Supporting Information

Shevela et al. 10.1073/pnas.1014249108

SI Text

The Level of Dissolved N_2 of the Reaction Mixture Under Elevated N_2 Pressure. Fig. S2 shows that the level of $^{14}N_2$ (detected at $m/z = 28$) in the reaction mixture at an N_2 pressure of 20 bars raised ≈13–14 times over ambient. This lower increase as compared to 20 bars O_2 has two reasons: (*i*) The partial pressure of N_2 in air is ∼0.78, whereas that of O_2 is ∼0.21. This about 4-fold higher partial pressure leads to a higher starting level at ambient air pressure. (*ii*) N_2 is about 2-fold less soluble in water than O_2 (0.019 g/L versus 0.043 g/L at 20 °C, respectively) (1). So when comparing pure N_2 versus pure O_2 gas at the same pressure, the signal for N_2 is expected to be about 50% of that of O_2. In addition, the ionization efficiencies of N_2 and O_2 in the mass spec source differ slightly. Therefore, our observation of a lower increase in signal level for N_2 versus O_2 after applying 20 bars pressure is in full agreement with theoretical expectations.

Comparison of Experimental Boundary Conditions. The experimental conditions between the UV experiments and the membrane-inlet mass spectrometry (MIMS) experiments were identical except for (*i*) the addition of the electron acceptor 2,5-dichloro-*p*-benzoquinone (DCBQ) in ethanol instead of DMSO, (*ii*) the use of ^{18}O-enriched water instead of water with normal isotope composition, (*iii*) 0.1 M sucrose in the buffer as compared to 1 M, and (*iv*) the UV-measuring light (for a detailed comparison of experimental conditions also to other previous studies, see Table S1). These minor differences may affect the stability of the electron acceptor 2,5-DCBQ. UV-visible (VIS) spectra displayed in Fig. S5 show that 2,5-DCBQ is indeed less stable in the measuring buffer at higher sucrose concentration. This effect was noticed previously and, therefore, 2,5-DCBQ was injected in the transient UV measurements 5 min prior to the data collection (2). Further experiments (Fig. S6) under ambient and O_2-depleted conditions did not reveal any indication for an O_2 sensitivity of this reaction. This latter finding makes it very improbable that a lack of electron acceptor can be blamed for the apparent block of photosystem II (PSII) in the third flash in the transient UV measurements when O_2 pressure was applied. Fig. S7 further supports this view by showing that inside the MIMS cell sufficient DCBQ was present (at 0.1 M sucrose) even after the exposure for 1 h to 20 bars O_2 in the gas phase.

The UV-measuring light used in previous work (see ref. 2 and Fig. S8) was switched on only 100 ms before sampling started. It had impinged on the sample for only 300 ms when the third flash was fired. Given the low extinction coefficient of oxidized 2,5-DCBQ at this wavelength, the illumination was not expected to impair its stability either. Given the similar sample handling [media, incubation, and dark adaptation (for details, see Table S1)], and the fact that PSII-core complexes (PSII-cc) from the same frozen stock were used as in ref. 2, there was no obvious difference between the experimental boundary conditions between refs. 2–4 and this work.

UV-Absorption Transients. For the discussion of the discrepancy between the present MIMS data and previous UV-absorption data, it is worthwhile to look back at flash-induced UV-absorption transients at 360 nm (2). Fig. S8 shows the transients elicited by short laser flashes no. 2 and 3 (6-ns half-duration) that were fired to suspension of dark-adapted PSII-cc. The rapid rise of absorption was mainly attributable to the oxidation of Mn and to the reduction of intrinsic and extrinsic quinone. The decay was attributable to the dismutation of the quinone electron acceptor 2,5-DCBQ, and the pronounced millisecond decay following the third flash to the reduction of Mn (by water) (2). Whereas the rapid rise of absorption after both flashes was the same under 1 bar air and under 20 bars O_2, the millisecond decay phase upon the third flash was suppressed by 20 bars O_2. The high absorption at 360 nm was then stable for some 100 ms (see Fig. S8).

