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## **Alternating electron and proton transfer steps in photosynthetic water oxidation**

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**Abbreviations:** ET, electron transfer; KIE, kinetic isotope effect; PBD, photothermal beam deflection; PSII, photosystem II;  $S_i$ , intermediate state of the water oxidation cycle; XAS, X-ray absorption spectroscopy,  $Y_Z$ , redox-active tyrosine residue (Tyr161) in the D1 subunit of PSII.

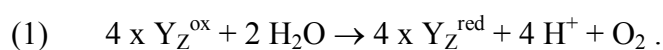
## Abstract

Water oxidation by cyanobacteria, algae, and plants is pivotal in oxygenic photosynthesis, the process that powers life on earth, and is the paradigm for engineering solar fuel production systems. Each complete reaction cycle of photosynthetic water oxidation requires the removal of 4 electrons and 4 protons from the catalytic site, a manganese-calcium complex and its protein environment in photosystem II (PSII). In time-resolved photothermal beam deflection (PBD) experiments, we monitored the apparent volume changes of the PSII protein that are associated with charge creation by light-induced electron transfer (contraction) and charge-compensating proton relocation (expansion). Two previously invisible proton removal steps were detected, thereby filling two gaps in the basic reaction-cycle model of photosynthetic water oxidation. In the  $S_2 \rightarrow S_3$  transition of the classical S-state cycle, an intermediate is formed by deprotonation clearly *before* electron transfer to the oxidant ( $Y_Z^{\text{ox}}$ ). The rate-determining elementary step ( $\tau \approx 30 \mu\text{s}$  at  $20^\circ\text{C}$ ) in the long-distance proton relocation toward the protein-water interface is characterized by a high activation energy ( $E_a = 0.46 \pm 0.05 \text{ eV}$ ) and strong H/D kinetic isotope effect ( $\text{KIE} \approx 6$ ). The characteristics of a proton transfer step during the  $S_0 \rightarrow S_1$  transition are similar ( $\tau \approx 100 \mu\text{s}$ ,  $E_a = 0.34 \pm 0.08 \text{ eV}$ ,  $\text{KIE} \approx 3$ ), however the proton removal from the Mn-complex proceeds *after* electron transfer to  $Y_Z^{\text{ox}}$ . By discovery of the transient formation of two further intermediate states in the reaction cycle of photosynthetic water oxidation, a temporal sequence of strictly alternating removal of electrons and protons from the catalytic site is established.

## Introduction

In oxygenic photosynthesis, plants, algae, and cyanobacteria oxidize water at the manganese-calcium ( $\text{Mn}_4\text{Ca}$ ) complex of photosystem II (PSII) (1-3). This process has shaped the atmosphere by massive  $\text{O}_2$ -formation (from water) and the biosphere by facilitating the large-scale production of primary biomass and energy-rich carbohydrates (4), but still is only insufficiently understood. Improved insight into photosynthetic water oxidation could promote the development of biomimetic systems for direct production of solar fuels (3, 5-9).

In PSII, the absorption of a light quantum results in oxidation of a specific tyrosine (10),  $\text{Y}_Z$ , which functions as the oxidant in the redox chemistry of water oxidation (9, 11-12):



The actual catalyst facilitating the reaction described by Eqn. 1 is the  $\text{Mn}_4\text{Ca}$  complex bound to the proteins of PSII (Fig. 1A, (13-15)). Four electrons are removed sequentially from the Mn-complex (that is, the  $\text{Mn}_4\text{Ca}(\mu\text{-O})_n$  core and its ligand environment) by electron transfer to  $\text{Y}_Z^{\text{ox}}$ , resulting in accumulation of four oxidizing equivalents before the onset of O-O bond formation and  $\text{O}_2$  liberation, as described by Kok's classical S-state cycle (Fig. 1B, inner circle of S-states) (16-17). Four protons are removed by deprotonation of the Mn-complex and relocation toward the aqueous phase of the thylakoid lumen (18-20).

The location of the Mn-complex at the interface between the membrane-intrinsic part of PSII and the extrinsic luminal proteins (Fig. 1A) (13-15) implies long-distance proton relocation toward the aqueous phase ( $\sim 30 \text{ \AA}$ ), occurring within tens or hundreds of microseconds along chains of water molecules and ionic residues (21). The interrelation between electron transfer and protonation dynamics (that is, the relocation of protons on various time and length scales) is functionally crucial (1, 22-25). Our study aims at identification of the basic sequence of electron transfer (ET) and long-distance proton relocation in the water oxidation cycle.

The electron transfer from the Mn-complex to  $\text{Y}_Z^{\text{ox}}$  (25-29) and the proton release, i.e. the appearance of protons in the aqueous phase (18-20), have been investigated extensively. It was found (inter alia) that the observable proton release often does not reflect the removal of a proton from the Mn-complex because electrostatically induced deprotonation of residues at the luminal periphery of the protein masks the protein-intrinsic proton removal (20, 30). Nonetheless it was possible to determine the 'intrinsic proton-release pattern', i.e. the number of protons removed in each of the classical S-state transitions from the Mn-complex, which is: 1  $\text{H}^+$  in  $\text{S}_0 \rightarrow \text{S}_1$ , 0  $\text{H}^+$  in  $\text{S}_1 \rightarrow \text{S}_2$ , 1  $\text{H}^+$  in  $\text{S}_2 \rightarrow \text{S}_3$ , and 2  $\text{H}^+$  in  $\text{S}_3 \rightarrow \text{S}_0$  (18-19, 31). The

appearance of protons in the aqueous bulk phase rapidly after  $Y_Z^{\text{ox}}$  formation and prior to its reduction by ET from the Mn-complex has been established firmly (19-20, 30), but likely does *not* reflect the deprotonation of a chemical group (e.g., a substrate water molecule) at the Mn-complex (18-19, 31). This implies that the time-resolved detection of proton-release into the aqueous phase cannot be employed to decide whether the proton removal from the Mn-complex precedes the ET to  $Y_Z^{\text{ox}}$ .

The temporal sequence of electron and proton removal steps after rapid  $Y_Z^{\text{ox}}$  formation therefore has remained largely obscure, with one notable exception. Today there is strong experimental support that after formation of the  $S_3Y_Z^{\text{ox}}$  state in the  $S_3 \rightarrow S_0$  transition, a proton is removed from the Mn-complex before onset of the electron transfer to  $Y_Z^{\text{ox}}$  (25-26, 32). This finding has led to an extension of the S-state cycle involving formation of a distinct  $S_4$  state by deprotonation (inner circle of Fig. 1B) before  $S_4'$  formation by electron transfer (17, 25). Later this reaction cycle was extended further to include each of the 4 protons (outer circle of Fig. 1B) (23, 33). However the proposed sequence of events has remained hypothetical, in particular because the proton removal from the Mn-complex in the  $S_0^+ \rightarrow S_1^n$  and  $S_2^+ \rightarrow S_2^n$  transitions could not be tracked in time-resolved experiments.

