Review

Cite this article: Cox N, Pantazis DA, Neese F, Lubitz W. 2015 Artificial photosynthesis: understanding water splitting in nature. Interface Focus 5: 20150009.
http://dx.doi.org/10.1098/rsfs.2015.0009

One contribution of 11 to a theme issue ‘Do we need a global project on artificial photosynthesis (solar fuels and chemicals)?’.

Subject Areas:
chemical biology, bioenergetics, biophysics

Keywords:
photosynthesis, photosystem II, water splitting, H2 production

Authors for correspondence:
Nicholas Cox
e-mail: nicholas.cox@cec.mpg.de
Wolfgang Lubitz
e-mail: wolfgang.lubitz@cec.mpg.de

1. The need for a global project on artificial photosynthesis

The world’s power supply is based predominantly on the use of non-renewable energy resources such as oil, coal and natural gas. These geological resources have been accumulated over millions of years from photosynthetic activity and thus constitute ‘stored solar energy’. The steep rise in energy consumption over the last century has led to an inevitable shortage of these valuable resources with the economic, social and political consequences already felt today. Human population and economic growth, particularly in fast-developing countries, will lead to a further increase in energy demand. Furthermore, the burning of carbon-rich fuels has increased CO2 concentration in the atmosphere. This increase is associated with anthropogenic climate changes, with projected adverse effects for our planet and human society (http://www.ipcc.ch/report). It is therefore one of the great challenges of mankind to identify and develop alternative sustainable energy sources [1–5].

Society’s energy needs come in many forms. The dominant fraction is related to liquid fuels used for example in transportation (approx. 80%); whereas electrical energy only accounts for a small part of our energy consumption (approx. 20%) (https://www.bdw.de/internet.nsf/id/DE_Energiedaten). This is particularly problematic as there currently exists no satisfactory method which would be able to supply our demand for renewable liquid fuels. One possible solution would be to generate electricity using alternative, clean physical energy conversion technologies such as photovoltaics, wind turbines and hydropower and subsequently convert it into a chemical storage form. Such technologies already represent an increasing share of electrical energy generation worldwide (in Germany in 2013 16%) (https://www.bdw.de/internet.nsf/id/DE_Energiedaten). The question that then arises is what represents the best feedstock for a synthetic, renewable fuel. Here, biology provides an answer: photosynthesis uses water, a cheap, non-toxic, abundant material. Thus, in an idealized scenario ‘clean’ electricity would be used in the electrolysis of water, generating hydrogen (H2), as a fuel [6]. Hydrogen itself is a very important base chemical and can be converted to many other important energy-rich materials for storage and/or transport, e.g. with CO2 to methane, methanol or formic acid, and with molecular nitrogen to ammonia (figure 1).
A simple calculation shows that the yearly energy needs of a single person can be met by decomposition of 12 000 l of water, i.e. the contents of a small water pool (4 × 3 × 1 m). In addition, such a scenario eliminates the need for an independent power supplier, allowing energy production at the site of use [7], reducing transportation losses. A bottleneck though for such a ‘hydrogen economy’ blueprint is the relatively high price of the hydrogen produced. The high cost is derived from the materials used currently in commercially viable electrolysers: noble metals such as platinum or precious metal oxides (Ru and Ir oxides). These need to be replaced by less expensive metal catalysts before this model for the hydrogen economy becomes viable.

The generation of H\textsubscript{2} from water requires two catalytic steps: (i) the oxidation of two water molecules into O\textsubscript{2} and protons and (ii) the subsequent reduction of protons to molecular H\textsubscript{2}. For proton reduction interesting approaches already exist (e.g. based on biomimetic catalysts from hydrogenase research) [8] that are close to enabling feasible commercial/industrial applications [9]. By contrast, there exists no completely satisfactory water oxidation catalyst. First-row transition metal based materials used for this reaction display typically a limited stability and durability (low turnover number, TON) or have an intrinsically low turnover frequency (TOF), precluding their wide range application. Another problem is often the excessive voltages needed to drive such catalysts (overpotential), which are due to the lack of suitable interfaces between the catalysts and the photo-active semiconductors.