Calibration of MIMS O_2 Signals. Air-saturated water samples (∼0.285 mM O_2 at 20 °C) were used for calibration of the O_2 signal. From these O_2 was removed by bubbling with N_2 within the MIMS cell (Fig. 1). The difference between the air-saturated level and the zero level was taken to correspond to 0.285 mM $^{32}O_2$. The $m/z = 34$ peak could be calibrated the same way, by taking the natural abundance of $^{34}O_2$ (0.4%) into account. The $m/z = 36$ peak cannot be calibrated in this manner due to the much larger presence of the ^{36}Ar isotope in air as compared to $^{36}O_2$. We therefore simply accepted the 10-fold higher cup sensitivity of $m/z = 36$ as compared to $m/z = 34$ to be a good estimate.

Chl/RC (Reaction Center) Ratio of *Synechocystis* Samples. On the basis of (*i*) the above calibration of the $^{16}O^{18}O$ signal, (*ii*) the Chl concentration of the suspension of PSII-cc, and (*iii*) the amplitude of the $^{16}O^{18}O$ signal (extrapolated to the start of illumination) induced by a train of 30 Xe flashes (2 Hz), the ratio of chlorophyll molecules per PSII RC in *Synechocystis sp.* PCC 6803 (*Syn. sp.*) PSII-cc was estimated to be 50 Chl/RC. Other conditions: pH 6.7, 0.25 mM DCBQ.

Spectrophotometry. UV-absorption transients were measured at a wavelength of 360 nm (2). Non-time-resolved spectra of 2,5-DCBQ were acquired on a Varian Cary 50 Bio UV-Visible spectrophotometer at room temperature using a 0.1-cm pathlength cuvette.

1. Atkins PW, de Paula J (2006) *Physical Chemistry* (Oxford University Press, Oxford, UK), 8th Ed, p 1064.
2. Clausen J, Junge W (2004) Detection of an intermediate of photosynthetic water oxidation. *Nature* 430:480–483.
3. Clausen J, Junge W, Dau H, Haumann M (2005) Photosynthetic water oxidation at high O_2 backpressure monitored by delayed chlorophyll fluorescence. *Biochemistry* 44:12775–12779.
4. Haumann M, Grundmeier A, Zaharieva I, Dau H (2008) Photosynthetic water oxidation at elevated dioxygen partial pressure monitored by time-resolved X-ray absorption measurements. *Proc Natl Acad Sci USA* 105:17384–17389.

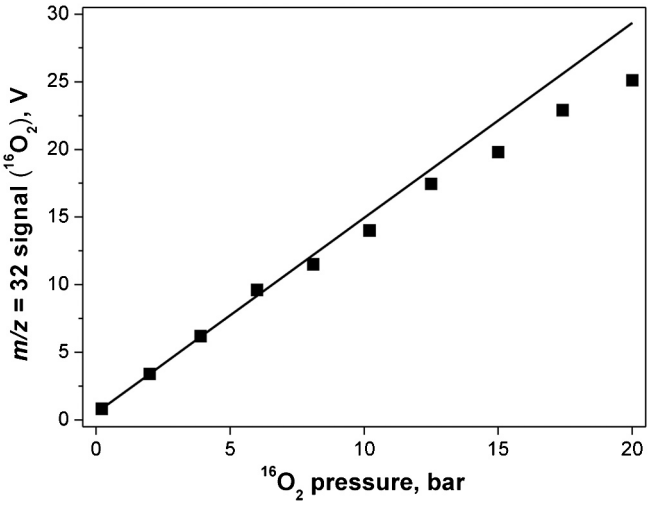

Fig. S1. Sensitivity of the MIMS setup to various applied pressures of $^{16}O_2$. The indicated values gives the O_2 pressure above the stirred buffer solution, whereas the $m/z = 32$ signal represents the signal amplitude reached after full equilibration.

