and subsequent sedimentation of the active particles resulted in the images appearing on the surface of the solution (see Fig. 5 of the paper). This layered segregation of active and passive particles enabled the authors to embed a series of images one after the other, because each image was generated solely in response to the reorganization of particles activated by the projection.

With their innovative use of light-sensitive particles, Zheng *et al.* might well have laid the foundations for future technologies such as electronic paper and camouflage materials. But the timescale on which the particles segregate is around one to two minutes, which makes their application to electronic paper possible, but not yet feasible. Similarly, electronic camouflage materials need to be made from components that respond to light intensities that are much lower than that required to activate these particles. However, Zheng and colleagues say they think that the segregation times and sensitivity of their light-responsive ink can be improved, so these exciting applications might soon be realized.

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Structural biology

Clues to how water splits during photosynthesis

Dimitrios A. Pantazis

The tools of crystallography, spectroscopy and quantum chemistry are pulling back the curtain on photosynthesis, probing previously elusive catalytic intermediates that arise when water splits to form oxygen. **See p.623 & p.629**

The step during photosynthesis in which water is split through a light-driven reaction to generate oxygen (O_2) is a fundamental process for life on Earth. Grasping how this occurs might offer inspiration for the development of water-splitting technologies to produce solar fuels. Bhowmick *et al.*[1] (page 629) and Greife *et al.*[2] (page 623) now shed light on this process, paving the way for a more complete understanding of the underlying mechanism.

Research efforts over the past four decades have refined our understanding of crucial catalytic steps that occur in a cluster of four manganese ions and one calcium ion that are connected by bridging oxygens (Mn_4CaO_5). This cluster of ions is known as the oxygen-evolving complex (OEC), and it is embedded in an enzyme called photosystem II. To oxidize water, the OEC first needs to lose up to four electrons from its manganese ions, generating a series of intermediate states that are termed S-states (Fig. 1). During the transitions between S-states, the removal of electrons from the OEC is coupled with the removal of hydrogen ions (H^+, a single proton) from the water, and with the binding of water to the OEC, all of which is regulated by the functionally important protein that surrounds the OEC.

> **“This tremendous achievement comes as close as possible to real-time observation of the oxygen-evolving complex at work.”**

The first four S-states in the catalytic cycle (S_0, S_1, S_2 and S_3) can be captured and studied, and scientists have a reasonably advanced, but incomplete, understanding of their nature. By contrast, it has been exceedingly difficult to resolve the complex cascade of events in the final part of the cycle, the $S_3 \rightarrow S_4 \rightarrow S_0$ transition that involves: the formation of a reactive S_4 intermediate through the loss of an electron; the formation and release of O_2; the release of two protons; and the binding of one water molecule to return the OEC to the starting position (S_0) for the next catalytic cycle.

Bhowmick *et al.* used a technique called serial femtosecond crystallography using X-ray free electron lasers (XFELs) to probe structural changes at the enzyme's active site during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. In contrast to conventional protein crystallography methods, this technique allows rapid collection of time-resolved structural data at room temperature. Snapshots obtained at different microsecond-to-millisecond intervals after light activation of the S_3 state document time-dependent structural changes at the tetramanganese cluster of the OEC and its protein environment.

Bhowmick and colleagues measured changes in the electron density indicating that after light exposure of the S_3 state, oxidation (electron loss) of a nearby tyrosine amino-acid residue, termed Y_Z, occurs within 50 microseconds (μs). Y_Z mediates electron transfer from the OEC to a special pair of chlorophyll molecules. Formation of the resulting tyrosine radical triggers an early deprotonation event (at 200–500 μs) that involves a manganese-bound water ligand and two amino-acid residues, Asp61 and Glu65. This is a remarkable observation that points directly to a specific hydrogen-bond network as the preferred route for proton removal.

The Y_Z radical subsequently extracts an electron from the manganese cluster, which then progresses to the transient S_4 state. This process begins after approximately 500 μs; by about 700 μs, the signal for an oxygen atom that was incorporated in the OEC during the previous catalytic step ($S_2 \rightarrow S_3$) begins to disappear. This is interpreted to be one of the two O_2-forming atoms. The source of the other oxygen atom is not clear, but it might be an adjacent oxygen in the manganese-containing cluster of atoms.

The subsequent data do not enable the generation of well-defined structural models until the S_0 state begins to be reconstituted (at about 2 milliseconds; ms), but the shortening (at approximately 1.2 ms) of the distance between the two manganese ions that are farthest apart is suggested to signify the presence of a partially formed O_2 molecule. This is presumably a bound peroxide ion (O_2^{2-}), and hence the shortening coincides with the onset of O_2 formation and release. This tremendous scientific achievement comes as close as possible to real-time observation of the OEC at work.