Clearly renewed public investment should be targeted towards the scientific, technological and economic challenges needed to initiate the energy transition described above, e.g. the ‘Energiewende’ policy adopted by the current German government. The central discipline in this endeavour is chemical catalysis in all its facets, allowing energy to be efficiently used, stored, transported and interconverted. In this endeavour much can be learned from nature, which has developed many highly efficient metallo-enzymes [10] for the chemical conversion of small molecules with high TONs, long lifetimes and low overpotentials, and which even have built-in protection and repair mechanisms.

To solve the energy problems of the future requires initiatives such as the Global Artificial Photosynthesis (GAP) project [5], which bring together the efforts of many researchers across a wide range of scientific disciplines. Ideally, technologies that use nature as a blueprint, such as photosynthesis, should be considered part of the common heritage of mankind. We should aim that such technologies are affordable and accessible throughout the world with maximum benefit to all. We endorse the spirit of the GAP project: ‘Our goal is to work cooperatively and with respect for basic ethical principles to produce the scientific breakthroughs that allow development and deployment of an affordable, equitably accessed, economically and environmentally sustainable, non-polluting global energy and food system that also contributes positively to our biosphere’, as discussed at the Chicheley Hall conference in July 2014.

2. Design strategies for artificial photosynthesis, learning from nature

The term ‘artificial photosynthesis’ or equally ‘synthetic photosynthesis’ describes technologies that attempt to capture
the energy of sunlight to make energy-rich molecules, i.e. ‘solar fuels’. This relies on the thesis that chemical bonds allow energy to be efficiently stored. This is essentially what nature does. It performs a photocatalytic water oxidation reaction which is coupled to carbon dioxide fixation (CO2 reduction).

In oxygenic photosynthesis, light-induced water oxidation occurs in a biological supercomplex, called photosystem II (PSII) that is found in all green plants, algae and cyanobacteria. It harbours a protein-embedded oxygen-bridged Mn4Ca cluster with four bound water-derived ligands [11], as shown in figure 2. This cluster, called the water oxidizing complex (WOC) or oxygen evolving complex (OEC), is coupled to a pigment–protein reaction centre, which successively extracts electrons from it. After four such extractions, the complex splits water into protons and molecular oxygen, which is released as by-product, returning to its resting state (for reviews, see [13–16]). Protons generated via this process could, in principle, be used by another enzyme found in many green algae and bacteria, hydrogenase, which catalyses the conversion of protons to molecular hydrogen (for a recent review, see [8]). In figure 2 next to PSII, the structure of an [FeFe] hydrogenase is also depicted. The active site of this hydrogenase is a six-iron cluster, made up from a classical [4Fe–4S] cubane linked to a di-iron complex that carries uncommon ligands (3 CO, 2 CN−, azadithiolate) [8]. Under certain metabolic conditions, this enzyme is activated and produces hydrogen from excess protons. Other types of hydrogenases are known that harbour a different binuclear metal cluster ([NiFe] hydrogenases) that converts H2 into protons and electrons, thereby generating energy for the organism [8,17,18]. In principle, both types of hydrogenases catalyse both reactions, dihydrogen splitting and production, albeit with different rates.

The efficient coupling of these two biological processes, water oxidation and hydrogen production (shown in figure 2), is currently being investigated in many laboratories aiming at producing (bio)hydrogen [19–23]. An interesting feature of the enzymes is the high turnover rate, which is about 500 s−1 for the WOC and up to 10 000 s−1 for the hydrogenase [24]. Unfortunately, both enzymes suffer from limited stability: the WOC has a half-life of only 30 min in average light under working conditions [25] and the [FeFe] hydrogenase is very sensitive to oxygen [26,27]; in the living cell, protection and repair mechanisms exist that efficiently resynthesize and replace the damaged parts of the enzymes. Among the [NiFe] hydrogenases, there is also a class of oxygen-tolerant enzymes [28–32].

An understanding of the principles and mechanisms of water oxidation and hydrogen conversion in nature would allow us to better design artificial systems capable of (bio)hydrogen production, be they biological photosynthetic organisms or purely synthetic systems, such as the hypothetical device in figure 1. For this latter system, the ultimate aim is to synthesize new catalysts for large-scale water splitting, hydrogen production and energy storage in chemical compounds. Such compounds may also be of importance for a future sustainable energy economy. In this short review, the current knowledge about the enzyme water oxidase (PSII) is described as an example. It is not intended to describe in depth the biological system but instead focus on the active metal cluster, and the recent insight gained about its function by structural, spectroscopic and theoretical results. Design criteria for the construction of respective biomimetic and bioinspired model systems for water splitting are discussed. We note that the reversible conversion of protons to molecular hydrogen is described in recent reviews (e.g. [8]).

