] hypothesize that the deprotonation step triggers the shift of Mn1 and Mn4. For example, if the proton is removed from W1, this can be coupled to proton transfer from W3 to O5 which will weaken the Mn1-O5-Mn4 interaction resulting in elongation of the Mn1-Mn4 distance [83,149] (see Fig. 1d and Fig. 4). The subsequent insertion of O_x close to Mn1 is coupled with the oxidation of Mn1 and to a proton transfer from O5 to W1 (Fig. 4).

The formation of Mn1- O_x -Ca bridge suggests that the Mn_4CaO_5 cluster does not form a stable closed cubane in any of the S-states. Instead, dynamic structural changes are observed thus likely excluding the rather static model of the nucleophilic attack of terminal water molecules.

In 2019, Suga, Ago and Shen's groups [137] collected improved resolution dataset allowing to identify the position of the newly inserted O atom by altering the O5-O6 distance and examining the residual densities in the $F_{obs}-F_{calc}$ difference Fourier map. The results suggested an O5-O6 distance of 1.9 Å (longer than the one proposed in 2017 [77] and closer to the 2.1 Å distance determined by the Berkeley group [76]). This distance excludes the formation of superoxo, peroxo, and hydroxo/oxo species and points towards

a formation of oxyl/oxo species. This recent study [137] indicated that the flipping of the Glu189 side chain is related to the movements in a short loop of CP43, including CP43 Val410, that restricts the size of the O1 channel. In the S_3 state, paired positive and negative densities around CP43 Val410 suggests a movement of this residue toward Glu189 by 0.5 Å. This change widens the channel, which may induce binding of water molecules to the WOC during the $S_2 \rightarrow S_3$ -state transition and coincides with the incorporation of O6 into the WOC during the S_2 to S_3 transition by formation of an open cubane Mn_4CaO_6 cluster with an oxyl/oxo bridge. The results show that Glu189 has an important role in coupling oxidation of the WOC with the opening of the water channel and delivery of the substrate into the WOC.

An additional complication in elucidating the mechanism of biological water oxidation is the possible presence of structural isomers of the Mn_4CaO_5 cluster in some of its S-states [9,81,84,121,150]. Spin isomers were observed by EPR in the S_2 state at cryogenic temperatures [151,152]. The isomers have often been suggested to differ structurally, e.g., open cubane (or right-open cubane, where Mn1 is 5-coordinated with long distance Mn1-O5, compatible with low-spin structure) vs closed cubane (or left-open high-spin cubane with no undercoordinated Mn site but dangling Mn4 connected only via mono- μ -oxo bridge to Mn3)

[81,84]. It was suggested that only one form may proceed towards S_3 state and/or structural change from low-spin to high-spin S_2 state is required to proceed to S_3 state [9,84,153]. As opposite to cryogenic temperatures, different isomers under physiological conditions were not experimentally confirmed so far [154]. In the recent crystallography experiment at room temperature (pH 6.5) [76,137], the dominant form of the cluster in the S_2 state is similar to the low spin isomer (right-open cubane) and the high spin configuration of the S_2 state at this pH was not observed.

The results above suggest that to elucidate the mechanism of water-oxidation information about the electronic structure of the WOC would be beneficial but such information cannot be provided solely by crystallographic data. To get details about the electronic structure of the Mn_4CaO_5 complex in its different S-states, often X-ray emission spectroscopy (Mn K_{β} emission spectra) are recorded separately [100,155–157] or simultaneously with the X-ray diffraction data [90,92,158,159]. Based on the analysis of Mn β emission spectra Pushkar and coworkers [155] suggested that O–O bond formation occurs before the transfer of the fourth electron from the Mn_4CaO_5 cluster to the Y_Z and developed a model in line with a recent X-ray crystallographic study of S_3 state proposed by Shen [77]. Recently the Magnetic Circular Dichroism, as a complementary method to the EPR analysis [139,151] has also been introduced as a probe of the electronic structure of the Mn_4CaO_5 cluster [160]. With this technique, both dynamic electronic structure and magnetic coupling between Mn ions can be studied in the WOC. However, at present, the modeling/computational parts are still under development and further studies will be needed.

In summary, a lot of information related to the mechanism of biological water oxidation is currently available. It refers to the sequence of the deprotonation and oxidation events, the role of the near-by Tyr (Y_Z) as redox co-factor, and the structure of the Mn_4CaO_5 complex in the different S-states. The role of the protein side chains surrounding the cluster is also investigated and water binding sites have been proposed but not yet unambiguously proven. However the information presently available is still insufficient to construct a full picture of the process of water oxidation with all mechanistic details. A large amount of the information available is not confirmed by independent methods. The origin of protons (and electrons) removed from the cluster at the different steps remains unclear. The quickly increasing amount of data, however, brings closer the complete understanding of biological water oxidation. Based on the available knowledge, the surprising similarity was recently pointed out by James Barber between the Mn_4CaO_5 cluster in PSII and Fe_4NiS_5 cluster in enzyme carbon monoxide dehydrogenase (CODH) found in anaerobic prokaryotic organisms that use carbon monoxide as an energy source to split water [161]. The structural similarity between these clusters, both catalyzing the production of reducing equivalents from water molecules and found in organisms with no direct evolutionary link known so far, suggests that other structural models can potentially mimic the functionality of the biological water-oxidizing cluster.