To detect proton removal from the Mn-complex, we employ a photothermal beam deflection (PBD) experiment exploiting the high sensitivity of the PBD signal to density changes (34-38), which in the following are discussed in terms of apparent volume changes of the protein. Expansion and contraction of the PSII complex were monitored with microsecond resolution at a precision of about  $2 \text{ \AA}^3$  per PSII. For comparison, the average volume of one water molecule in aqueous solution is  $\sim 30 \text{ \AA}^3$ . In photoacoustic or photothermal measurements, it is usually found that a volume contraction results from the (light-induced) deposition of charges at the electron donor and/or acceptor, as has been shown for synthetic molecules (39-40) and for photosystems (41-42). Also in PSII, the light-induced formation of  $Q_A^-$  and  $Y_Z^{\text{ox}(+)}$  is associated with a volume contraction (37, 43). The de-charging of the donor side by removal of a proton results in an expansion that reverts the  $Y_Z^{\text{ox}(+)}$  contribution to the preceding contraction. This enables monitoring of proton removal from the Mn-complex by measuring the concomitant volume expansion in the PBD experiment, thereby revealing the temporal sequence of electron and proton removal steps in the classical  $S_0 \rightarrow S_1$  and  $S_2 \rightarrow S_3$  transitions.

## Results

For insight in the individual S-state transitions, PBD measurements were combined with the following laser-flash protocol (Fig. S1): Dark-adapted PSII membrane particles were excited by a sequence of  $n$  saturating ns-laser flashes (532 nm,  $10 \text{ mJ cm}^{-2}$ ). Each flash populated predominantly a specific (semi-)stable S-state of Kok's classical reaction cycle (16-17), namely  $S_1$  ( $n = 0$ , dark-adapted PSII),  $S_2$  ( $n = 1$ ),  $S_3$  ( $n = 2$ ),  $S_0$  ( $n = 3$ ), and again  $S_1$  ( $n = 4$ ). Subsequently a single sub-saturating ns-flash ( $0.1 \text{ mJ cm}^{-2}$ ) was applied and the thereby induced PBD signal was recorded. The sub-saturating flash initiated predominantly the following transitions:  $S_1 \rightarrow S_2$  (flash 1),  $S_2 \rightarrow S_3$  (flash 2),  $S_3 \rightarrow S_0$  (flash 3), and  $S_0 \rightarrow S_1$  (flash 4). The measured PBD signals were corrected for imperfect advancement in the S-state cycle (S-state mixing) using previously established procedures (26, 44) (Fig. S2). We note that all central conclusions of this work are independent of the details of the correction procedure. Corrected PBD transients for each of the four transitions between semi-stable S-states are shown in Fig. 2 and discussed in the following.

*The instantaneous rise* observed after each flash (at 25 °C) is attributable to the rapid light-induced processes that result in reduction of the primary quinone acceptor ( $Q_A^-$ ) and oxidation of the tyrosine donor ( $Y_Z^{\text{ox}(+)}$ ) (11). At 12 °C, the magnitude of the instantaneous rise was smaller than at 25 °C, and at 1 °C, a decay rather was observed (Fig. 2B). This behavior results from the temperature dependent thermal ( $\Delta Q$ ) and temperature-independent non-thermal ( $\Delta V$ ) contributions to the PBD signal (34-36) associated with the  $Y_Z^{\text{ox}(+)}$  $Q_A^-$  formation. Evaluation of the temperature dependence of the rapid phase in comparison to a calorimetric standard (Fig. S3) yielded an apparent volume contraction ( $\Delta V$ ) by about  $-12 \text{ \AA}^3$ , in reasonable agreement with previous estimates (37, 43). For a possible contribution to the PBD signals associated with interquinone electron transfer see Figs. S6 and S7. (We note that all herein presented  $\Delta V$  values were not corrected for the effective quantum yield,  $\Phi_{\text{eff}}$ , of the light-induced transition, which could be as low as 50% (see SI). This means that the volume changes per PSII complex may be twice as large as the herein documented values.)

*In the  $S_1 \rightarrow S_2$  transition (flash 1)*, the instantaneous  $Y_Z^{\text{ox}(+)}$  $Q_A^-$  signal ( $<10 \text{ \mu s}$ ) was followed by an exponential decay with a time constant of about  $100 \text{ \mu s}$  at 20 °C (Figs. 2, 3; Table 1). The amplitude of the decay phase depended only weakly on the temperature suggesting that it mostly originates from a volume change ( $\Delta V$ ) of the PSII sample; its negative amplitude (PBD signal decrease) indicates a contraction. The weak temperature dependence of the signal magnitude suggests a small contribution to the signal from a positive  $\Delta Q$  (heat release) (Table

1). The decay became only slightly slower at lower temperatures and the Arrhenius plot of the rate constants (Fig. 3B) revealed a small activation energy (Table 2). From PBD transients measured in D<sub>2</sub>O (Fig. 4), a minor H/D kinetic isotope effect (KIE) of 1.3 was determined. (The KIE is the ratio of time constants determined in D<sub>2</sub>O or H<sub>2</sub>O;  $KIE = \tau_D/\tau_H = k_H/k_D$ .) Time constant, activation energy, and KIE agree well with figures previously determined for the ET from the Mn-complex to  $Y_Z^{ox}$  in the  $S_1 \rightarrow S_2$  transition (Fig. 1B) (25-27).

The large contraction associated with the ET from the Mn-complex to  $Y_Z^{ox}$  is specifically observed in the  $S_1 \rightarrow S_2$  transition. For  $S_2 \rightarrow S_3$ , such a contraction paralleling the ET step was not detectable (Figs. 2B and S7). In the  $S_0 \rightarrow S_1$  transition, a small contraction might be coupled to the ET step (Fig. S4). We note that the contraction associated with the ET in the  $S_1 \rightarrow S_2$  transition, which likely is reversed in the  $S_3 \rightarrow S_0$  transition (see below), could reflect an interesting new mode of coupling the ET step to nuclear rearrangements, possibly related to changes in the protein backbone conformation suggested by FTIR data (45).

*In the  $S_2 \rightarrow S_3$  transition (flash 2)*, the instantaneous  $Y_Z^{ox(+)}Q_A^-$  rise was followed by an exponentially rising phase with a time constant of about 30  $\mu$ s (at 20 °C, Figs. 2 and 3). This rise was roughly ten times faster than the ET from the Mn-complex to  $Y_Z^{ox}$  (30  $\mu$ s versus 300  $\mu$ s, Table 2) and thus is assignable to a process that precedes the ET step. Its almost temperature-independent signal amplitude (Fig. 3A) indicates that the rising phase originates mostly from a volume expansion (Table 1). A striking feature of the rapid rise was its large KIE of close to 6 (Fig. 4, Table 2), facilitating the assignment to a process involving proton movements. We emphasize that at all temperatures and also in D<sub>2</sub>O, the apparent volume expansion clearly preceded the  $Mn_4Ca \rightarrow Y_Z^{ox}$  ET step (Table 2). Thus we conclude that in the classical  $S_2 \rightarrow S_3$  transition, a proton relocation precedes the ET from the Mn-complex to  $Y_Z^{ox}$ .

