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Recent developments in biological water oxidation

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Rapid progress has been made in the last five years towards resolution of the structure of nature's water splitting catalyst — a Mn_4O_5Ca cofactor embedded in Photosystem II — especially in the field of X-ray crystallography. In addition, recent magnetic resonance data have allowed the structure of the cofactor to be accessed in its last metastable intermediate state, prior to O–O bond formation. This activated form of the catalyst is geometrically similar to that seen by X-ray crystallography, which represents the resting state of the cofactor, but requires the coordination of an additional water molecule to the cofactor, rendering all Mn ions six coordinate. Importantly, it locates two water derived, Mn bound oxygen ligands in close proximity. It is these two oxygen ligands that likely form the product O_2 molecule, as proposed earlier by quantum chemical modeling. Current views on the molecular level events that facilitate catalyst activation, that is, catalyst/substrate deprotonation, Mn oxidation and water molecule insertion are briefly described.

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The structure of nature's water splitting cofactor in its resting state

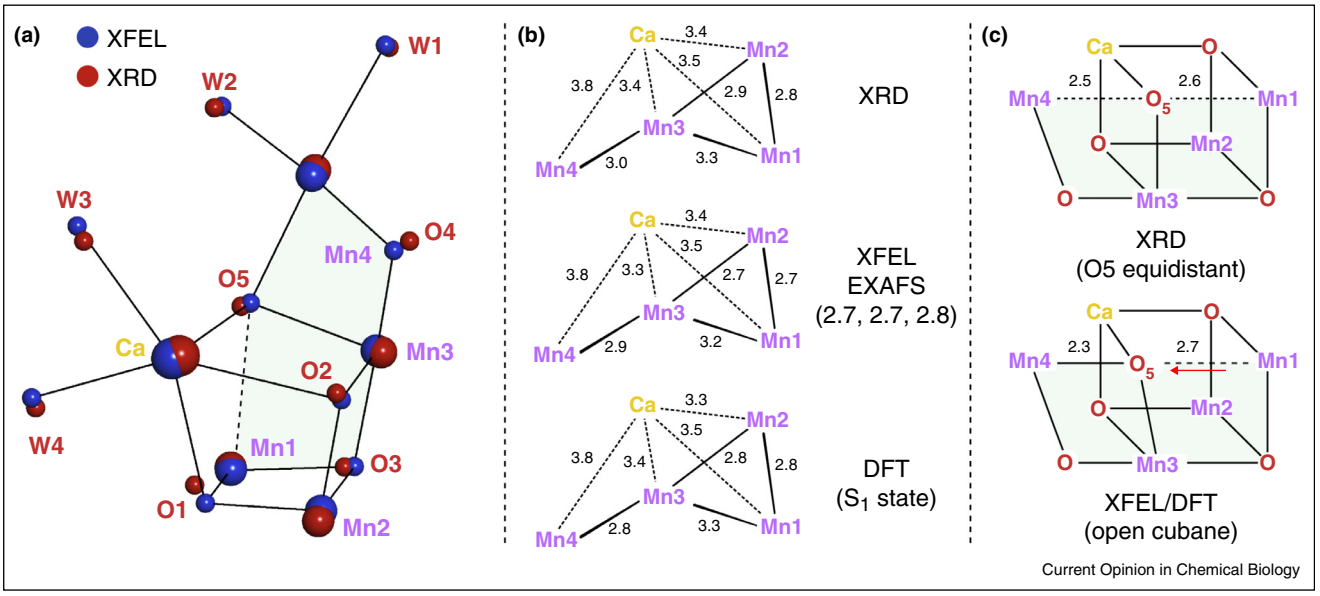
Crystallographic advances, including the introduction of free electron laser sources, have fundamentally altered the trajectory of research on the mechanism of biological water splitting [1••]. These new methods have recently provided an atomic resolution crystal structure of the resting state of the catalyst. Specifically, the XFEL (X-ray free electron laser) structure: (i) reproduces earlier EXAFS constraints (e.g. Mn–Mn distances) [2,3,4•]; (ii) clarifies the location of all oxygen bridges of the catalyst including the O5 bridge, which in earlier X-ray models (XRD) was placed in a chemically unprecedented bonding position; and (iii) constrains the net oxidation state of the cofactor in the resting state (III, IV, IV, III) by resolving the Jahn–Teller axes of two Mn^{III} ions, Mn1 and Mn4 (see Figure 1). This novel structure allows the information content of complementary spectroscopies to be fully realized and together these data now provide a solid basis for the development of robust chemical models throughout its entire catalytic cycle [5•].