Figure 2. Light-induced water splitting into molecular oxygen and hydrogen by the enzymes PSII and [FeFe] hydrogenase. This cascade reaction (water oxidation and subsequent proton reduction) is for example exploited by green algae, e.g. Chlamydomonas reinhardtii. The structure of the proteins ((a) PDB 3ARC [11], (b) PDB 1HFE [12]) and the structure of the active sites are shown (see text for details).
3. Nature’s water splitting catalyst

Nature’s water oxidizing catalyst represents a penta-oxygen tetramanganese–calcium cofactor (MnO₄Ca) [11,13,16] (figure 2). Its catalytic cycle comprises five distinct redox intermediates, the Sₙ states, where the subscript indicates the number of stored oxidizing equivalents (n = 0–4) required to oxidize two water molecules and release dioxygen [33] (figure 3b). It is embedded in a unique pigment–protein supercomplex, PSII, which acts as a photosensitizer. Outer light harvesting complexes absorb visible light and funnel excitation energy to the reaction centre of PSII, where charge separation takes place. The reaction centre comprises chlorophyll, pheophytin and quinone molecules arranged about a twofold symmetry axis [35]. The radical cation generated upon charge separation, termed the primary oxidant (P680⁺), is predominantly associated with the chlorophylls P₃D₁ and P₄D₂ (figure 3a). The photochemically generated electron is passed in succession to the pheophytin (PheoD₁) and subsequently to the bound plastoquinone (QB), which acts as a mobile charge carrier. It accepts two electrons and two protons, before leaving its protein pocket to be exchanged with a new plastoquinone. In this way, electrons are passed to the next photosynthetic protein pocket to be exchanged with a new plastoquinone. Reaction centre photochemistry occurs on a picosecond to nanosecond time scale [35].

Subsequently, the electron is passed from QA to a second plastoquinone (Qₐ), which acts as a mobile charge carrier. It accepts two electrons and two protons, before leaving its protein pocket to be exchanged with a new plastoquinone. In this way, electrons are passed to the next photosynthetic supercomplex, the cytochrome bₙf complex.

The photogenerated cation P680⁺ is coupled to the MnO₄Ca cofactor via an intermediary redox-active tyrosine residue Yₐ, which acts as a single-electron gate (figure 3a). Initial electron (hole) transfer between P680⁺ and Yₐ occurs on a nanosecond time scale, whereas the subsequent electron (hole) transfer between Yₐ and the MnO₄Ca cofactor occurs on a microsecond to millisecond time scale, depending on the intermediate (S-state). Fast reversible oxidation of Yₐ is a property of the protein pocket. A nearby histidine residue acts as an intermolecular base, stabilizing the deprotonated (Tyr-O⁻) state, while structured waters (bound to the Ca²⁺ ion) act to tune its redox potential [11,36]. After four charge separation events, the transiently formed S₄Yₐ state [S₄] rapidly decays to the Sₐ state with the concomitant release of molecular triplet dioxygen and probably the rebinding of one substrate water molecule [37,38].

4. Key design features of the oxygen evolving complex

Nature’s ‘photosynthetic cell’—the water splitting cofactor coupled to the pigment–protein reaction centre photosynthesizer—has a number of unique design features, including the following.

(i) Light-induced single-electron transfer on a picosecond time scale coupled to a much slower millisecond four-electron process, the oxidation of two water molecules to molecular oxygen.

(ii) The temporary storage of oxidation equivalents required for this reaction, i.e. transient oxidation of the Mn ions.

(iii) Decoupling of O–O bond formation and proton release. Protons are released continuously throughout the S-state cycle [34], whereas the O–O bond forms in a single concerted step. Cofactor oxidation coupled with deprotonation (charge balancing/redox leveling) ensures that the redox potential of each of the four-electron withdrawal steps is similar, allowing all transitions to be driven by a single oxidant, P680, i.e. it lowers the overpotential needed to drive catalysis.

(iv) Efficient interfacing of the catalyst and the photosensitizer via a redox-active tyrosine Yz.