*In the transition  $S_3 \rightarrow S_0 + O_2$  (flash 3)*, a prominent millisecond rise ( $\tau$  around 2 ms at 20 °C) was visible that resulted mostly from a volume expansion by about 15  $\text{\AA}^3$  (Figs. 2 and 3A, Table 1). The moderate activation energy and small KIE of the millisecond phase (Figs. 3 and 4, Table 2) were similar to the respective values for the ET step ( $S_3^n \rightarrow S_4^+$ ) and the concomitant dioxygen formation ( $S_4^+ \rightarrow S_0^+ + O_2$ ) (26-27, 46-47).

Using time-resolved X-ray spectroscopy and near-UV measurements to monitor the oxidation state of the Mn-complex, it has been found that an apparent lag phase of about 200  $\mu$ s duration precedes the millisecond rise of O<sub>2</sub> formation. (25-27, 32). A similar lag phase was not discernable in the PBD transients. Instead, a rising phase with a similar  $\tau$ -value as the

previously observed lag phase could be detected by simulation of summed PBD transients (Fig. S5). However, this phase was not sufficiently well resolved for analysis of its temperature dependence and quantitative determination of  $\Delta Q$  and  $\Delta V$ . Conservatively, we conclude that the PBD data is compatible with a volume expansion associated with proton release in the  $S_3^+ \rightarrow S_3^n$  transition. Thus we propose that the overall extent of the expansion in the  $S_3 \rightarrow S_0$  transition is explainable by three additive contributions, namely proton removal from the Mn-complex prior to the ET, reversal of the contractions associated with previous manganese oxidation (in the  $S_1 \rightarrow S_2$  transition), and removal of a second proton from the Mn-complex after the ET (see SI for a more quantitative consideration).

*In the  $S_0 \rightarrow S_1$  transition (flash 4)*, the initial signal increase was followed by an exponentially rising phase with a time constant of  $\sim 100 \mu\text{s}$  (at  $20^\circ\text{C}$ ). The amplitude of this signal rise was almost temperature independent indicating a volume expansion (Fig. 3A), similar to the signal rise in the  $S_2^+ \rightarrow S_2^n$  transition. Also further parameters of this phase in the  $S_0 \rightarrow S_1$  transition were similar, namely its large activation energy, its large KIE ( $\sim 3$ ), and the  $\Delta V$  magnitude (Tables 1 and 2, see also Table S2). The  $100 \mu\text{s}$  rise in the PBD signal was slower than the ET from the Mn-complex to  $Y_Z^{\text{ox}}$  ( $\sim 40 \mu\text{s}$  in (25, 29)) (Table 2), implying that the underlying process occurred after the ET step, that is after the  $S_0^n \rightarrow S_1^+$  transition (Fig. 1B).

We note that vastly different values have been reported for the ET rate constant of the  $S_0 \rightarrow S_1$  transition ranging from about  $40 \mu\text{s}$  to  $300 \mu\text{s}$  (at  $\sim 20^\circ\text{C}$ ) (27-29, 32, 48). For the same PSII samples used herein, we have previously determined a value of  $\sim 40 \mu\text{s}$  (25), suggesting that the ET step in the  $S_0 \rightarrow S_1$  transition is faster than the proton-removal step already at room temperature and even more so at lower temperatures and in  $\text{D}_2\text{O}$  (27-28). In conclusion, the volume expansion observed in the classical  $S_0 \rightarrow S_1$  transition results from a proton relocation (the  $S_1^+ \rightarrow S_1^n$  transition in Fig. 1B) that takes place after manganese oxidation by ET to  $Y_Z^{\text{ox}}$ .

## Discussion

*In the course of the classical  $S_2 \rightarrow S_3$  transition*, a volume expansion precedes the ET step and can be assigned to the removal of a proton from the Mn-complex in the  $S_2^+ \rightarrow S_2^n$  transition of the extended S-state cycle shown in Fig. 1B. This is a central finding of our investigation, as it directly supports the proton-first ET in the  $S_2 \rightarrow S_3$  transition and establishes a close analogy to the first steps ( $S_3^+ \rightarrow S_3^n \rightarrow S_4^+$ ) in the  $S_3 \rightarrow S_0 + \text{O}_2$  transition (25). This finding implies that a

previously undetected intermediate state ( $S_2^n$ ) is transiently formed by deprotonation. Why this proton removal step is associated with a volume expansion, is explained straightforwardly: the removal of a positive charge ( $H^+$ ) from the donor side of PSII reverts the preceding volume contraction caused by charging of the donor side by  $Y_Z^{ox(+)}$  formation. Detection of this proton removal step ( $t_{1/2}$  of only  $\sim 20 \mu s$  at pH 6.2) by analysis of electrochromic absorption changes of the PSII pigments may be feasible, but has not been achieved yet (28-29, 32), presumably because of technical limitations.

*In the course of the classical  $S_0 \rightarrow S_1$  transition*, a volume expansion occurs after the ET step and is assigned to proton removal from the Mn-complex in the  $S_1^+ \rightarrow S_1^n$  transition. In analogy to the  $S_2^+ \rightarrow S_2^n$  transition, this expansion is explained by removal of a positive charge ( $H^+$ ) from the donor side of PSII thereby reversing the contraction due to  $Y_Z^{ox(+)}$  formation. As opposed to the  $S_2^+ \rightarrow S_2^n$  transition, the deprotonation in the course of the classical  $S_0 \rightarrow S_1$  transition proceeds *after* oxidation of the Mn-complex (after  $S_1^+$  formation). This result supports the extended S-state cycle scheme (Fig. 1B) directly by revealing transient formation of the  $S_1^+$  intermediate.

*The kinetic parameters determined for proton removal* from the Mn-complex relate to the slowest step in the sequence of all elementary proton transfer steps of the long-distance relocation of a proton from the Mn-complex toward the aqueous phase. Presently the site and physico-chemical nature of the rate-determining steps are unknown. We determined similarly large activation energies and KIE values for proton removal from the  $Y_Z^{ox}S_2^+$  and  $Y_Z^{red}S_1^+$  intermediates, pointing to the formation of a transition state of relatively high energy and proton tunneling across a sizeable energetic barrier; a more elaborate analysis of the thermodynamic and kinetic parameters may provide deeper insight (49-50). Interestingly, activation energy and KIE for proton removal from the  $Y_Z^{ox}S_3^+$  intermediate are smaller, suggesting that site or/and nature of the rate determining proton transfer may differ. Identification of pathways and modes of proton transfer within PSII could be approached experimentally (e.g.) by time-resolved infrared spectroscopy (51-52).

*In extension of the classical S-state cycle model*, we have suggested a basic reaction-cycle model (23, 33) that involves 9 intermediate states and describes the temporal sequence of light-induced  $Y_Z$ -oxidation, electron transfer to  $Y_Z^{ox}$ , proton removal from the Mn-complex, and  $O_2$  formation (Fig. 1B). However with respect to the proton removal steps, the supporting evidence remained largely circumstantial. Only the  $S_3^+ \rightarrow S_3^n$  transition (or  $S_3 \rightarrow S_4$  (25)) had



become detectable in time-resolved experiments (25-27, 32, 53-54). Now we have followed the charge-compensating proton removal in the classical  $S_0 \rightarrow S_1$  and  $S_2 \rightarrow S_3$  transitions and have determined the kinetic parameters of these essential reaction steps (see Table 2).

