## **Supporting Information**

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SI Text

**Calculation of the CO<sub>2</sub> and HCO\_3^- Fractions in the Buffer Solutions.** For estimation of the fractions of carbonates in particular form at pH 6.3 and 5.5 the following equations were used:

$$\alpha_{CO_2} = \left(1 + K_1 / [H^+] + K_1 K_2 / [H^+]^2\right)^{-1}$$

1. Harned HS, Davis R (1943) The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0 to 50°. J Am Chem Soc 65(10):2030–2037.

 $\alpha_{HCO_3^-} = (1 + [H^+]/K_1 + K_2/[H^+])^{-1}.$ 

The  $K_1$  and  $K_2$  values for 20 °C were taken from ref. 1. The results are compiled in Table S1.



**Fig. S1.** Dependence of water splitting in photosystem II (PSII) on the inorganic carbon ( $C_i$ ) concentration of the medium. PSII membranes were illuminated with continuous white light of a slide projector for 10 s inside of gas-tight syringes and then injected into the membrane-inlet mass spectrometry (MIMS) cell (20 °C). The  $C_i^+$  trace was obtained at ambient  $C_i$  concentration, and the  $C_i^-$  trace was recorded at a 60-times-reduced  $C_i$  concentration that was achieved by purging sample solution with N<sub>2</sub> inside an N<sub>2</sub>-filled glove box [based on the depletion factor for <sup>16</sup>O<sub>2</sub> (m/z 32)]. Rapid reversibility was demonstrated by addition NaHCO<sub>3</sub> powder (to give a final concentration of 0.5 mM) to a  $C_i^-$  sample ( $C_i^-$  + 0.5 mM HCO<sub>3</sub><sup>--</sup> trace). The time between NaHCO<sub>3</sub> addition and illumination was about 2 min owing to the sample handling in the dark in an N<sub>2</sub>-filled glove box. The chlorophyll concentration was 50 µg (Chl)·mL<sup>-1</sup>, the H<sub>2</sub><sup>18</sup>O-enrichment was 10%, and the medium contained 1 mM MES (pH 6.3) and 15 mM NaCl. The measurements were done in the presence of the following electron acceptors: 2 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>] (dissolved in H<sub>2</sub>O) and 0.25 mM 2-phenyl-*p*-benzoquinone (dissolved in DMSO). The average result of two repeats is presented.



**Fig. S2.** Relative signal intensities of nonlabeled (m/z 32,  ${}^{16}O_2$  and m/z 44,  ${}^{12}C^{16}O_2$ ) and  ${}^{18}O$ -singly labeled (m/z 34,  ${}^{16}O^{18}O$  and m/z 46,  ${}^{12}C^{16}O^{18}O$ ) isotopologues of  $O_2$  and  $CO_2$  and argon signal (m/z 40,  ${}^{40}Ar$ ) that were monitored simultaneously with the  ${}^{18}O$ -doubly labeled isotopologues shown in traces 1 of Fig. 3. The spectra are colored individually according to their m/z values. The onsets of flash illumination (50 saturating xenon flashes, 2 Hz) are marked by arrows. The values shown in parentheses at the traces represent the relative amplification of the Faraday cups of the mass spectrometer.



## Time after sample injection (s)

**Fig. S3.** Off-line time-resolved (TR)-MIMS measurements of the  $O_2$  and  $CO_2$  content in dark-adapted PSII sample suspension before (trace 1) and rapidly (<3 s) after illumination (trace 2) with 100 xenon flashes (2 Hz) at pH 6.3 and 20 °C. Fifty-microliter aliquots of the PSII sample suspension ([Ch]], 1 mM) containing H<sub>2</sub><sup>18</sup>O (15%) and 2 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>] were injected into MES buffer (pH 6.3) that was thoroughly degassed in the MIMS cell at 20 °C. The levels of  $O_2$  and  $CO_2$  were monitored simultaneously as doubly labeled isotopologues at m/z 36 (<sup>18</sup>O<sub>2</sub>, blue traces) and at m/z 48 (C<sup>18</sup>O<sub>2</sub>, green traces), respectively. The TR-MIMS difference signals (Fig. 4) were obtained by subtraction of the dark traces (1) from the ones obtained after illumination (2).



**Fig. S4.** Difference MIMS signals of  $O_2$  level in PSII membrane fragments measured as <sup>18</sup> $O_2$  (at *m/z* 36) rapidly (<3 s) (solid line 1) and 1 min (dashed line 2) after illumination with 100 xenon flashes (2 Hz) at pH 6.3 and 20 °C. The average result out of two to three repeat measurements is presented. The signals were normalized to trace 1. The zero levels of the traces are off set for clarity of presentation.



**Fig. S5.** Simultaneous off-line TR-MIMS measurement of  $O_2$  and  $CO_2$  production by PSII. Dark-adapted PSII membranes (0.3 mg Chl/mL) were illuminated inside a gas-tight syringe with 100 xenon flashes and then injected into the MIMS cell with a delay of 1 ms (*A*), 500 ms (*B*), and 3,000 ms (*C*) after the last flash. Displayed are the light-minus-dark difference signals of  $O_2$  (blue traces) and  $CO_2$  (green traces). The amplitudes of the traces for  $CO_2$  and  $O_2$  can be directly compared, because our set-up detects both gases with nearly equal sensitivity. In addition to 1 mM MES (pH 6.3), the sample medium contained 15 mM NaCl, 20% H<sub>1</sub><sup>18</sup>O, and 2 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>] as electron acceptor. Before the experiments sample medium was equilibrated at room temperature for 30 min. Zero levels are offset for clarity of presentation. In all panels the average of two to three repeats is presented.

Table S1.	Fractions of inorga	nic carbon species	at pH 5.5 and 6.3

Inorganic carbon species	pH 5.5	pH 6.3
CO <sub>2</sub>	0.884	0.546
HCO <sub>3</sub> <sup>-</sup>	0.116	0.454