Substrate binding and delivery to the catalyst

An understanding of the mechanism of biological water splitting is greatly aided by resolving what oxygen sites of the cofactor participate in the reaction [12•,13]. Recent magnetic resonance data has implicated O5 as one of the two substrates owing to its fast exchange with bulk solvent water [14•,15•,16]. This is a unique property not seen for the other four bridges, or for oxygen bridges in simpler synthetic model systems [17–19]. The identity of the second substrate remains more contentious as it is unclear if it is bound in all intermediate states of the catalyst, or if bound in all states, whether its position changes during the cycle [12•,20]. Water analogs such as ammonia and methanol provide a means to address this question by probing how the substrates first access the cofactor. Substrate access forms part of a larger debate on the dynamics of the solvent/substrate interface, namely how does the catalyst selectively ***activate*** two solvent water molecules (when it is surrounded by solvent water) and ***regulate*** solvent water access to the site of the catalyst, to avoid deleterious side products such as H_2O_2.

Although ammonia and methanol do not drastically alter the binding/exchange kinetics of the two substrates, their addition is associated with a decrease of the catalyst's efficiency under steady-state conditions suggesting that they hinder or interfere with substrate access to the site(s) of catalysis. The crystal structure identifies at least three water pathways leading towards/away from the cofactor: (i) a channel which includes the Ca^{2+} ion and the redox active tyrosine residue (Y_Z); (ii) a channel which includes the outer Mn4 and the Asp61 residue [1,6]; and (iii) a similar channel which also includes the Cl^- ion (Figure 2**a**). The location of ammonia binding was recently deduced from chemical modeling [14•,15•,22] and mutagenesis [23•], displacing the water ligand (W1) of the outer manganese (Mn4). The binding location of methanol, however, is less well defined, with one option placing it close to the site of ammonia binding and another favoring its binding at the Ca^{2+} ion [24]. Competitive

Figure 1



(A) Comparison of the new XFEL structure with the earlier synchrotron (XRD) structure of the Mn_4O_5Ca cofactor [1,6]. The cofactor resembles a distorted chair with the back of the chair shown by the light blue plane. The XFEL structure better reproduces EXAFS constraints [2,3] (panel B) and places O5 in a more chemically realistic position (panel C), more in line with earlier chemical models [5•,7–9]. The cofactor contains three coordinately saturated Mn ions (Mn2, Mn3 and Mn4) with a potential substrate binding site located at Mn1, the only five coordinate Mn ion. Mn4 and the Ca^{2+} ion both carry two water derived ligands. All remaining ligands are derived from the protein backbone and oxygen bridge network. Substitution of the Ca^{2+} ion with Sr^{2+} has little effect on the structure of the catalyst [10], in line with spectroscopic results [11].

binding of small molecules at/near the position of W1 indicate that the water channel associated with this site (Asp61 channel) is important for water delivery [25••]. As a whole, these results favor that one of the water derived ligands of the Mn4 is the second substrate. Water binding via the Asp61 channel involves a rotation of the waters on the Mn4, initiated by the facile shift of the O5 bridge [9,21]. This cascade of structural rearrangements results in a five coordinate, trigonal bipyramidal Mn ion, shown in Figure 2 [25••]. Within this model water binding occurs on the back-face of the cofactor. As such the water molecule when first delivered is most likely not the substrate of the reaction – this is instead one of the existing water ligands (W2) – but form the substrate in the next catalytic cycle, see [12•,20].

The structure of the activated catalyst — structural evolution during the S-state

It is well known that the cofactor cycles through a series of five intermediate states: S_0, S_1 (dark stable), S_2, S_3, and S_4 where the subscript refers to the number of oxidizing equivalents stored by the cofactor. The S_4 state spontaneously decays upon release of triplet O_2 returning to the S_0 state. Oxidation of the cofactor is driven by light excitation of the Photosystem II reaction center, via a redox active tyrosine residue (Y_Z). Coupled with oxidation, protons are successively removed from the cofactor, to ensure its total charge/redox potential remains constant, termed redox tuning or redox leveling. As such the cycle can more accurately be described in terms of nine states which differ in terms of their net electron and proton count [26,27•] (Figure 3). An important observation is that at different points in the cycle the order in which electrons and protons are removed from the cofactor reverses; in the early S-state transitions (e.g. S_0 to S_1) electron transfer precedes proton transfer whereas in the late transitions (e.g. S_2 to S_3) proton transfer precedes electron transfer [26,27•]. As a consequence, Y_Z can be though of having a dual role: first, it acts as an electron carrier; and second, it promotes deprotonation of the catalyst which is presumably facilitated via the intervening H-bonding network.

The XFEL structure described above represents the resting state of the catalyst (S_1) [1••], although it may contain some admixture of the S_0 state [5•]. It is also thought to be a good model for the S_2 state as EXAFS results for the S_0, S_1 and S_2 are all very similar [2,3] and progression from the S_1 to the S_2 state can occur at low temperatures (<200 K), where the protein's conformation should be fixed. The same however cannot be said of the S_3 state, the last metastable intermediate of the reaction cycle. Recent pulse EPR data has demonstrated that in this state all four Mn ions are structurally and electronically *similar:* they all exhibit a formal oxidation state 4+ and octahedral local geometry [28••]. This requires the inclusion of an additional water-derived ligand at the