7. Implications for the synthetic MnO_x catalysts

The core water-oxidizing cluster in PSII is the oxide-like Mn_4CaO_5 cluster but it is the protein environment with the specific amino acid ligands, protein channels, and hydrogen-bonding network that ensure the flexibility of the Mn_4CaO_5 cluster and its high efficiency. The individual steps in the reaction cycle are triggered by complex short- and long-range electrostatic interactions that are practically impossible to be directly copied in the synthetic catalysts for water oxidation. That is why the efforts in the development of artificial catalysts are focused at mimicking of basic features of the biological catalyst while maintaining lower price

and higher stability, thus reducing the performance in comparison to the biological catalyst.

A basic requirement to the artificial catalysts is to oxidize water at low overpotentials. This is also needed to avoid the possible degradation of the catalyst at high oxidizing potentials. A huge number of manganese oxide catalysts for water oxidation were synthesized in the late decade [66]. Most of them could be seen as “biomimetic rocks” [162] as they are robust and purely inorganic materials that mimic some of the key features of the atomic arrangement of the biological catalyst. As a common feature, they contain extensive di- μ -oxo bridging between the Mn ions, which seems to be required for the catalytic activity [117,163,164]. To allow for structural rearrangements of the metal ions during the reaction cycle, similar flexibility as found in PSII Mn_4CaO_5 cluster should be allowed in the oxide structure and it is likely achieved by ensuring of the catalyst, where also mono- μ -oxo bridges between Mn ions can be found. Recently, using quasi *in situ* X-ray absorption spectroscopy, it has been shown that an amorphous synthetic Mn hydroxide catalyst, obtained by electrosynthesis, when exposed to oxidation potential, shows Mn oxidation state changes and structural rearrangements similar to those observed in the biological water-oxidation catalyst [163]. These observations, which include comparable intrinsic rates for the O–O bond formation compared to PSII, suggest similar mechanisms in both the artificial and the biological catalysts [163].

Besides flexibility, the Mn oxidation state in the oxide catalysts also plays an important role in the catalytic activity. The average oxidation state of the Mn ions in the resting state of the catalysts is found to be between III and IV [163] and often the important role of Mn ions in oxidation state III is emphasized [165,166]. Recently several studies suggested that a prerequisite for this catalytic activity is a structure that allows for a fraction of Mn(III) ions stable not only in the resting state but also during operation at oxidation potentials relevant for O_2 evolution that are typically higher than those required to oxidize Mn(III) to Mn(IV) ions [46,118,165,167,168]. This means that in any design of artificial Mn-based catalyst for water oxidation, the target structure should be an amorphous material where application of oxidizing potential does not lead to a formation of a well-ordered crystalline phase, but instead to a rather flexible structure where fast oxidation-state changes coupled to the protonation/deprotonation events and flexibility of the atoms remain possible.

Interestingly, several studies on the role of the redox-inert cations in the structure of the inorganic catalysts suggest that Ca^{2+} ions are not essential for the catalytic activity of Mn oxides [169,170], although Ca^{2+} could improve their activity.

Most recent studies confirm that there are structural and functional similarities between the biological Mn_4CaO_5 catalyst for water oxidation and the synthetic Mn oxide catalysts. The key to improve their activity and relevance for the technological application for solar fuel synthesis is the better understanding the photosystem II function by investigations involving the application of a combination of state-of-the-art techniques, rather than single diffraction or spectroscopic methods.

8. Conclusions

A highly efficient Mn–Ca oxide-based cluster (Mn_4CaO_5) has been selected by Nature to extract electrons and protons from water and, coincidentally, to supply the atmosphere with molecular oxygen. Surprisingly, in contrast to many other enzymes, the catalyst structure has been highly conserved from cyanobacteria, through various eukaryotic algae to embryophyte land plants during >2 billion years of evolution. Recent results provided a firm structural basis to understand the mechanism of the biological

water oxidation. Such data can serve as a blueprint for the synthesis of artificial photosynthetic model compounds, where significant progress has recently been achieved towards the development of Mn-based catalysts with the future technological application.

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Conflict of interest

The authors declare no conflict of interest.