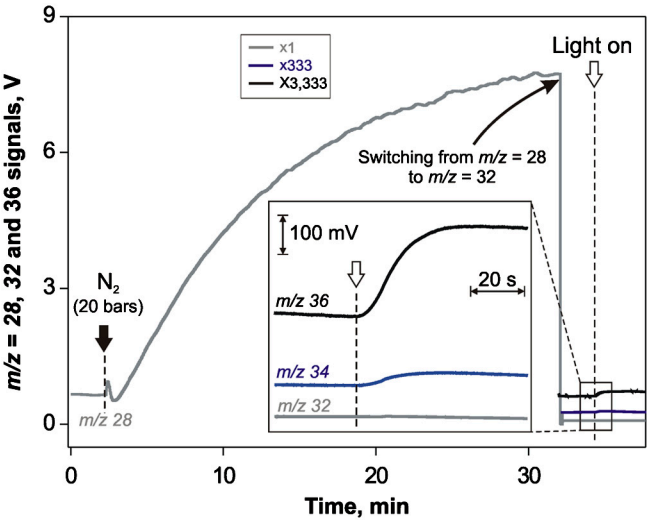

Fig. S2. MIMS measurements of the level of dissolved $^{14}N_2$ ($m/z = 28$) in the medium containing PSII-cc upon application of 20 bars N_2 pressure (closed arrow) before flash illumination (open arrows) at pH 6.7 and 20 °C. (*Inset*) The zoomed region of O_2 evolution (Fig. 2) detected as doubly- ($m/z = 36$), singly- ($m/z = 34$), and unlabeled ($m/z = 32$) oxygen that was induced by excitation of PSII-cc from *Syn. sp.* with 30 Xe flashes (2 Hz) after 35-min incubation in the MIMS cell. Shortly before the illumination the instrument settings were switched from the detection of $^{14}N_2$ ($m/z = 28$) to the detection of the isotopologues of molecular oxygen ($m/z = 32$, $m/z = 34$, and $m/z = 36$). The values shown for traces represent the amplification of the Faraday cups of the MS. The final $H_2{}^{18}O$ enrichment was ~50%. The measurements were carried out with a Chl concentration of 5 μM in the presence of 250 μM 2,5-DCBQ.

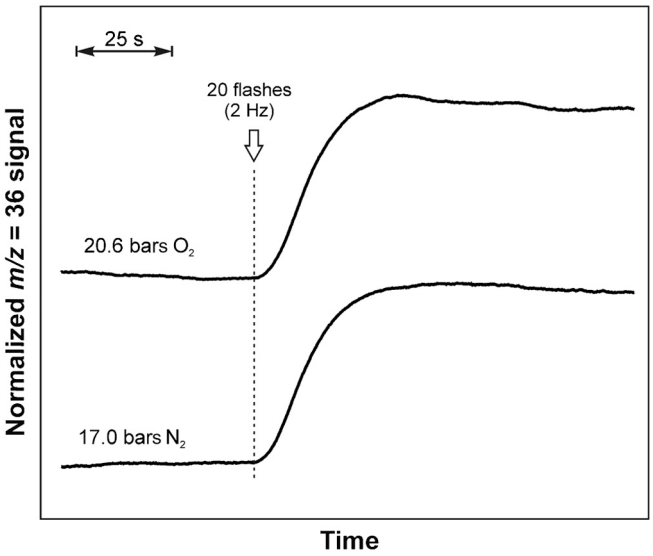

Fig. S3. Flash-induced $^{18}O_2$ evolution measured by MIMS in the PSII-cc from *Syn. sp.* as doubly ^{18}O-labeled oxygen ($m/z = 36$) after 40-min of dark incubation in the MIMS cell under elevated O_2 (20.6 bars) or N_2 (17.0 bars) at pH 5.5 and 20 °C. $^{18}O_2$ evolution was induced by a series of 20 saturating Xe flashes at the repetitive frequency of 2 Hz. The arrow indicates the initial time of illumination. The $H_2{}^{18}O$ enrichment was ~50%. All measurements were carried out with a Chl concentration of 5 μM in the presence of 250 μM 2,5-DCBQ as electron acceptor.