On these grounds and based on earlier investigations (reviewed in (2, 23)), we propose the basic reactions sequence for the transitions from  $S_0^n$  to the  $S_4^+$  state outlined in the following (Figs. 1B and 5). **(I)** Starting in the most reduced semi-stable S-state,  $S_0^n$ , absorption of a photon by PSII induces rapid  $Y_Z$  oxidation ( $< 1 \mu\text{s}$ ) followed by ET from the Mn-complex to  $Y_Z^{\text{ox}}$  (40  $\mu\text{s}$  in Fig. 1B), resulting in  $S_1^+$  formation. Mn oxidation in the  $S_0^n \rightarrow S_1^+$  transition lowers the  $pK$  of a Mn ligand, possibly a bridging hydroxide (44, 55-56), to a value around 3.3 (57). The proton is removed from the Mn-complex and relocated toward the lumen only after  $S_1^+$  formation, in the  $S_1^+ \rightarrow S_1^n$  transition. **(II)** The next absorbed photon induces  $Y_Z^{\text{ox}}$  oxidation followed by a  $\text{Mn}^{\text{III}} \rightarrow \text{Mn}^{\text{IV}}$  oxidation in the  $S_1^n \rightarrow S_2^+$  transition. The oxidation of the Mn-complex lowers the  $pK$  values of ligand groups, but not to an extent sufficient for deprotonation. **(III)** Oxidation of the Mn-complex without any charge-compensating chemical change raises its redox potential to a level that prohibits a second oxidation by  $Y_Z^{\text{ox}}$  (1-2, 7). In the  $S_2^+$  state, this redox-potential problem initially prevents oxidation of the Mn-complex by  $Y_Z^{\text{ox}}$ . However,  $Y_Z^{\text{ox}(+)}$  drives the removal of a proton from the Mn-complex, resulting in formation of the  $S_2^n$  state within about 30  $\mu\text{s}$ . We propose that the proton is removed from the cluster of water molecules indicated in Fig. 5. Thereby a proton vacancy is created which is effectively delocalized within the water cluster (on the  $\mu\text{s}$ -timescale), but likely resides mostly on the water molecule close to  $Y_Z^{\text{ox}}$  that is coordinated to the Ca ion of the Mn-complex. In the subsequent ET step ( $S_2^n \rightarrow S_3^+$ ),  $\text{Mn}^{\text{III} \rightarrow \text{IV}}$  oxidation of Mn4 (58-60) is directly coupled to proton transfer to the previously deprotonated water cluster. Such a concerted electron-proton transfer is in line with a comparably large KIE for this ET step and well-suited to solve the redox-potential problem mentioned above. In the proton-removal step preceding the ET, deprotonation of D1-Asp<sub>61</sub> can be excluded as the  $S_2 \rightarrow S_3$  transition is not severely affected in the Asp-Asn mutant (61-62). A central role of CP43-Arg357 (63) is unlikely as this residue is neither close to  $Y_Z$  nor to Mn4. Thus we consider deprotonation of the water cluster interconnecting  $Y_Z$  and the Mn-complex to be the most plausible option, which is supported by the importance of the  $pK$ -value of the water molecules coordinated to the Ca ion (64). **(IV)** In the  $S_3^+ \rightarrow S_3^n \rightarrow S_4^+$  transitions, the basic temporal sequence of proton removal and electron transfer is similar to the one described above for the  $S_2^+ \rightarrow S_2^n \rightarrow S_3^+$  transitions. However, the key players are others. As opposed to the  $S_2 \rightarrow S_3$  transition, the proton-removal step likely

involves D1-Asp<sub>61</sub> (62). Therefore and in line with the clear differences in H/D-isotope effect (KIE) and activation energy (Table 2), we propose that proton removal in the  $S_3^+ \rightarrow S_3^n$  transition proceeds along a path that includes D1-Asp<sub>61</sub> (14, 21) whereas proton removal in the  $S_2^+ \rightarrow S_2^n$  transition proceeds along another path starting close to D1-Tyr<sub>161</sub>/His<sub>190</sub> (15, 65). In comparison to the ET of the  $S_2^n \rightarrow S_3^+$  transition, the  $S_3^n \rightarrow S_4^+$  transition may be coupled to more extensive chemical changes suitable to initiate O-O bond formation. Gaining insight in the presently merely hypothetical  $S_4^+$  and  $S_0^+$  intermediates represents a central challenge in future research on photosynthetic water oxidation.

*In conclusion*, the basic sequence of events in the reaction cycle of water oxidation now has been established for the 6 transitions leading from  $S_0^n$  to  $S_4^+$ . This sequence is characterized by the strictly alternating removal of electrons and protons from the Mn-complex. Three intermediate states of the catalytic metal center ( $S_0^+$ ,  $S_2^n$ ,  $S_3^n$ ) and its immediate ligand environment are transiently formed. On these grounds, strategies can be developed for characterization of the new reaction intermediates at the atomistic level. The extended S-state cycle model (Fig. 1B) may serve as a framework for the future design of experimental and theoretical investigations on photosynthetic water oxidation. It may be worthwhile to scrutinize also synthetic systems for catalysis of water oxidation within a conceptual framework that involves redox-potential leveling and local accumulation of 4 oxidizing equivalents by alternating electron and proton removal from the catalytic site.

## Materials and Methods

PSII membrane particles prepared from spinach were resuspended in a buffered solution (80  $\mu\text{g}$  chlorophyll per mL; pH/pD of 6.2) and 20  $\mu\text{M}$  of 2,6-dichloro-p-benzoquinone (DCBQ) was added as an artificial electron acceptor. The PBD experiments were carried out and analyzed as described previously (37-38). In the PBD experiments presented herein, 100 mL of PSII suspension were kept in a dark reservoir on ice (gently stirred) and pumped first through a thermostated laboratory-built heat exchanger and then into the sample compartment of the thermostated flow-through cuvette (3 mm optical path). Then the respective laser flash protocol was applied (0-4 saturating ns-laser flashes plus a single sub-saturating flash; 532 nm; see Fig. S1). The PBD signal induced by the non-saturating laser flash was recorded. In the next pump cycle, the flow-through cuvette was filled again with a fresh sample of dark-

adapted PSII. Extensive signal averaging was applied; about 4000 measurements were needed for obtaining the data of Fig. 2. Further details are provided as Supporting Information.