References

- [1] B. Ke, *Photosynthesis Photobiology and Photobiophysics*, Springer Science & Business Media, 2001.
- [2] R.E. Blankenship, *Molecular Mechanisms of Photosynthesis*, John Wiley & Sons, 2014.
- [3] J.-R. Shen, *Annu. Rev. Plant Biol.* 66 (2015) 23–48.
- [4] H. Dau, I. Zaharieva, M. Haumann, *Curr. Opin. Chem. Biol.* 16 (2012) 3–10.
- [5] B.A. Diner, F. Rappaport, *Annu. Rev. Plant Biol.* 53 (2002) 551–580.
- [6] J.P. Dekker, R. Van Grondelle, *Photosynth. Res.* 63 (2000) 195–208.
- [7] Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, *Nature* 473 (2011) 55.
- [8] J.-R. Shen, Structure-function relationships in the Mn₄CaO₅ water-splitting cluster, in: *The Biophysics of Photosynthesis*, Springer, 2014, pp. 321–349.
- [9] N. Cox, M. Retegan, F. Neese, D.A. Pantazis, A. Boussac, W. Lubitz, *Science* 345 (2014) 804–808.
- [10] M. Suga, F. Akita, K. Hirata, G. Ueno, H. Murakami, Y. Nakajima, T. Shimizu, K. Yamashita, M. Yamamoto, H. Ago, J.-R. Shen, *Nature* 517 (2015) 99–103.
- [11] D.J. Vinyard, G.M. Ananyev, G. Charles Dismukes, *Annu. Rev. Biochem.* 82 (2013) 577–606.
- [12] Y. Tachibana, L. Vayssieres, J.R. Durrant, *Nat. Photonics* 6 (2012) 511–518.
- [13] M.D. Karkas, O. Verho, E.V. Johnston, B.R. Åkermark, *Chem. Rev.* 114 (2014) 11863–12001.
- [14] J.M. Bockris, *Int. J. Hydrogen Energy* 24 (1999) 1–15.
- [15] M.W. Kanan, D.G. Nocera, *Science* 321 (2008) 1072–1075.
- [16] A.J. Bard, M.A. Fox, *Acc. Chem. Res.* 28 (1995) 141–145.
- [17] H. Dau, C. Limberg, T. Reier, M. Risch, S. Roggan, P. Strasser, *ChemCatChem* 2 (2010) 724–761.
- [18] P. Joliet, G. Barbieri, R. Chabaud, *Photochem. Photobiol.* 10 (1969) 309–329.
- [19] M. Haumann, P. Liebisch, C. Müller, M. Barra, M. Grabolle, H. Dau, *Science* 310 (2005) 1019–1021.
- [20] H. Dau, M. Haumann, *Photosynth. Res.* 92 (2007) 327–343.
- [21] A. Grundmeier, H. Dau, BBA (BBA)-Bioenerg. 1817 (2012) 88–105.
- [22] H. Dau, M. Haumann, *Coord. Chem. Rev.* 252 (2008) 273–295.
- [23] M. Haumann, C. Müller, P. Liebisch, L. Iuzzolino, J. Dittmer, M. Grabolle, T. Neisius, W. Meyer-Klaucke, H. Dau, *Biochemistry* 44 (2005) 1894–1908.
- [24] H. Dau, M. Haumann, *Photosynth. Res.* 84 (2005) 325–331.
- [25] A. Klauß, M. Haumann, H. Dau, *Proc. Natl. Acad. Sci. USA* 109 (2012) 16035–16040.
- [26] I. Zaharieva, M. Grabolle, P. Chernev, H. Dau, Water oxidation in photosystem II: Energetics and kinetics of intermediates formation in the S₂ → S₃ and S₃ → S₀ transitions monitored by delayed chlorophyll fluorescence, in: *Photosynthesis Research for Food, Fuel and the Future*, Springer, 2013, pp. 234–238.
- [27] I. Zaharieva, J.M. Wichmann, H. Dau, *J. Biol. Chem.* 286 (2011) 18222–18228.
- [28] I. Zaharieva, H. Dau, *Front. Plant Sci.* 10 (2019) 386.
- [29] L. Gerencsér, H. Dau, *Biochemistry* 49 (2010) 10098–10106.
- [30] I. Zaharieva, H. Dau, M. Haumann, *Biochemistry* 55 (2016) 6996–7004.