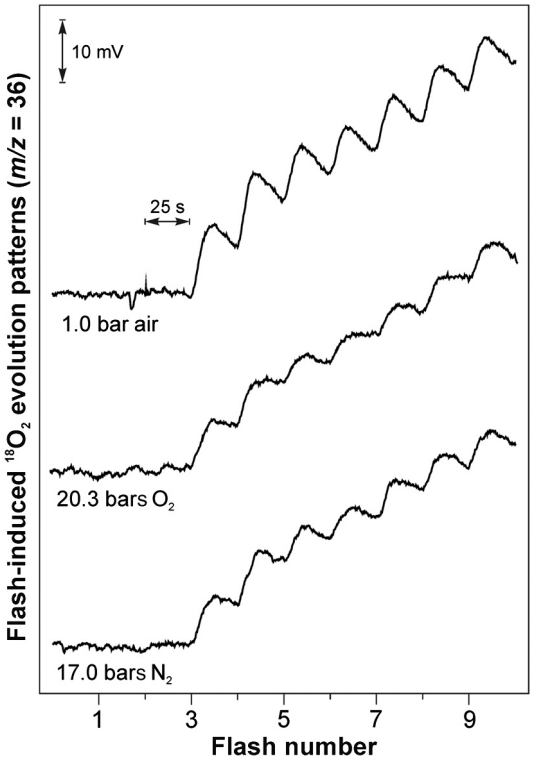

Fig. S4. Flash-induced-oxygen production measured by MIMS in the $S_1Y_D{}^{ox}$-PSII-cc from *Syn. sp.* as doubly ^{18}O-labeled oxygen ($m/z = 36$) after 35 min of dark incubation in the MIMS cell under atmospheric air pressure, elevated O_2 (20.3 bars) or elevated N_2 pressure (17.0 bars) at pH 6.5 and 20 °C. $^{18}O_2$ evolution was induced by a series of saturating Xe flashes separated by dark times of 25 s. The final $H_2{}^{18}O$ enrichment was ~50%. All measurements were carried out with a Chl concentration of 5 μM in the presence of 250 μM 2,5-DCBQ as electron acceptor.

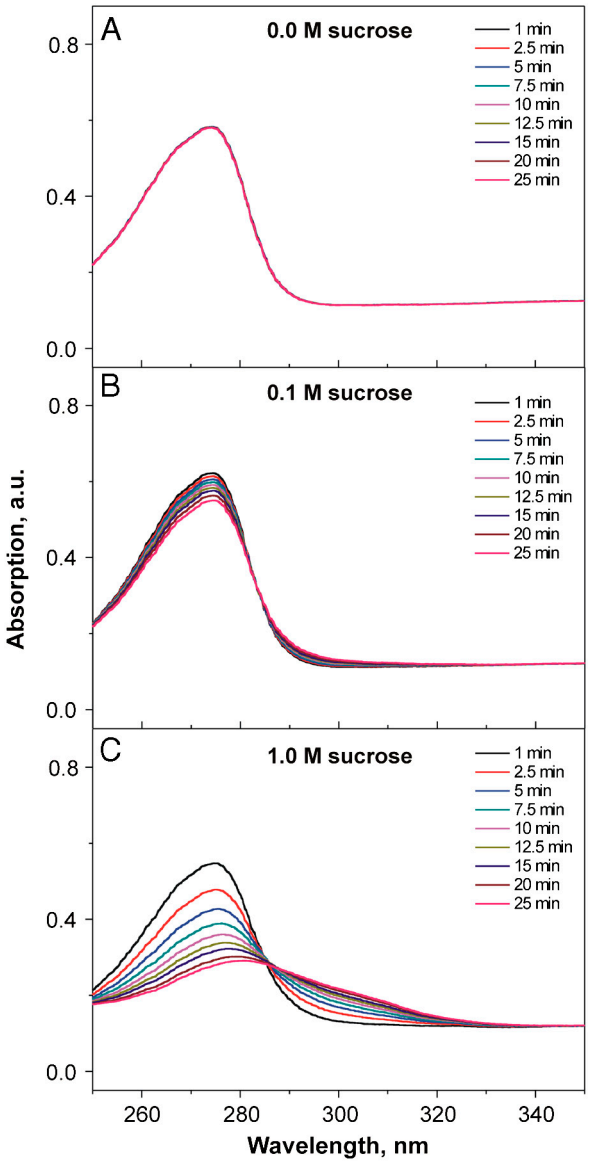

Fig. S5. UV-absorption changes of 2,5-DCBQ/DMSO (250 μM) as a function of dark incubation time in medium (pH 6.7; for composition, see *Materials and Methods*) containing 0.0 M (*A*), 0.1 M (*B*), and 1.0 M sucrose (*C*).