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## References

1. McEvoy JP & Brudvig GW (2006) Water-splitting chemistry of photosystem II. *Chem. Rev.* 106(11):4455-4483.
2. Dau H & Haumann M (2008) The manganese complex of photosystem II in its reaction cycle—basic framework and possible realization at the atomic level. (Translated from English) *Coord. Chem. Rev.* 252(3-4):273-295 (in English).
3. Barber J (2009) Photosynthetic energy conversion: natural and artificial. *Chem. Soc. Rev.* 38(1):185-196.
4. Blankenship RE (2002) *Molecular mechanisms of photosynthesis* (Blackwell Science, Oxford, England).
5. Rutherford AW & Moore TA (2008) Mimicking photosynthesis, but just the best bits. *Nature* 453(7194):449.
6. Magnuson A, *et al.* (2009) Biomimetic and microbial approaches to solar fuel generation. *Acc. Chem. Res.* 42(12):1899-1909.
7. Dau H, *et al.* (2010) The mechanism of water oxidation: from electrolysis via homogeneous to biological catalysis. *ChemCatChem* 2(7):724-761.
8. Lubitz W, Reijerse EJ, & Messinger J (2008) Solar water-splitting into H<sub>2</sub> and O<sub>2</sub>: design principles of photosystem II and hydrogenases. *Energy Environ. Sci.* 1(1):15-31.
9. Dau H & Zaharieva I (2009) Principles, efficiency, and blueprint character of solar-energy conversion in photosynthetic water oxidation. *Acc. Chem. Res.* 42(12):1861-1870.
10. Barry BA & Babcock GT (1987) Tyrosine radicals are involved in the photosynthetic oxygen-evolving system. *Proc. Natl. Acad. Sci. USA* 84(20):7099-7103.
11. Rappaport F & Diner BA (2008) Primary photochemistry and energetics leading to the oxidation of the (Mn)<sub>4</sub>Ca cluster and to the evolution of molecular oxygen in Photosystem II. *Coord. Chem. Rev.* 252(3-4):259-272.
12. Styring S, Sjöholm J, & Mamedov F (Two tyrosines that changed the world: Interfacing the oxidizing power of photochemistry to water splitting in photosystem II. *Biochim. Biophys. Acta* -doubled version - (0).
13. Zouni A, *et al.* (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* 409(6821):739-743.
14. Ferreira KN, Iverson TM, Maghlaoui K, Barber J, & Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. *Science* 303:1831-1838.
15. Umena Y, Kawakami K, Shen J-R, & Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473:55-60.

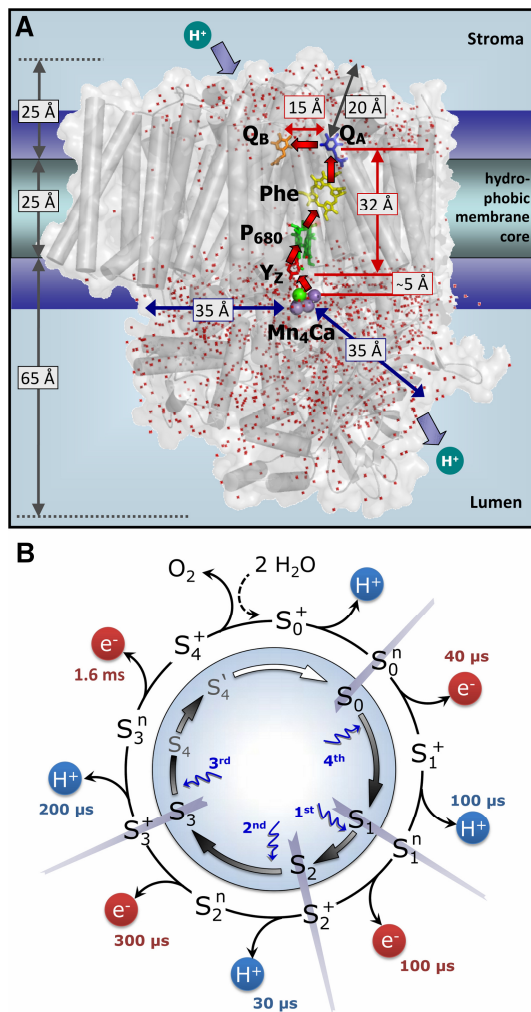
16. Kok B, Forbush B, & McGloin M (1970) Cooperation of charges in photosynthetic O<sub>2</sub> evolution - I. A linear four-step mechanism. *Photochem. Photobiol.* 11:457-475.
17. Dau H & Haumann M (2007) Time-resolved X-ray spectroscopy leads to an extension of the classical S-state cycle model of photosynthetic oxygen evolution. *Photosynth. Res.* 92:327-343.
18. Lavergne J & Junge W (1993) Proton release during the redox cycle of the water oxidase. *Photosynth. Res.* 38:279-296.
19. Junge W, Haumann M, Ahlbrink R, Mulkidjanian A, & Clausen J (2002) Electrostatics and proton transfer in photosynthetic water oxidation. *Phil. Trans. R. Soc. B* 357(1426):1407 - 1418.
20. Rappaport F & Lavergne J (1991) Proton release during successive oxidation steps of the photosynthetic water oxidation process: stoichiometries and pH dependence. *Biochemistry* 30(41):10004-10012.
21. Ho FM (2008) Uncovering channels in photosystem II by computer modelling: current progress, future prospects, and lessons from analogous systems. (Translated from eng) *Photosynth Res* 98(1-3):503-522 (in eng).
22. Hoganson CW & Babcock GT (1997) A metalloradical mechanism for the generation of oxygen from water in photosynthesis. *Science* 277(5334):1953-1956.
23. Dau H & Haumann M (2007) Eight steps preceding O-O bond formation in oxygenic photosynthesis—a basic reaction cycle of the photosystem II manganese complex. *Biochim. Biophys. Acta* 1767:472-483.
24. Meyer TJ, Huynh MHV, & Thorp HH (2007) The possible role of proton-coupled electron transfer (PCET) in water oxidation by photosystem II. *Angew. Chem. Int. Ed.* 46(28):5284-5304.
25. Haumann M, *et al.* (2005) Photosynthetic O<sub>2</sub> formation tracked by time-resolved x-ray experiments. *Science* 310(5750):1019-1021.
26. Gerencsér L & Dau H (2010) Water Oxidation by Photosystem II: H<sub>2</sub>O–D<sub>2</sub>O Exchange and the Influence of pH Support Formation of an Intermediate by Removal of a Proton before Dioxygen Creation. *Biochemistry* 49(47):10098-10106.
27. Haumann M, Bögershausen O, Cherepanov D, Ahlbrink R, & Junge W (1997) Photosynthetic oxygen evolution: H/D isotope effects and the coupling between electron and proton transfer during the redox reactions at the oxidizing side of Photosystem II. *Photosynth. Res.* 51(3):193-208.
28. Renger G & Hanssum B (1992) Studies on the reaction coordinates of the water oxidase in PS II membrane fragments from spinach. *FEBS Lett.* 299(1):28-32.
29. Dekker JP, Plijter JJ, Ouwehand L, & van Gorkom HJ (1984) Kinetics of manganese redox transitions in the oxygen evolving apparatus of photosynthesis. *Biochim. Biophys. Acta* 767:176-179.