- [31] P.L. Dilbeck, H.J. Hwang, I. Zaharieva, L. Gerencsér, H. Dau, R.L. Burnap, *Biochemistry* 51 (2012) 1079–1091.
- [32] J.P. McEvoy, G.W. Brudvig, *Phys. Chem. Chem. Phys.* 6 (2004) 4754–4763.
- [33] T.A. White, R.A. Kirian, A.V. Martin, A. Aquila, K. Nass, A. Barty, H.N. Chapman, *J. Appl. Crystallogr.* 45 (2012) 335–341.
- [34] M. Hundelt, A.-M.A. Hays, R.J. Debus, W. Junge, *Biochemistry* 37 (1998) 14450–14456.
- [35] J. Clausen, R.J. Debus, W. Junge, *Biochim. Biophys. Acta, (BBA)-Bioenerg.* 1655 (2004) 184–194.
- [36] P.L. Dilbeck, H. Bao, C.L. Neveu, R.L. Burnap, *Biochemistry* 52 (2013) 6824–6833.
- [37] F. Rappaport, M. Blanchard-Desce, J. Lavergne, *Biochim. Biophys. Acta, Bioenerg.* 1184 (1994) 178–192.
- [38] A. Klauß, M. Haumann, H. Dau, *J. Phys. Chem. B* 119 (2014) 2677–2689.
- [39] H. Suzuki, M. Sugiura, T. Noguchi, *Biochemistry* 44 (2005) 1708–1718.
- [40] G. Bernát, F. Morvarid, Y. Feyziyev, S. Styring, *Biochemistry* 41 (2002) 5830–5843.
- [41] F. Rappaport, N. Ishida, M. Sugiura, A. Boussac, *Energy Environ. Sci.* 4 (2011) 2520–2524.
- [42] P.O. Sandusky, C.F. Yocum, *Biochim. Biophys. Acta, Bioenerg.* 766 (1984) 603–611.
- [43] G.W. Brudvig, W.F. Beck, J. Paula, *Annu. Rev. Biophys. Biophys. Chem.* 18 (1989) 25–46.
- [44] X. Li, P.E. Siegbahn, *Phys. Chem. Chem. Phys.* 17 (2015) 12168–12174.
- [45] M. Askerka, D.J. Vinyard, J. Wang, G.W. Brudvig, V.S. Batista, *Biochemistry* 54 (2015) 1713–1716.
- [46] E.M. Sproviero, J.A. Gascón, J.P. McEvoy, G.W. Brudvig, V.S. Batista, *J. Chem. Theory Comput.* 2 (2006) 1119–1134.
- [47] P.E. Siegbahn, *Phys. Chem. Chem. Phys.* 20 (2018) 22926–22931.
- [48] M. Askerka, D.J. Vinyard, G.W. Brudvig, V.S. Batista, *Biochemistry* 54 (2015) 5783–5786.
- [49] E.M. Sproviero, J.A. Gascón, J.P. McEvoy, G.W. Brudvig, V.S. Batista, *J. Am. Chem. Soc.* 130 (2008) 3428–3442.
- [50] P.E. Siegbahn, *Acc. Chem. Res.* 42 (2009) 1871–1880.
- [51] R.I. Cukier, D.G. Nocera, *Annu. Rev. Phys. Chem.* 49 (1998) 337–369.
- [52] S. Hammes-Schiffer, *Acc. Chem. Res.* 42 (2009) 1881–1889.
- [53] M.H.V. Huynh, T.J. Meyer, *Chem. Rev.* 107 (2007) 5004–5064.
- [54] C.W. Hoganson, G.T. Babcock, *Science* 277 (1997) 1953.
- [55] T.J. Meyer, M.H.V. Huynh, H.H. Thorp, *Angew. Chem. Int. Ed.* 46 (2007) 5284–5304.
- [56] C.J. Gagliardi, A.K. Vannucci, J.J. Concepcion, Z. Chen, T.J. Meyer, *Energy Environ. Sci.* 5 (2012) 7704–7717.
- [57] C.J. Gagliardi, B.C. Westlake, C.A. Kent, J.J. Paul, J.M. Papanikolas, T.J. Meyer, *Coord. Chem. Rev.* 254 (2010) 2459–2471.
- [58] A. Pirson, *Z. Bot.* 31 (1937) 193–267.
- [59] V.K. Yachandra, K. Sauer, M.P. Klein, *Chem. Rev.* 96 (1996) 2927–2950.
- [60] R.J. Debus, *Biochim. Biophys. Acta, (BBA)-Bioenerg.* 269 (1992) 352.
- [61] C.F. Yocum, V.L. Pecoraro, *Curr. Opin. Chem. Biol.* 3 (1999) 182–187.
- [62] T.A. Roelofs, W. Liang, M.J. Latimer, R.M. Cinco, A. Rompel, J.C. Andrews, K. Sauer, V.K. Yachandra, M.P. Klein, *Proc. Natl. Acad. Sci. USA* 93 (1996) 3335–3340.