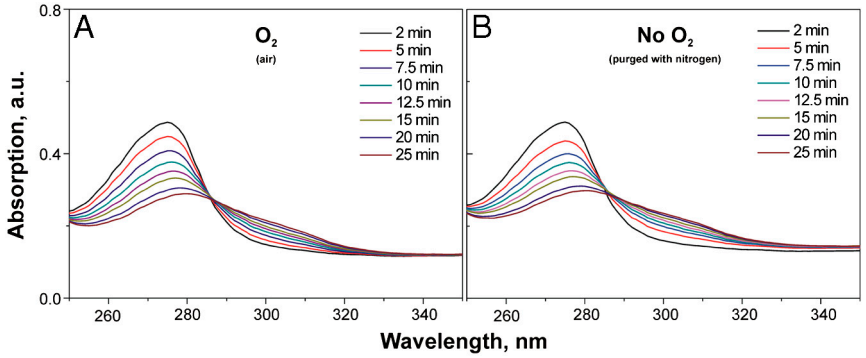

Fig. S6. UV-absorption changes of DCBQ/DMSO (250 μM) as a function of dark incubation time in air-saturated (*A*) and O_2-depleted (*B*) buffers (pH 6.7) containing 1 M sucrose (for detailed composition, see *Materials and Methods* in the main paper). The buffer was depleted of O_2 by intensive flushing with nitrogen in septum sealed vials for 20 min. Afterward, 2,5-DBCQ was added to the O_2-depleted medium from stock solution (20 mM) to give final concentration of 250 μM. To avoid O_2 contamination when handling samples, all transfers were made with gas-tight syringes that had been preflushed with nitrogen. The measurements were performed in a sealed cuvette.

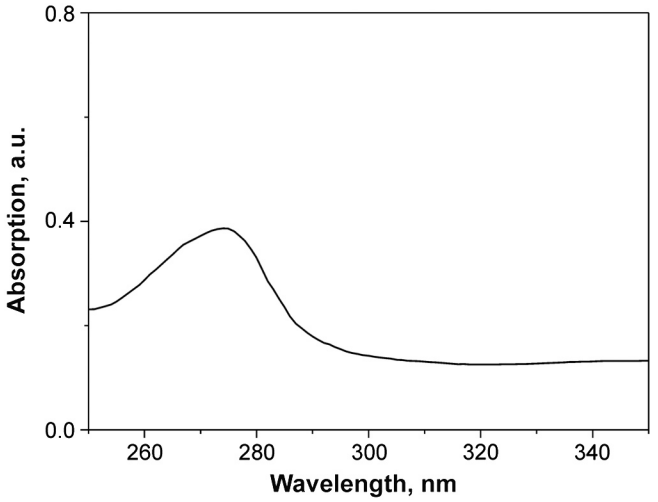

Fig. S7. UV-absorption spectrum of 2,5-DCBQ/Ethanol (250 μM) after 60-min incubation of the medium (pH 6.7) in the MIMS cell under 20 bars O_2. The incubation was done under the identical conditions that were used for the current MIMS study in the presence of PSII-cc from *Syn. Sp.* ([Chl] = 5 μM). Standard medium contained 100 mM sucrose (for full composition, see *Materials and Methods*).