30. Haumann M & Junge W (1994) Extent and rate of proton release by photosynthetic water oxidation in thylakoids: electrostatic relaxation versus chemical production. *Biochemistry* 33(4):864-872.
31. Schlodder E & Witt HT (1999) Stoichiometry of proton release from the catalytic center in photosynthetic water oxidation. Reexamination by a glass electrode study at pH 5.5-7.2. *J. Biol. Chem.* 274(43):30387-30392.
32. Rappaport F, Blanchard-Desce M, & Lavergne J (1994) Kinetics of electron transfer and electrochromic change during the redox transition of the photosynthetic oxygen-evolving complex. *Biochim. Biophys. Acta* 1184:178-192.
33. Dau H & Haumann M (2006) Reaction cycle of photosynthetic water oxidation in plants and cyanobacteria (response letter). *Science* 312:1471-1472.
34. Braslavsky SE & Heibel GE (1992) Time-resolved photothermal and photoacoustic methods applied to photoinduced processes in solution. *Chem. Rev.* 92(6):1381 - 1410.
35. Falvey DE (1997) Photothermal beam deflection calorimetry in solution photochemistry: recent progress and future prospects. *Photochem Photobiol* 65(1):4-9.
36. Gensch T & Viappiani C (2003) Time-resolved photothermal methods: accessing time-resolved thermodynamics of photoinduced processes in chemistry and biology. *Photochem. Photobiol. Sci.* 2:699 - 721.
37. Krivanek R, Dau H, & Haumann M (2008) Enthalpy changes during photosynthetic water oxidation tracked by time-resolved calorimetry using a photothermal beam deflection technique. *Biophys. J.* 94(5):1890-1903.
38. Klauss A, Krivanek R, Dau H, & Haumann M (2009) Energetics and kinetics of photosynthetic water oxidation studied by photothermal beam deflection (PBD) experiments. (Translated from English) *Photosynth. Res.* 102(2-3):499-509 (in English).
39. Feitelson J & Mauzerall D (2002) Enthalpy and Electrostriction in the Electron-Transfer Reaction between Triplet Zinc Uroporphyrin and Ferricyanide. *J. Phys. Chem. B* 106(37):9674-9678.
40. Rizzi AC, *et al.* (2008) Entropic Changes Control the Charge Separation Process in Triads Mimicking Photosynthetic Charge Separation. *J. Phys. Chem. A* 112(18):4215-4223.
41. Hou HJM & Mauzerall D (2011) Listening to PS II: Enthalpy, entropy, and volume changes. *J. Photochem. Photobiol. B: Biol.* 104(1-2):357-365.
42. Mauzerall D, Hou JM, & Boichenko VA (2002) Volume changes and electrostriction in the primary photoreactions of various photosynthetic systems: estimation of dielectric coefficient in bacterial reaction centers and of the observed volume changes with the Drude-Nernst equation. *Photosynth. Res.* 74(2):173-180.
43. Hou JM, Boichenko VA, Diner BA, & Mauzerall D (2001) Thermodynamics of electron transfer in oxygenic photosynthetic reaction centers: volume change, enthalpy,

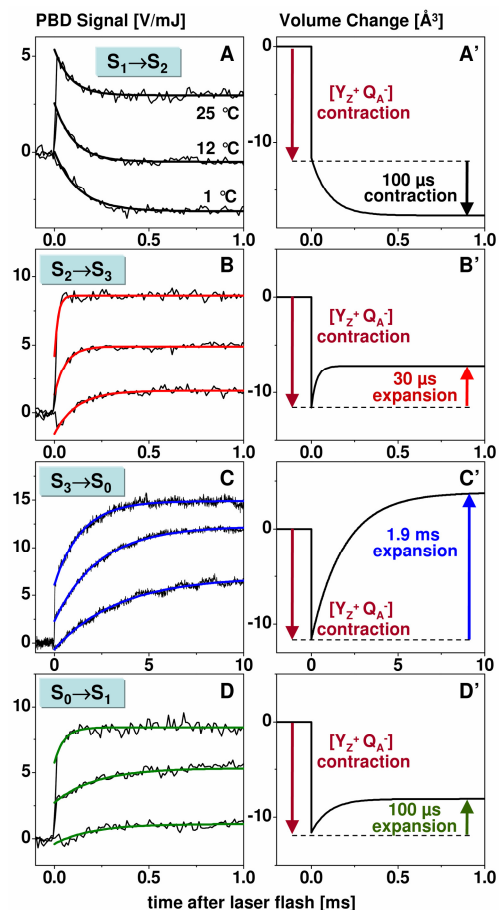
- and entropy of electron-transfer reactions in manganese-depleted photosystem II core complexes. *Biochemistry* 40(24):7117-7125.
44. Haumann M, *et al.* (2005) Structural and oxidation state changes of the photosystem II manganese complex in four transitions of the water oxidation cycle ( $S_0 \rightarrow S_1$ ,  $S_1 \rightarrow S_2$ ,  $S_2 \rightarrow S_3$ , and  $S_{3,4} \rightarrow S_0$ ) characterized by X-ray absorption spectroscopy at 20 K and room temperature. *Biochemistry* 44(6):1894-1908.
  45. Noguchi T (2008) Fourier transform infrared analysis of the photosynthetic oxygen-evolving center. *Coord. Chem. Rev.* 252(3-4):336-346.
  46. Buchta J, Grabolle M, & Dau H (2007) Photosynthetic dioxygen formation studied by time-resolved delayed fluorescence measurements--method, rationale, and results on the activation energy of dioxygen formation. *Biochim. Biophys. Acta* 1767:565-574.
  47. Karge M, Irrgang KD, & Renger G (1997) Analysis of the reaction coordinate of photosynthetic water oxidation by kinetic measurements of 355 nm absorption changes at different temperatures in photosystem II preparations suspended in either H<sub>2</sub>O or D<sub>2</sub>O. (Translated from English) *Biochemistry* 36(29):8904-8913 (in English).
  48. Razeghifard MR, Klughammer C, & Pace RJ (1997) Electron paramagnetic resonance kinetic studies of the S states in spinach thylakoids. (Translated from eng) *Biochemistry* 36(1):86-92 (in eng).
  49. Hatcher E, Soudackov A, & Hammes-Schiffer S (2005) Comparison of dynamical aspects of nonadiabatic electron, proton, and proton-coupled electron transfer reactions. *Chem. Phys.* 319(1-3):93-100.
  50. Krishtalik LI (2000) The mechanism of the proton transfer: an outline. *Biochim. Biophys. Acta* 1458(1):6-27.
  51. Garczarek F & Gerwert K (2006) Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy. *Nature* 439(7072):109-112.
  52. Barry BA, *et al.* (2006) Time-resolved vibrational spectroscopy detects protein-based intermediates in the photosynthetic oxygen-evolving cycle. *Proc. Natl. Acad. Sci. USA* 103(19):7288-7291.
  53. Razeghifard MR & Pace RJ (1999) EPR kinetic studies of oxygen release in thylakoids and PSII membranes: an intermediate in the  $S_3$  to  $S_0$  transition. *Biochemistry* 38:1252-1257.
  54. Rappaport F, Ishida N, Sugiura M, & Boussac A (2011) Ca<sup>2+</sup> determines the entropy changes associated with the formation of transition states during water oxidation by Photosystem II. *Energy Environ. Sci.* 4(7):2520-2524.
  55. Dau H, Iuzzolino L, & Dittmer J (2001) The tetra-manganese complex of photosystem II during its redox cycle: X-ray absorption results and mechanistic implications. *Biochim. Biophys. Acta* 1503(1-2):24-39.
  56. Robblee JH, *et al.* (2002) The Mn cluster in the  $S_0$  state of the oxygen-evolving complex of photosystem II studied by EXAFS spectroscopy: are there three di- $\mu$ -oxo-