- [63] S.K. Chapman, *Appl. Organomet. Chem.* 13 (1999) 475–476.
- [64] R.M. Cinco, A. Rompel, H. Visser, G. Aromí, G. Christou, K. Sauer, M.P. Klein, V. K. Yachandra, *Inorg. Chem.* 38 (1999) 5988–5998.
- [65] J.B. Vincent, G. Christou, *Inorg. Chim. Acta* 136 (1987) L41–L43.
- [66] M.M. Najafpour, G. Renger, M. Holyńska, A.N. Moghaddam, E.-M. Aro, R. Carpentier, H. Nishihara, J.J. Eaton-Rye, J.-R. Shen, S.I. Alkhalidiev, *Chem. Rev.* 116 (2016) 2886–2936.
- [67] D.N. Hendrickson, G. Christou, E.A. Schmitt, E. Libby, J.S. Bashkin, S. Wang, H.L. Tsai, J.B. Vincent, P.D. Boyd, J. Am. Chem. Soc. 114 (1992) 2455–2471.
- [68] K. Dimitrou, K. Folting, W.E. Streib, G. Christou, *J. Am. Chem. Soc.* 115 (1993) 6432–6433.
- [69] A. Singh, L. Spiccia, *Coord. Chem. Rev.* 257 (2013) 2607–2622.
- [70] G.W. Brudvig, R.H. Crabtree, *Proc. Natl. Acad. Sci. USA* 83 (1986) 4586–4588.
- [71] J.M. Peloquin, K.A. Campbell, D.W. Randall, M.A. Evanchik, V.L. Pecoraro, W.H. Armstrong, R.D. Britt, *J. Am. Chem. Soc.* 122 (2000) 10926–10942.
- [72] A. Zouni, H.-T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, *Nature* 409 (2001) 739.
- [73] N. Kamiya, J.-R. Shen, *Proc. Natl. Acad. Sci.* 100 (2003) 98–103.
- [74] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, *Science* 303 (2004) 1831–1838.
- [75] K. Kawakami, Y. Umena, N. Kamiya, J.-R. Shen, *J. Photochem. Photobiol., B* 104 (2011) 9–18.
- [76] J. Kern et al., *Nature* 563 (2018) 421–425.
- [77] M. Suga, F. Akita, M. Sugahara, M. Kubo, Y. Nakajima, T. Nakane, K. Yamashita, Y. Umena, M. Nakabayashi, T. Yamane, J.-R. Shen, *Nature* 543 (2017) 131–135.
- [78] F.H.M. Koua, Y. Umena, K. Kawakami, J.-R. Shen, *Proc. Natl. Acad. Sci. USA* 110 (2013) 3889–3894.
- [79] R. Chatterjee, S. Milikisijants, C.S. Coates, F.H. Koua, J.-R. Shen, K. Lakshmi, *Phys. Chem. Chem. Phys.* 16 (2014) 20834–20843.
- [80] E.Y. Tsui, T. Agapie, *Proc. Natl. Acad. Sci. USA* 110 (2013) 10084–10088.
- [81] H. Isobe, M. Shoji, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, K. Yamaguchi, *Dalton Trans.* 41 (2012) 13727–13740.
- [82] M. Shoji, H. Isobe, S. Yamanaka, M. Suga, F. Akita, J.-R. Shen, K. Yamaguchi, *Chem. Phys. Lett.* 627 (2015) 44–52.
- [83] P. Chernev, I. Zaharieva, E. Rossini, A. Galstyan, H. Dau, E.-W. Knapp, *J. Phys. Chem. B* 120 (2016) 10899–10922.
- [84] D.A. Pantazis, W. Ames, N. Cox, W. Lubitz, F. Neese, *Angew. Chem. Int. Ed.* 51 (2012) 9935–9940.
- [85] D. Bovi, D. Narzi, L. Guidoni, *Angew. Chem. Int. Ed.* 52 (2013) 11744–11749.
- [86] K. Hirata, K. Shinzawa-Itoh, N. Yano, S. Takemura, K. Kato, M. Hatanaka, K. Muramoto, T. Kawahara, T. Tsukihara, E. Yamashita, *Nat. Methods* 11 (2014) 734–736.
- [87] H.N. Chapman et al., *Nature* 470 (2011) 73–77.
- [88] J. Hattne, N. Echols, R. Tran, J. Kern, R.J. Gildea, A.S. Brewster, R. Alonso-Mori, C. Glöckner, J. Hellmich, H. Laksmono, *Nat. Methods* 11 (2014) 545.
- [89] W. Kabsch, *Acta Crystallogr., Sect. D: Biol. Crystallogr.* 70 (2014) 2204–2216.