(v) Regulated substrate (water) delivery and product release (O₂, H⁺) via protein channels.

(vi) Protection and repair mechanisms that limit oxidative damage of the protein pocket and replace the protein scaffold every 30 min under full sunlight [39]. It is noted that the water splitting catalyst binds to the protein and assembles spontaneously from free Mn4O5Ca and Ca2⁺ in solution under visible light, a process called photoactivation [40].

**Figure 3.** (a) Electron transport chain of PSII linking reaction centre photochemistry to the site of water splitting catalysis. (b) The catalytic (Kok) [33] cycle of the cofactor showing the sequence of electron and proton withdrawals and the time constant for each transition [34].
5. The structure of the catalyst

The arrangement of the five metal ions and the connectivity of the cofactor (oxo bridges) have been resolved by conventional X-ray crystallography using synchrotron radiation (XRD) [11] supplemented by femtosecond X-ray crystallography using a free electron laser (XFEL) [41]. The structure represents a distorted chair where the back of the chair is defined by a plane containing Mn₁, Mn₃, and Mn₄. A second plane containing the Ca ion and Mn₂ defines the front of the chair. The Mn–Mn distances seen in the XRD structure are systematically longer than those seen in extended X-ray absorption fine structure (EXAFS) and in model complexes [42–46]. This is not the case for the XFEL structure, where the same Mn–Mn distances as seen in EXAFS are observed. The longer Mn–Mn distances in the XRD structure are thought to reflect reduction [47–49] of the Mn ions, and as such the XFEL structure is considered more reliable.

Our own work has concentrated on the development of methods for calculation of spectroscopic observables from magnetic resonance and X-ray spectroscopies, to further refine structural models of the cofactor and precisely describe the electronic structure of the catalyst in all catalytic states [50,51]. Our starting point was the structure of the cofactor in the S₂ state, for which extensive spectroscopic data exist, in which the net oxidation state of the cofactor is Mn⁴⁺[52]. Theoretical modelling has shown that the cofactor can adopt two interconvertible core topologies in this state [52–54], an open cubane (S₂⁰) and a closed cubane motif (S₂¹) (figure 4). The S₂⁰ form is slightly lower in energy by ca 1 kcal mol⁻¹ and displays an electronic ground state of spin S_G = 1/2. In this structure, the only Mn⁴⁺ ion of the cofactor, which is five-coordinate, is found within the cuboidal unit (Mn₁, figure 5a). In the S₂¹ form, the cofactor instead displays an electronic ground state of spin S_G = 1/2. In this structure, the five-coordinate Mn³⁺ ion is now located at the terminal Mn₄ (figure 5b) [52–54]. Movement of the Mn³⁺ site is thus coupled to a reorganization of the core connectivity of the cofactor, involving the movement of a single µ-oxo bridge (OS) (figures 4 and 5a). In the open structure (S₂⁰), OS represents a bis-µ-oxo linkage between Mn₃ and Mn₄ (open form, S₂⁰), whereas in the closed structure it becomes (S₂¹) a vertex of the heterobimetallic cubane unit. These two structures show a one-to-one correspondence with the two well-known electron paramagnetic resonance (EPR) signals for the S₂ state, the multiline (S = 1/2) and g₄ (S = 5/2) signals (figure 5a) [52].

Recent multifrequency EPR measurements from our laboratory have now resolved the net oxidation state of the S₃ state (figure 5b). We find the net oxidation state of the cofactor is Mn⁴⁺[56] categorically ruling out a ligand centred oxidation during the S₂–S₁ transition as has been proposed in the literature. Concomitant double resonance data suggest that the cofactor adopts a single configuration (unlike the S₂ state), which is best described as an open cubane (S₂⁰) structure (figures 4 and 5b). Importantly, these results require that all four Mn ions are six coordinate in the S₃ state. This constraint necessitates the binding of a new water molecule to the open coordination site of the cofactor in the form of a hydroxide (figures 4 and 5b) [56,59]. Further evidence for water binding during the S₂–S₁ transition comes from work of the Noguchi laboratory [60]; their Fourier transform infrared spectroscopic data suggest that the solvation of the cluster increases during the S₂–S₁ transition. Interpretations of X-ray absorption near-edge structure data of the S₂–S₃ state are also consistent with a water binding event, where the absence of a large edge shift may reflect the five-coordinate Mn³⁺ ion of the S₂ state becoming six coordinate in the S₃ state [61].