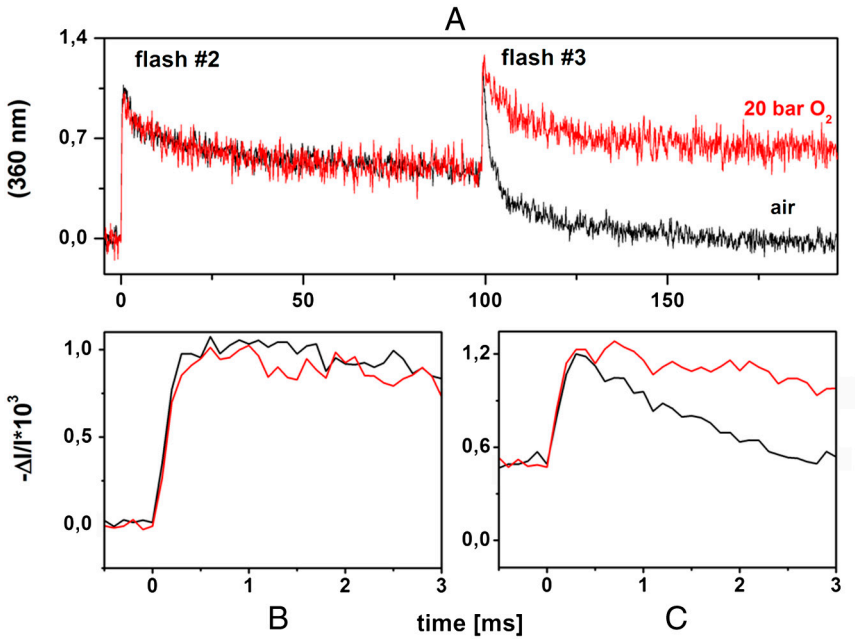

Fig. S8. UV-absorption transients at 360 nm induced by the excitation of a dark-adapted suspension of PSII-cc from *Syn. sp.* with a series of short laser flashes (pH 6.5) with time resolution 200 ms (*A*) and 3 ms for the flash #2 (*B*) and flash #3 (*C*). The suspension was equilibrated with air (black trace; average of 3 exp.), and with 20 bars O_2 (red trace; average of 2 exp.), respectively. The transients represent the response to flashes #2 and #3. The extent of the black and the red absorption transient was normalized to yield the same amplitude upon flash #2.

Table S1. Measuring conditions for previous and the present studies

Study, reference	Sample, concentration	Buffer, pH	Acceptor(s)	O_2 pressure
UV absorption (1)	PSII-cc from *Syn. sp.*, 3–5 μM Chl.	1 M sucrose, 25 mM $CaCl_2$, 10 mM NaCl, 1 M glycine betaine, 0.06% β-DM, 50 mM MES (pH 6.7)	200 μM 2,5-DCBQ in DMSO; added 5 min prior to measurement	20 bars; buffer stirred strongly for 40–60 min, then sample added and gentle stirring for 20 min
DF (2)	Spinach BBY,20 μM Chl	1 M glycine betaine, 10 mM NaCl, 5 mM $CaCl_2$, 25 mM MES, pH 6.5	No electron acceptor added	20 bars; buffer stirred strongly for 40–60 min, then sample added and gentle stirring for 20 min
Time-resolved K-edge X-ray absorption spectroscopy (3)	Spinach BBY/thin partially dried film of 15 mg/mL; 10 μL	15 mM NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 1 M betaine, 10% vol/vol glycerol, and 25 mM MES, pH 6.2	PPBQ in DMSO (1% final)	13 bars; 10–20 min at indicated pressure; diffusion into thin film
VIS fluorescence (4)	Spinach thylakoids (and various whole cells); 0.6 mm film of 40 μL sample; 50 μg/mL	300 mM sucrose, 35 mM NaCl, 50 mM MES (pH 6.5)	2,6-DCBQ in DMSO (<1% final)	10 bars (spinach thylakoids); 43 bars (*A. maxima* cells); 5-min exposure at indicated pressure; diffusion into thin film
MIMS (present study)	PSII-cc from *Syn. sp.* and spinach BBY; 5–50 μM Chl, 600 μL	100 mM sucrose, 25 mM $CaCl_2$, 10 mM NaCl, 1 M glycine betaine, 50 mM MES (pH 6.7 or 5.5) for *Syn. sp.*; 150 mM sucrose, 35 mM NaCl, 40 mM MES (pH 6.5) for spinach	2,5-DCBQ in ethanol (1.75% final) for PSII-cc; 1 mM Fecy for PSII-mf	20 bars; 30–50 min stirring

BBY (Berthold, Babcock, and Yocum), PSII membrane fragments (5); PPBQ, 2-phenyl-p-benzoquinone.

1. Clausen J, Junge W (2004) Detection of an intermediate of photosynthetic water oxidation. *Nature* 430:480–483.
2. Clausen J, Junge W, Dau H, Haumann M (2005) Photosynthetic water oxidation at high O_2 backpressure monitored by delayed chlorophyll fluorescence. *Biochemistry* 44:12775–12779.
3. Haumann M, Grundmeier A, Zaharieva I, Dau H (2008) Photosynthetic water oxidation at elevated dioxygen partial pressure monitored by time-resolved X-ray absorption measurements. *Proc Natl Acad Sci USA* 105:17384–17389.
4. Kolling DRJ, Brown TS, Ananyev G, Dismukes GC (2009) Photosynthetic oxygen evolution is not reversed at high oxygen pressures: mechanistic consequences for the water-oxidizing complex. *Biochemistry* 48:1381–1389.
5. Berthold DA, Babcock GT, Yocum CF (1981) A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes. *FEBS Lett* 134:231–234.