- [90] J. Kern, R. Alonso-Mori, R. Tran, J. Hattne, R.J. Gildea, N. Echols, C. Glöckner, J. Hellmich, H. Laksmono, R.G. Sierra, *Science* 340 (2013) 491–495.
- [91] C. Kupitz, S. Basu, I. Grotjohann, R. Fromme, N.A. Zatsepin, K.N. Rendek, M.S. Hunter, R.L. Shoeman, T.A. White, D. Wang, *Nature* 513 (2014) 261.
- [92] J. Kern, R. Tran, R. Alonso-Mori, S. Koroidov, N. Echols, J. Hattne, M. Ibrahim, S. Gul, H. Laksmono, R.G. Sierra, *Nat. Commun.* 5 (2014) 4371.
- [93] I.D. Young et al., *Nature* 540 (2016) 453–457.
- [94] F.A. Cotton, G. Wilkinson, C.A. Murillo, M. Bochmann, R. Grimes, *Advanced Inorganic Chemistry*, Wiley, New York, 1988.
- [95] R.J. Pace, L. Jin, R. Stranger, *Dalton Trans.* 41 (2012) 11145–11160.
- [96] S. Petrie, R.J. Pace, R. Stranger, *Angew. Chem. Int. Ed.* 54 (2015) 7120–7124.
- [97] W. Ames, D.A. Pantazis, V. Krewald, N. Cox, J. Messinger, W. Lubitz, F. Neese, *J. Am. Chem. Soc.* 133 (2011) 19743–19757.
- [98] J. Yano, V. Yachandra, *Chem. Rev.* 114 (2014) 4175–4205.
- [99] A. Grundmeier, P. Loja, M. Haumann, H. Dau, The manganese complex of photosystem II: Extended-range EXAFS data and specific structural models for four S-states, in: *Photosynthesis. Energy from the Sun*, Springer, 2008, pp. 405–408.
- [100] I. Zaharieva, P. Chernev, G. Berggren, M. Anderlund, S.R. Styring, H. Dau, M. Haumann, *Biochemistry* 55 (2016) 4197–4211.
- [101] D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, *FEBS Lett.* 167 (1984) 127–130.
- [102] K.-C. Han, S. Katoh, *Plant Cell Physiol.* 34 (1993) 585–593.
- [103] P. Aedelroth, K. Lindberg, L.-E. Andreasson, *Biochemistry* 34 (1995) 9021–9027.
- [104] A. Boussac, F. Rappaport, P. Carrier, J.-M. Verbavatz, R. Gobin, D. Kirilovsky, A. W. Rutherford, M. Sugiura, *J. Biol. Chem.* 279 (2004) 22809–22819.
- [105] M. Shoji, H. Isobe, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, T. Nakajima, K. Yamaguchi, Large-scale QM/MM calculations of hydrogen bonding networks for proton transfer and water inlet channels for water oxidation—theoretical system models of the oxygen-evolving complex of photosystem II, in: *Advances in Quantum Chemistry*, Elsevier, 2015, pp. 325–413.
- [106] S. Nakamura, K. Ota, Y. Shibuya, T. Noguchi, *Biochemistry* 55 (2016) 597–607.
- [107] Z. Guo, J. He, B.A. Barry, *Proc. Natl. Acad. Sci. USA* 115 (2018) 5658–5663.
- [108] R.J. Debus, *Biochemistry* 53 (2014) 2941–2955.
- [109] H. Bao, R.L. Burnap, *Proc. Natl. Acad. Sci. USA* 112 (2015) E6139–E6147.
- [110] J. Yano, J. Kern, K.-D. Irrgang, M.J. Latimer, U. Bergmann, P. Glatzel, Y. Pushkar, J. Biesiadka, B. Loll, K. Sauer, *Proc. Natl. Acad. Sci. USA* 102 (2005) 12047–12052.
- [111] M. Grabolle, M. Haumann, C. Muller, P. Liebisch, H. Dau, *J. Biol. Chem.* 281 (2006) 4580–4588.
- [112] J. Yano, Y. Pushkar, P. Glatzel, A. Lewis, K. Sauer, J. Messinger, U. Bergmann, V. Yachandra, *J. Am. Chem. Soc.* 127 (2005) 14974–14975.
- [113] H. Popelková, C.F. Yocum, *Photosynth. Res.* 93 (2007) 111–121.
- [114] C. Glöckner, J. Kern, M. Broser, A. Zouni, V. Yachandra, J. Yano, *J. Biol. Chem.* 288 (2013) 22607–22620.
- [115] K. Kawakami, Y. Umena, N. Kamiya, J.-R. Shen, *Proc. Natl. Acad. Sci. USA* 106 (2009) 8567–8572.
- [116] H. Wincencjusz, H.J. van Gorkom, C.F. Yocum, *Biochemistry* 36 (1997) 3663–3670.