From this starting point, i.e. an understanding of the atomic structure of the S₂ and S₃ states that includes assignments of the energetically most favourable and spectroscopically consistent protonation states of all titratable ligands [55], we recently extended our spectroscopy-oriented theoretical modelling to the S₀ and S₁ states. The guiding principle for selecting the best models was again maximal consistency with experimental observables, including spin states (S_G = 1/2 for S₀ and S_G = 0 for S₁) [56,62,63], Mn hyperfine couplings, metal–metal distances from EXAFS and profiles of Mn K-pre-edge X-ray absorption spectra [55]. Figure 4 summarizes the results of this effort, showing the cofactor geometry, protonation states of titratable groups and individual Mn oxidation states for all spectroscopically consistent S-states models of the Kok cycle. 

**Figure 4.** Models for each S state of the Kok cycle, showing the optimized geometry, protonation pattern and Mn oxidation states of the inorganic core, modified from Krewald et al. [55]. The cofactor exists in two forms in the S₂ state, an open cubane (S₂⁰, S_G = 1/2) and a closed cubane (S₂¹, S_G = 5/2) structure which differ in their core connectivity by the reorganization of OS [52].
6. The substrate (water) molecules of the oxygen evolving complex

Experiments performed with isotopically labelled water ($^{17}$O/H$_2^{18}$O) provide a means to identify the two substrate binding sites of the water oxidizing cofactor. Earlier membrane-inlet mass spectrometry (MIMS) results [64], which monitor the uptake of H$_2^{18}$O into the product O$_2$ molecule, have demonstrated that the complex contains two chemically different substrate sites: an early ($W_s$) and late ($W_f$) binding substrate site, both of which exchange with bulk water in all catalytic states (S-states) [65–67]. Owing to the relatively slow rate of exchange of $W_s$ ($\approx 1$ s$^{-1}$), and its dependence on the oxidation state of the Mn tetramer, $W_s$ is usually considered to be an oxygen ligand of one of the Mn ions [38,67]. By using water labelled with the magnetic isotope ($^{17}$O, $I = 5/2$), the same substrate binding site ($W_s$) can be characterized spectroscopically, using the EPR technique ELDOR-detected NMR (EDNMR) [13,58,68]. These measurements identify a unique, exchangeable $\mu$-oxo bridge as a potential candidate for $W_s$ and the identity of the exchangeable $\mu$-oxo bridge has been inferred from small site perturbations of the cofactor (figure 5c).

Ammonia, a substrate water analogue, is known to bind to the water oxidizing cofactor in the S$_0$ state [69,70]. Its binding mode was recently determined as a terminal ligand to the Mn$_4$ ion, replacing the water ligand $W_1$ [71,72]. Such a binding motif readily explains the observed hyperfine and quadrupole couplings; the asymmetric quadrupole coupling is a property of the H-bonding network of the W1/NH$_3$ site (Asp61) [57,72]. $W_1$ displacement as opposed to other binding modes ($\mu$-oxo bridge displacement) is also preferred based on energetic considerations and an analysis of available vibrational data [71–73]. In samples where NH$_3$ is added, the hyperfine coupling of the exchangeable $\mu$-oxo bridge is strongly perturbed (ca 30%) [57,72]. This result is readily understood as due to a trans effect: the $W_s$ ligand is trans to the O$_5$ bridge. Displacement of $W_1$ by NH$_3$ elongates the O$_5$–Mn$_4$ distance perturbing the observed hyperfine coupling (figure 5c,d) [71].

The assignment that the exchangeable $\mu$-oxo bridge is $O_5$ is further supported by measurements on PSII, where the Ca$^{2+}$ ion is biosynthetically replaced with Sr$^{2+}$ [57]. In such samples, the hyperfine coupling of the exchangeable $\mu$-oxo bridge is perturbed (ca 5%, figure 5c). As O$_5$ is a ligand to the Ca$^{2+}$/Sr$^{2+}$ site this result is again easily understood. Furthermore, in samples where the Ca$^{2+}$ is replaced by Sr$^{2+}$ and NH$_3$ is bound to the cofactor, an additive effect is seen on the exchangeable $\mu$-oxo bridge hyperfine coupling (ca 35%) [57], again consistent with the notion that O$_5$ represents the exchangeable $\mu$-oxo bridge (figure 5c).