- bridged Mn<sub>2</sub> moieties in the tetranuclear Mn complex? *J. Am. Chem. Soc.* 124(25):7459-7471.
57. Zaharieva I, Wichmann JM, & Dau H (2011) Thermodynamic limitations of photosynthetic water oxidation at high proton concentrations. (Translated from Eng) *J Biol Chem* 286:18222-18228 (in Eng).
  58. Teutloff C, *et al.* (2009) Electronic structure of the tyrosine D radical and the water-splitting complex from pulsed ENDOR spectroscopy on photosystem II single crystals. (Translated from eng) *Phys Chem Chem Phys* 11(31):6715-6726 (in eng).
  59. Siegbahn PE (2008) A structure-consistent mechanism for dioxygen formation in photosystem II. *Chem. Eur. J.* 14(27):8290-8302.
  60. Ames W, *et al.* (2011) Theoretical evaluation of structural models of the S<sub>2</sub> state in the oxygen evolving complex of Photosystem II: protonation states and magnetic interactions. (Translated from eng) *J Am Chem Soc* 133(49):19743-19757 (in eng).
  61. Hundelt M, Hays AM, Debus RJ, & Junge W (1998) Oxygenic photosystem II: the mutation D1-D61N in *Synechocystis* sp. PCC 6803 retards S-state transitions without affecting electron transfer from YZ to P680+. *Biochemistry* 37(41):14450-14456.
  62. Dilbeck PL, *et al.* (2012) The mutation D1-D61N in *Synechocystis* sp. PCC 6803 allows the observation of pH-sensitive intermediates in the formation and release of O<sub>2</sub> from Photosystem II. *Biochemistry* 51(6):1079–1091.
  63. McEvoy JP & Brudvig GW (2004) Structure-based mechanism of photosynthetic water oxidation. *Phys. Chem. Chem. Phys.* 6:4754-4763.
  64. Vrettos JS, Stone DA, & Brudvig GW (2001) Quantifying the ion selectivity of the Ca<sup>2+</sup> site in photosystem II: evidence for direct involvement of Ca<sup>2+</sup> in O<sub>2</sub> formation. *Biochemistry* 40(26):7937-7945.
  65. Bondar AN & Dau H (2012) Extended protein/water H-bond networks in photosynthetic water oxidation. (Translated from Eng) *Biochim Biophys Acta* 1817:1177-1190 (in Eng).
  66. Zaharieva I, Grabolle M, Chernev P, & Dau H (2011) Water oxidation in photosystem II: Energetics and kinetics of intermediates formation in S<sub>2</sub>->S<sub>3</sub> and S<sub>3</sub>->S<sub>0</sub> transitions monitored by delayed chlorophyll fluorescence. *Proc. 15th Internatl. Conference on Photosynthesis, Beijing, China*:in press.

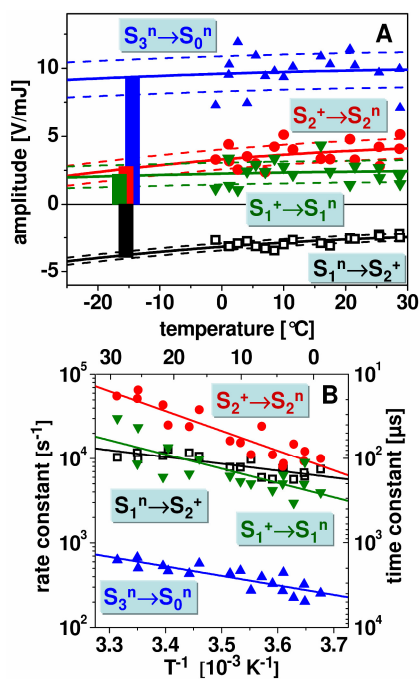




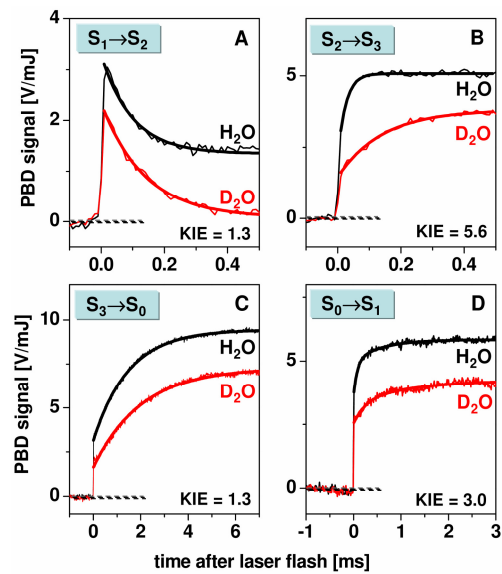
**Figure 1:** Photosystem II (A) and reaction cycle of water oxidation (B). In (A), crucial redox cofactors and dimensions of the PSII complex are shown (15). Red arrows connect redox cofactors of the ET chain including the primary electron donor ( $P_{680}$ ), the primary pheophytin acceptor (Phe), the primary ( $Q_A$ ) and secondary ( $Q_B$ ) quinone acceptors, and at the electron donor side, a redox-active tyrosine ( $Y_Z$ ) and the Mn-complex. Water molecules resolved in the crystallographic model (PDB entry 3ARC (15)) are shown as red dots; the indicated distances illustrate relevant dimensions. In (B), the classical Kok model (16) (inner circle, including states  $S_4$  and  $S_4'$  (25)) is extended to describe both oxidation of the Mn-complex by electron transfer (ET) to the  $Y_Z$  radical and proton removal from the Mn-complex or its ligand environment by long-distance proton transfer. Coupling of the ET step to local proton shifts is not covered by the shown framework model. The subscripts indicate the number of oxidation equivalents accumulated at the Mn-complex, the superscripts the charge relative to the dark-stable  $S_1$ -state (+, positive; n, neutral). The proton release steps in the  $S_0 \rightarrow S_1$  and  $S_2 \rightarrow S_3$  transitions have not been tracked in time-resolved experiments before, but now these steps are detected in the PBD experiments; the indicated time constants result from the present study.



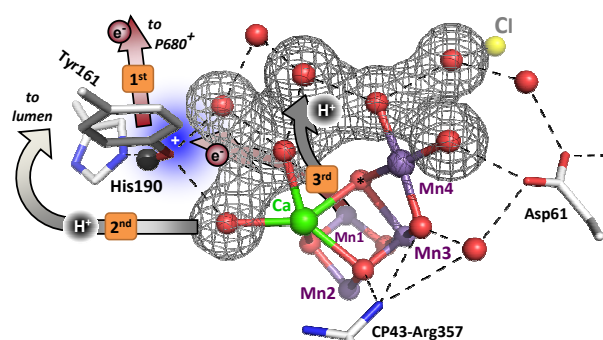
**Figure 2:** Flash-induced PBD signals and volume changes:  $S_1 \rightarrow S_2$  (A, A'),  $S_2 \rightarrow S_3$  (B, B'),  $S_3^+ \rightarrow S_0$  (C, C') and  $S_0 \rightarrow S_1$  (D; D'). Thin lines, experimental data; thick lines, simulations using a step-shaped function for the rapid jump due to  $Q_A^- Y_Z^+$  formation and single-exponential functions for the slower signal contributions. The right panels show schematically the volume changes deduced from the analysis of the temperature dependence of the PBD signals (time constants for about 20 °C, see Fig. 3).