Greife *et al.* used an approach known as time-resolved microsecond Fourier-transform

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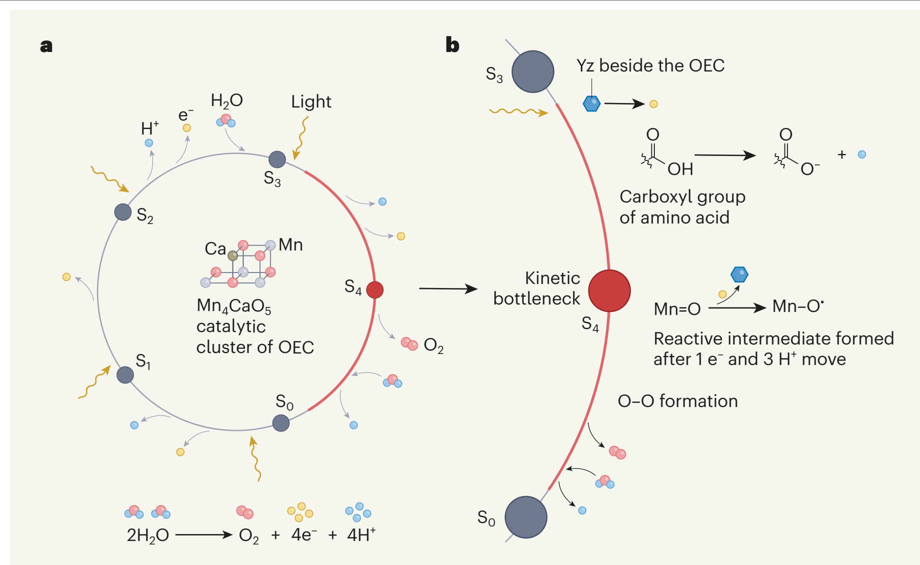

Figure 1 | Key steps in photosynthesis. a, A light-dependent reaction splits water into oxygen (O_2), hydrogen ions (H^+) and electrons (e^-). This occurs in a region of the enzyme photosystem II called the oxygen-evolving complex (OEC), which is a catalytic cluster of manganese (Mn), calcium (Ca) and oxygen ions. The OEC cycles between states termed S-states. **b**, Bhowmick *et al.*[1] and Greife *et al.*[2] reveal the sequence and timing of individual steps during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. These events include electron and H^+ movements (exits or transfers) in and around the OEC that involve Y_Z and other amino-acid residues. At the S_4 stage, a crucial reactive group (Mn–O$^\bullet$) is generated, and this step is rate-limiting (a kinetic bottleneck) for the entire reaction. The wiggly line across the bond attached to the carboxyl group indicates that the group is attached to an amino-acid residue.

infrared spectroscopy (FTIR) to study the kinetics of the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. Building on a remarkable experimental set-up that enabled detection of time-resolved vibrational spectra of the OEC over thousands of catalytic cycles, the authors identified distinct processes that occur during the transition. The results suggest that formation of the Y_Z radical after the S_3 state is followed by a deprotonation event at 340 µs, for which the spectral features can be associated with an amino-acid residue. The authors' quantum-chemical calculations attribute this to either Asp61 or a pair of residues (Glu65 and Glu312), in remarkable agreement with Bhowmick and colleagues' conclusion.

Formation of a reactive manganese-bound oxygen radical (Mn–O$^\bullet$, the S_4 transient reactive intermediate) involves an extraordinary simultaneous transfer of one electron and three protons, and is identified as the rate-limiting step (kinetic bottleneck) of the whole reaction. The subsequent O–O bond formation and O_2 release are faster, by comparison. This shows that preparing for O–O bond formation is harder than actually performing this step, which makes sense if, in this way, the enzyme avoids the accumulation of highly reactive intermediate products.

Both studies provide crucial insights into, and new constraints on, the mechanism of biological water oxidation. The studies complement each other as well as previous work[3,4] on the timing of the early deprotonation event, the proton-exit pathway and the stability of the S_4 state. The mechanistic interpretations are broadly in line with expectations from previous computational studies[5] but offer a much-needed experimental framing.

This does not mean that the mechanism is fully solved. The XFEL snapshots of Bhowmick and colleagues cannot be viewed as 'freeze-frames' of a movie depicting a reaction pathway, because the proposed chemical events and intermediates are mostly inferred, rather than observed. Furthermore, a large part of the mechanism derived from the quantum-chemical approach presented by Greife *et al.*, although consistent with the experimental data, is not the only possible explanation. Lack of direct information about where the electrons are in the catalyst during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition means that no mechanistic scenarios can be safely excluded at this point. In view of the central role of Y_Z and the necessity of understanding proton movement, these are obvious targets for future investigation using complementary experimental approaches[6,7] and by theoretical methods. The hardest questions in biological water-splitting seem much closer to a final answer than ever before.

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Cancer neuroscience

How thought itself can drive tumour growth

George M. Ibrahim & Michael D. Taylor

Tumour cells can form connections with neurons in the brain. Examination of a variety of types of evidence concerning human brain cancer sheds light on how these tumour–neuron interactions affect cognition and survival times. **See p.599**

Few effective treatments are available for a common and universally fatal type of adult brain tumour called a malignant glioma. Although these tumours exist exclusively in the central nervous system, the interactions between malignant glioma cells and the 86 billion neurons in the human brain are poorly understood. This is particularly relevant because most people with the disease develop progressive cognitive decline that robs them of quality of life during their final months[1]. Krishna *et al.*[2] show on page 599 that malignant gliomas can grow by modifying brain circuitry, thus taking cognitive function away from their host and ultimately leading to death. These insights might lead to fundamentally new approaches to glioma treatment and provide a means of limiting cognitive decline in affected individuals.

The human brain is a complex system that involves highly coordinated interactions between large-scale specialized groups of

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