The above results on Ca$^{2+}$/Sr$^{2+}$ exchange point to O$_5$ representing $W_c$. This hypothesis comes from correlation to MIMS results which show that Ca$^{2+}$/Sr$^{2+}$ replacement perturbs the rate at which $W_c$ exchanges with bulk water [38,67,74]. As Ca$^{2+}$/Sr$^{2+}$ replacement similarly affects the electronic properties of O$_5$ (but no other exchangeable oxygen ligand) [57], this result can again be easily understood. We note that the fast rate of exchange of O$_5$ when compared with that of $\mu$-oxo bridges in simple synthetic model systems is likely due to the unusual flexibility of its metal coordination (figures 4–6) [52,53].

Assignment of the second substrate site $W_i$ is less clear. The above description of the structure of the S$_0$ state suggests that the second substrate would represent the newly bound terminal hydroxide ligand, in close proximity to O$_5$/$W_s$.  

---

**Figure 5.** (a) CW-EPR data of the two forms of the S$_2$ state ($S_a^2$ and $S_b^2$) modified from Cox et al. [13]. (b) High field (W-band) data of the S$_2$ state consistent with an open $S_a^2$ type structure, modified from Cox et al. [56]. (c) $^{17}$O-EDNMR data of exchangeable oxygen sites of the Mn$_4$O$_5$Ca cofactor, modified from Lohmiller et al. [57]. The splitting of the largest doublet (green, O$_5$) is sensitive to both Ca$^{2+}$/Sr$^{2+}$ replacement and W$_1$/NH$_3$ exchange. (d) Replacement of W$_1$ by NH$_3$. Also shown are the three different sites where $^{17}$O labelled water is taken up by the cofactor: (i) as a ligand to the Ca$^{2+}$ ion, (ii) as a ligand to the outer Mn$_4$ ion and (iii) as a $\mu$-oxo bridge (O$_5$) [58].
This result though appears at odds with MIMS data that show that $W_f$'s exchange kinetics are almost identical for the $S_2$ and $S_3$ states [13,37,75], suggesting that it is associated with the Mn$_4$O$_5$Ca cofactor in the $S_2$ and $S_3$ states in the same way [38,75]. A possible solution is that $W_f$ represents a pre-existing water ligand of the cluster (W2 bound to the Mn4 ion or W3 bound to Ca$^{2+}$ ion) which is relocated in the $S_3$ state, suggesting a concerted mechanism for substrate delivery [38,71,75,76].

7. The reaction cycle of the catalyst

As described above, the water oxidizing cofactor takes two discrete conformations during its catalytic cycle and as such, its reaction cycle can be divided into two (figure 6). In the ‘resting’ $S$-states ($S_0$, $S_1$), the cofactor adopts a low-spin configuration ($S = 1/2$ and 0) [62,63]. Calculations demonstrate that this is a property of the incomplete cuboidal unit [52,55], i.e. a manganese unit with a vacant coordination site. In this form, the catalyst is inactivated. In the ‘activated’ $S$ states, the cofactor instead adopts a high-spin configuration ($S = 3$) [56], concomitant with the insertion of a water ligand into the cubodial unit. $S_2$ displays both low- and high-spin forms ($S = 1/2$ for the open cubane form $S^O_2$, $S = 5/2$ for the closed cubane form $S^C_2$) suggesting that it may represent a switching point in the catalytic cycle (figure 6). Spin-state interconversion, which involves the opening of the cubodial unit may facilitate water insertion/relocation into the heterobimetallic cubane. It is noted that EPR data suggest that Ca$^{2+}$ is necessary to proceed past the low-spin $S_2$ $YZ$ state, and thus Ca$^{2+}$ may play a role either in allowing efficient $S_2^O/S_2^C$ interconversion, or at least in allowing spin-state crossover [77–79]. It is then the high-spin topology that allows low barrier single-product formation to proceed, for example, via an oxo–oxyl coupling mechanism as proposed by Siegbahn [56,59,80]. Late second substrate binding may avoid deleterious two-electron chemistry (slow peroxide formation) in the resting states of the catalytic cycle [81].