**Figure 3:** Temperature dependence of the PBD signals of the four resolved transitions. (A) Temperature dependence of the amplitudes as obtained by an exponential simulation (symbols, experimental data; lines obtained by a fit). The dotted lines show the  $1\sigma$  error ranges of the fit curves. The bars represent the nonthermal part of the PBD signal (volume change  $\Delta V$ ) that corresponds to the PBD amplitude at  $-14^\circ\text{C}$  ( $T_0 = -14 \pm 1^\circ\text{C}$ ; see SI, PBD analysis). (B) Arrhenius plots of the rate constants ( $k = \tau^{-1}$ , left y-axis; time constants,  $\tau$ , on the right y-axis). The symbols indicate the experimentally determined values; the lines are fit curves used for determination of the respective activation energy shown in Table 2.



**Figure 4:** Comparison of PBD signals on the four S-transitions measured for PSII membranes in H<sub>2</sub>O (black) and D<sub>2</sub>O (red) at 20 °C. Thin lines, experimental data; thick lines, simulations with single exponential functions plus offset, except for the S<sub>0</sub>→S<sub>1</sub> transition, for which a double-exponential function plus offset was used (Fig. S7 and Table S2). The respective rate constant ratio is indicated (KIE =  $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$ ). The PBD amplitudes differ due to different thermoelastic properties of H<sub>2</sub>O and D<sub>2</sub>O.



**Figure 5:** Sequence of events in the classical  $S_2 \rightarrow S_3$  transition of photosynthetic water oxidation. The  $Mn_4CaO_5$  cluster, the redox-active tyrosine ( $Tyr_{161}$ ,  $Y_Z$ ), and the key groups of the surrounding hydrogen-bonded network are shown (coordinates from \_\_\_\_). All indicated amino-acid residues are from the D1-subunit of PSII, with exception of the CP43-Arg<sub>357</sub>. (Water molecules,  $H_xO$ , are indicated as red spheres, putative H-bonds as broken lines which connect H-bond donor and acceptor. Of all the protons, only the phenolic proton is shown as a grey sphere.) The grey mesh outlines a water cluster which includes 4  $H_xO$  in the first coordination sphere of manganese (Mn4) and calcium (Ca) as well as 3 second-sphere water molecules. (**1<sup>st</sup>**) Within less than 100 ns after absorption of a photon and oxidation of the primary chlorophyll donor of PSII (P680),  $Tyr_{161}$  ( $Y_Z$ ) is oxidized by  $P680^+$ . The  $Y_Z$  oxidation results in a rearrangement of the shown H-bonded network (completed within less than 1  $\mu$ s), likely involving a shift of the phenolic proton to His<sub>190</sub> and lowering of  $pK_a$ -values for deprotonation of the water molecules in the outlined cluster (grey mesh). (**2<sup>nd</sup>**) A proton is removed from the Mn-complex/ $Y_Z$  environment within about 30  $\mu$ s, as evidenced by the PBD results presented herein, and supposedly a proton vacancy is created within the outlined water cluster. (**3<sup>rd</sup>**) In ET to the oxidized  $Tyr_{161}$  (about 300  $\mu$ s), Mn oxidation is directly coupled to a proton transfer step involving the previously created proton vacancy of the water cluster (concerted electron-proton transfer).

Flash No.	Transition	$\tau$ [ $\mu\text{s}$ ]	$\Delta V$ [ $\text{\AA}^3$ ]	$\Delta Q$ [meV]
1	$S_1^n \rightarrow S_2^+$	$98 \pm 3$	$-6.1 \pm 0.4$	$160 \pm 50$
2	$S_2^+ \rightarrow S_2^n$	$29 \pm 2$	$+4.4 \pm 1.1$	$190 \pm 120$
3	$S_3 \rightarrow S_0$	$1960 \pm 90$	$+15 \pm 2.5$	$60 \pm 250$
4	$S_1^+ \rightarrow S_1^n$	$94 \pm 8$	$+3.5 \pm 1.3$	$40 \pm 140$

**Table 1:** Time constants ( $\tau$  at 20°C), volume changes ( $\Delta V$ ), and heat release ( $\Delta Q$ ) of four transitions resolved in the PBD measurements. The given parameters were determined from the data shown in Fig. 3; the error ranges correspond to the uncertainty of the fit result at the 1 $\sigma$ -level. The value of  $\Delta V$  denotes apparent volume changes calculated using Eqn. S1. Negative or positive signs correspond to contractions or expansions, respectively. The figures given for  $\Delta V$  and  $\Delta Q$  were calculated for a single PSII complex after absorption of one light quantum (without correction for non-unity quantum yield).

S-State Transition		ET/PT	$\tau$ [ $\mu$ s]	$E_a$ [meV]	$k_H/k_D$
$S_1 \rightarrow S_2$	$S_1^n \rightarrow S_2^+$	$e^-$	$100^{a,(b,c,d)}$	$160 \pm 30^{a,(d,e)}$	$1.3^a, (1.2^b)$
$S_2$	$S_2^+ \rightarrow S_2^n$	$H^+$	$30^a$	$470 \pm 50^a$	$5.6^a$
$\rightarrow S_3$	$S_2^n \rightarrow S_3^+$	$e^-$	$300^{b-e}$	$\sim 360^{d,e}$	$1.7^b$
$S_3$	$S_3^+ \rightarrow S_3^n$	$H^+$	$200^{b,c}$	$180^h$	$2.4^b$
	$S_3^n \rightarrow S_0^+$	$e^-$	$\sim 1700^{b-h}$	$230 \pm 40^{a,(e,f)}$	$1.3^a, (1.2^b)$
$\rightarrow S_0 + O_2$	$S_0^+ \rightarrow S_0^n$	$H^+$	—	—	—
$S_0$	$S_0^n \rightarrow S_1^+$	$e^-$	$40^{c,g}$	$50^e$	$1.3^d$
$\rightarrow S_1$	$S_1^+ \rightarrow S_1^n$	$H^+$	$100^a$	$340 \pm 75^a$	$3.0^a$

**Table 2:** Kinetic parameters of electron transfer (ET,  $e^-$ ) and rate-determining proton transfer (PT,  $H^+$ ) during the reaction cycle of PSII water oxidation (at  $\sim 20^\circ\text{C}$ ). Activation energies ( $E_a$ ) were derived from the temperature dependence of rate constants (Fig. 3B). The kinetic parameters were mostly determined for plant PSII (membrane particles): <sup>a</sup>PBD results of this work; <sup>b</sup>(26); <sup>c</sup>(25); <sup>d</sup>(27); <sup>e</sup>(28); <sup>f</sup>(46); <sup>g</sup>(29); <sup>h</sup>(66) and for thermophilic cyanobacteria, in (54).