- [117] C. Hureau, G. Blondin, M.-F. Charlot, C. Philouze, M. Nierlich, M. Césario, E. Anxolabéhère-Mallart, *Inorg. Chem.* 44 (2005) 3669–3683.
- [118] A. Boussac, A.W. Rutherford, *J. Biol. Chem.* 269 (1994) 12462–12467.
- [119] I. Rivalta, M. Amin, S. Lubner, S. Vassiliev, R. Pokhrel, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, D. Bruce, G.W. Brudvig, M.R. Gunner, V.S. Batista, *Biochemistry* 50 (2011) 6312–6315.
- [120] M. Broser, C. Glöckner, A. Gabdulkhakov, A. Guskov, J. Buchta, J. Kern, F. Müh, H. Dau, W. Saenger, A. Zouni, *J. Biol. Chem.* 286 (2011) 15964–15972.
- [121] G. Renger, *Biochim. Biophys. Acta (BBA)-Bioenerg.* 1817 (2012) 1164–1176.
- [122] B.A. Barry, *Biochim. Biophys. Acta (BBA)-Bioenerg.* 1847 (2015) 46–54.
- [123] K. Saito, J.-R. Shen, T. Ishida, H. Ishikita, *Biochemistry* 50 (2011) 9836–9844.
- [124] T. Noguchi, M. Sugiura, *Biochemistry* 41 (2002) 15706–15712.
- [125] H. Suzuki, M. Sugiura, T. Noguchi, *Biochemistry* 47 (2008) 11024–11030.
- [126] M. Shoji, H. Isobe, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, K. Yamaguchi, *Catal. Sci. Technol.* 3 (2013) 1831–1848.
- [127] F.M. Ho, *Photosynth. Res.* 98 (2008) 503.
- [128] F.M. Ho, S. Styring, *Biochim. Biophys. Acta (BBA)-Bioenerg.* 1777 (2008) 140–153.
- [129] N. Cox, W. Lubitz, Molecular concepts of water splitting: Nature's approach, in: R. Schlögl (Ed.), *Chemical Energy Storage*, Walter de Gruyter GmbH, Berlin/Boston, 2013, pp. 185–215.
- [130] J. Messinger, M. Badger, T. Wydrzynski 92 (1995) 3209–3213.
- [131] W. Hillier, T. Wydrzynski, *Phys. Chem. Chem. Phys.* 6 (2004) 4882–4889.
- [132] G. Hendry, T. Wydrzynski, *Biochemistry* 41 (2002) 13328–13334.
- [133] W. Hillier, T. Wydrzynski, *Coord. Chem. Rev.* 252 (2008) 306–317.
- [134] B. Demmig, K. Winter, *Planta* 159 (1983) 66–76.
- [135] J. Messinger, J.H. Robblee, U. Bergmann, C. Fernandez, P. Glatzel, H. Visser, R. M. Cinco, K.L. McFarlane, E. Bellacchio, S.A. Pizarro, *J. Am. Chem. Soc.* 123 (2001) 7804–7820.
- [136] M. Retegan, V. Krewald, F. Mamedov, F. Neese, W. Lubitz, N. Cox, D.A. Pantazis, *Chem. Sci.* 7 (2016) 72–84.
- [137] M. Suga, F. Akita, K. Yamashita, Y. Nakajima, G. Ueno, H. Li, T. Yamane, K. Hirata, Y. Umena, S. Yonekura, L.-J. Yu, H. Murakami, T. Nomura, T. Kimura, M. Kubo, S. Baba, T. Kumasaka, K. Tono, M. Yabashi, H. Isobe, K. Yamaguchi, M. Yamamoto, H. Ago, J.-R. Shen, *Science* 366 (2019) 334–338.
- [138] P.E.M. Siegbahn, *Phys. Chem. Chem. Phys.* PCCP 20 (2018) 22926–22931.
- [139] R.D. Britt, K.A. Campbell, J.M. Peloquin, M.L. Gilchrist, C.P. Aznar, M.M. Dicus, J. Robblee, J. Messinger, *Biochim. Biophys. Acta (BBA) Bioenerg.* 1655 (2004) 158–171.
- [140] T. Noguchi, M. Sugiura, *Biochemistry* 39 (2000) 10943–10949.
- [141] J. Messinger, *Phys. Chem. Chem. Phys.* 6 (2004) 4764–4771.
- [142] J. Barber, K. Ferreira, K. Maghlaoui, S. Iwata, *Phys. Chem. Chem. Phys.* 6 (2004) 4737–4742.
- [143] S. Yamanaka, H. Isobe, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura, *Chem. Phys. Lett.* 511 (2011) 138–145.
- [144] V.L. Pecoraro, M.J. Baldwin, M.T. Caudle, W.Y. Hsieh, N.A. Law, *Pure Appl. Chem.* 70 (1998) 925–929.
- [145] J. Barber, *Q. Rev. Biophys.* 50 (2017) e13.