8. Conclusion and outlook

The results described above place new, important restrictions on the structure of nature’s water oxidizing cofactor, its catalytic cycle and the mechanism of water splitting and O$_2$ evolution. These are listed below with reference to design criteria for the development of biomimetic and bioinspired water splitting catalysts using manganese.

(i) The oxidation states of all individual Mn sites in all $S$-states are now resolved: $S_0$ (Mn$^{III}_3$)Mn$^{IV}$, $S_1$ (Mn$^{III}_3$)(Mn$^{IV}$)$_2$, $S_2$ Mn$^{III}_2$(Mn$^{IV}$)$_2$, $S_3$ (Mn$^{IV}_4$) and ligand oxidation can be excluded prior to formation of the $S_3$ state. This assignment is in agreement with the average Mn oxidation state inferred for heterogeneous manganese oxide water oxidation catalysts [82].

(ii) An exchangeable \(\mu\)-oxo bridge (O5) is likely the first substrate of the water splitting reaction. This result, coupled with the observation that the cofactor gains a new hydroxide ligand in the $S_3$ state supports the notion that the O–O bond forms between oxygen ligands of neighbouring redox-active Mn ions (Mn$_1$ and Mn$_4$).

(iii) Structural flexibility of the cofactor appears to be critical for function, namely catalyst activation, i.e. the formation of the $S_3$ state, which is coupled to second substrate inclusion. This flexibility is only readily apparent in the $S_2$ state, with the other $S$-states adopting a single preferred configuration. This property seems to be conferred in part by the Ca$^{2+}$ ion, which also may act to tune the redox/magnetic properties of the cuboidal (Mn$_3$Ca) unit [83], or of the tyrosine radical [36] or both. The inclusion of calcium has been correlated with enhanced water splitting activity of manganese oxide water oxidation catalysts [82,84].

The heterobimetallic nature of the cluster and the choice of Mn as the redox active metal for biological water
splitting must therefore reflect a confluence of effects. Key properties are

(i) that it can stabilize multiple oxidation states to be accessed over a narrow range of redox potentials. This property is derived from protein/cofactor interactions being able to tune the $pK_a$ of cofactor bound water (substrate) molecules allowing sequential $\text{H}^+$ release upon each Mn oxidation event;

(ii) the possibility that the heterobimetallic cubane allows the formation of an energetically accessible Mn$^{IV}$-oxo or Mn$^{V}$-oxyl radical in the transition state [85–88]; and

(iii) that the Mn ions represent trapped valence states of local high-spin states, which magnetically couple together in a precise way in each S-state.

While the points above pose interesting theoretical questions, from a more practical perspective, the static properties of heterobimetallic cubane appear to be universal and can be reproduced in simpler model compound systems [89–91]. Similarly, heterogeneous Mn/Ca materials already show reasonable, if somewhat slow water splitting capacity normalized to Mn content [82,92,93]. Although it cannot be excluded that such Mn/Ca materials adopt lower efficiency pathways for O–O bond formation, the alternative explanation, that such materials only contain an intrinsically small number of ‘active’ heterobimetallic cubane units, seems equally reasonable. If, as figure 6 suggests, nature’s catalyst essentially toggles between an ‘inactive’ and ‘active’ form, the latter having a substrate bound within the heterobimetallic cubane, the challenge in designing a better catalyst amounts to introducing this property (structural flexibility) or stabilizing a solvent accessible cuboidal structure that is stable/robust. These key design criteria should form the basis for new synthetic complex development—akin to the progress recently made in $\text{H}_2$ production using hydrogenase mimics [9]—for water splitting catalysts using manganese.

Acknowledgements. We thank Dr Alain Boussac (Saclay, France) and Prof. Johannes Messinger (Umeå, Sweden) for helpful discussions.

Funding statement. This work was supported by the MPG, the Cluster of Excellence RESOLV (EXC 1069) and the DIP (Lu 315/17-1), both funded by the Deutsche Forschungsgemeinschaft (DFG).

References


43. Haumann M et al. 2005 Structural and oxidation state changes of the photosystem II manganese complex in four transitions of the water oxidation cycle: S0 → S1 → S2 → S3 + S5 and S5 + S6 → S1 characterized by X-ray absorption spectoscopy at 20 K and room temperature. *Biochemistry* 44, 1894 – 1908. (doi:10.1021/bi040679e)