- [146] C. Tommos, G.T. Babcock, *Acc. Chem. Res.* 31 (1998) 18–25.
- [147] J.P. McEvoy, G.W. Brudvig, *Chem. Rev.* 106 (2006) 4455–4483.
- [148] A. Klaus, M. Haumann, H. Dau, *J. Phys. Chem. B* 119 (2015) 2677–2689.
- [149] E. Rossini, E.-W. Knapp, *Coord. Chem. Rev.* 345 (2017) 16–30.
- [150] H. Isobe, M. Shoji, S. Yamanaka, H. Mino, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, K. Yamaguchi, *Phys. Chem. Chem. Phys.* 16 (2014) (1923) 11911–11921.
- [151] A. Haddy, *Photosynth. Res.* 92 (2007) 357–368.
- [152] R. Pokhrel, G.W. Brudvig, *Phys. Chem. Chem. Phys.* 16 (2014) (1821) 11812–11821.
- [153] D. Narzi, D. Bovi, L. Guidoni, *Proc. Natl. Acad. Sci. USA* 111 (2014) 8723–8728.
- [154] R. Chatterjee, L. Lassalle, S. Gul, F.D. Fuller, I.D. Young, M. Ibrahim, C. de Lichtenberg, M.H. Cheah, A. Zouni, J. Messinger, *Physiol. Plant.* (2019).
- [155] K.M. Davis, B.T. Sullivan, M.C. Palenik, L. Yan, V. Purohit, G. Robison, I. Kosheleva, R.W. Henning, G.T. Seidler, Y. Pushkar, *Phys. Rev. X* 8 (2018) 041014.
- [156] 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, V.K. Yachandra, *J. Am. Chem. Soc.* 123 (2001) 7804–7820.
- [157] U. Bergmann, P. Glatzel, J.H. Robblee, J. Messinger, C. Fernandez, R. Cinco, H. Visser, K. McFarlane, E. Bellacchio, S. Pizarro, K. Sauer, V.K. Yachandra, M.P. Klein, B.L. Cox, K.H. Nealson, S.P. Cramer, *J. Synchrotron Radiat.* 8 (2001) 199–203.
- [158] T.A. Latimer, D. McQueen, A.R. DiFiore, M. Fry, A. Messerschmidt, D.W. Miahnahri, M.M. Schafer, D. Seibert, T.-C. Sokaras, P.H. Weng, W.E. Zwart, P.D. White, M.J. Adams, S. Bogan, G.J. Boutet, J. Williams, N.K. Messinger, A. Sauter, U. Zouni, J. Bergmann, V.K. Yano, Yachandra, *Proc. Natl. Acad. Sci. USA* 109 (2012) 9721–9726.
- [159] R. Tran, J. Kern, J. Hattne, S. Koroidov, J. Hellmich, R. Alonso-Mori, K. Sauter, Nicholas, U. Bergmann, J. Messinger, A. Zouni, J. Yano, K. Yachandra Vittal, *Philos. Trans. R. Soc. B: Biol. Sci.* 369 (2014) 20130324.
- [160] J. Morton, M. Chrysina, V.S.J. Craig, F. Akita, Y. Nakajima, W. Lubitz, N. Cox, J.-R. Shen, E. Krausz, *Biochim. Biophys. Acta (BBA) Bioenerg.* 1859 (2018) 88–98.
- [161] J. Barber, *Nat. Plants* 3 (2017) 17041.
- [162] M. Wiechen, H.-M. Berends, P. Kurz, *Dalton Trans.* 41 (2012) 21–31.
- [163] C. Zhang, C. Chen, H. Dong, J.-R. Shen, H. Dau, J. Zhao, *Science* 348 (2015) 690–693.
- [164] I. Zaharieva, D. Gonzalez-Flores, B. Asfari, C. Pasquini, M.R. Mohammadi, K. Klingan, I. Zizak, S. Loos, P. Chernev, H. Dau, *Energy Environ. Sci.* 9 (2016) 2433–2443.
- [165] Z.M. Chan, D.A. Kitchaev, J.N. Weker, C. Schnedermann, K. Lim, G. Ceder, W. Tumas, M.F. Toney, D.G. Nocera, *Proc. Natl. Acad. Sci. USA* 115 (2018) E5261–E5268.
- [166] M. Mahdi Najafpour, D. Jafarian Sedigh, S. Maedeh Hosseini, I. Zaharieva, *Inorg. Chem.* 55 (2016) 8827–8832.
- [167] I. Zaharieva, P. Chernev, M. Risch, K. Klingan, M. Kohlhoff, A. Fischer, H. Dau, *Energy Environ. Sci.* 5 (2012) 7081–7089.
- [168] M. Abrashev, P. Chernev, P. Kubella, M.R. Mohammadi, C. Pasquini, H. Dau, I. Zaharieva, *J. Mater. Chem. A* (2019).
- [169] M.M. Najafpour, T. Ehrenberg, M. Wiechen, P. Kurz, *Angew. Chem. Int. Ed.* 49 (2010) 2233–2237.
- [170] M. Wiechen, I. Zaharieva, H. Dau, P. Kurz, *Chem. Sci.* 3 (2012) 2330–2339.