Supporting Information

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Materials and Methods

Pressure Cell for X-Ray Experiments. A pressure cell for XAS on PSII samples was constructed and used in the measurements, which sustained O_2 pressures up to 20 bar (Fig. S1).

Characterization of X-Ray Photoreduction of Mn at Ambient and Elevated pO_2. The high intensity and relatively small focus spot of the X-ray beam at beamline ID26 in combination with the room temperature conditions, according to previous experiments (1–3), were expected to cause rapid X-ray photoreduction of the Mn complex. Furthermore, the rate of Mn photoreduction was predicted to be reduced at elevated pO_2 because of the increased absorption of the gas in the beam path in front of the sample. The rate of Mn photoreduction was monitored by recording time courses of X-ray fluorescence in the region of the Mn K-edge (1–3). At an excitation energy of 6549 eV, reduction of the Mn complex caused a pronounced increase of the X-ray fluorescence level because of a shift of the Mn K-edge by ≈6 eV to lower energies until all Mn ions initially in the Mn(III) and Mn(IV) oxidation states became reduced to the Mn(II) level. From the time courses and amplitudes of the fluorescence increase, the rate and extent of Mn photoreduction were determined (1–3).

We studied the rate of Mn photoreduction in dark-adapted PSII samples (*i*) as function of the incident X-ray intensity at 0.2 bar (ambient air pressure) and 11 bar O_2, (*ii*) at O_2 partial pressures ranging from 0.2 to 16 bar, and (*iii*) varying the O_2 exposure time of the PSII samples before the X-ray measurements between ≈1 min and 1 h, both at 0.2 and at 11 bar O_2. The respective data are shown in Fig. S2 (see legend).

The results can be summarized as follows. (*i*) Mn photoreduction at room temperature was biphasic as observed in ref. 3; a lag phase (of duration d_{lag}) during which the Mn oxidation state essentially remained unchanged was followed by the reduction to the all-Mn(II) level obeying single-exponential kinetics (with halftime t_{red}). (*ii*) The magnitudes of d_{lag} and t_{red} linearly increased with decreasing X-ray intensity both at 0.2 and 11 bar O_2; that d_{lag} and t_{red} at 11 bar O_2 both were by a factor of ≈2 larger compared with 1 bar of air was in line with the calculated decrease of the X-ray transmission (http://henke.lbl.gov/optical_constants) through the gas in the pressure cell in front of the sample also by a factor of ≈2 at 11 bar O_2. (*iii*) Within the noise limits of the data, d_{lag} and t_{red} linearly increased with increasing pO_2 between 0.2 and 16 bar. (*iv*) There was no effect of the preincubation time of samples at 11 bar O_2 on the rate of photoreduction.

In summary, there was no indication that the intrinsic rate of the Mn photoreduction at room temperature was affected by an increase of the pO_2 to up to 16 bar. The slower photoreduction at increased pO_2 can be explained by the reduced X-ray intensity on the PSII samples caused by the increased gas absorption in the path of the X-ray beam. However, quantitative comparison of X-ray absorption data obtained at ambient and elevated pO_2 (see below) required that Mn photoreduction was negligible under both conditions. Accordingly, the X-ray intensities on the samples were adjusted (by Al foils, attenuator box of ID26 from XIA), so that the duration of the lag phase, during which the Mn oxidation state remained unchanged, was similar at all pO_2 values and exceeded the duration of the XAS measurements described in the main text at least by a factor of 2.

EPR Measurements at high pO_2. One option to interpret the apparent double-hit factor in the time-resolved XAS measurements was oxidation of the S_1-state of the Mn complex to S_2 in the dark. The normal S_2-state is characterized by a well-known EPR signal, the so-called S_2-multiline signal (4). We searched for the multiline signal in PSII samples exposed to high-O_2 pressure. Pellets of PSII membranes in acrylic glass frames, which were prepared similarly to the XAS samples, were inserted in a plastic tube, and after flushing with O_2 gas, were set under O_2 pressure. After O_2 incubation, the tube was immersed in liquid nitrogen while retaining the pressure, for rapid freezing of samples. Then, the deep-frozen tube was cut off after pressure release, and samples were removed and stored in liquid nitrogen. EPR at X-band (9.5 GHz) was performed on PSII samples similar to those used in XAS (*i*) in the EPR-silent S_1-state (dark), (*ii*) after continuous light illumination at 200 K to maximize the S_2-state multiline signal (Ctrl) (5), and (*iii*) after incubation in the dark at room temperature at 15 bar of O_2 for 30 min and freezing of samples in liquid nitrogen under pressure (O_2). The Ctrl showed a pronounced multiline signal (Fig. S3), which is typical for the S_2-state in the used PSII membrane preparation (6). This signal amounted to ≥50% of the maximal signal magnitude. In O_2-treated samples, there was no indication for the presence of an S_2-multiline signal and also not for any other new EPR signals (Fig. S3, O_2) (the remaining signals mainly are caused by incomplete subtraction of the microwave cavity background). We estimate that a contribution of 10% of the maximal signal magnitude would have been visible but was not observed. Accordingly, if there was oxidation of the S_1-state in the dark by O_2, it did not result in formation of the normal S_2-state. Formation of an EPR-invisible state of the Mn complex under O_2, however, is not excluded.

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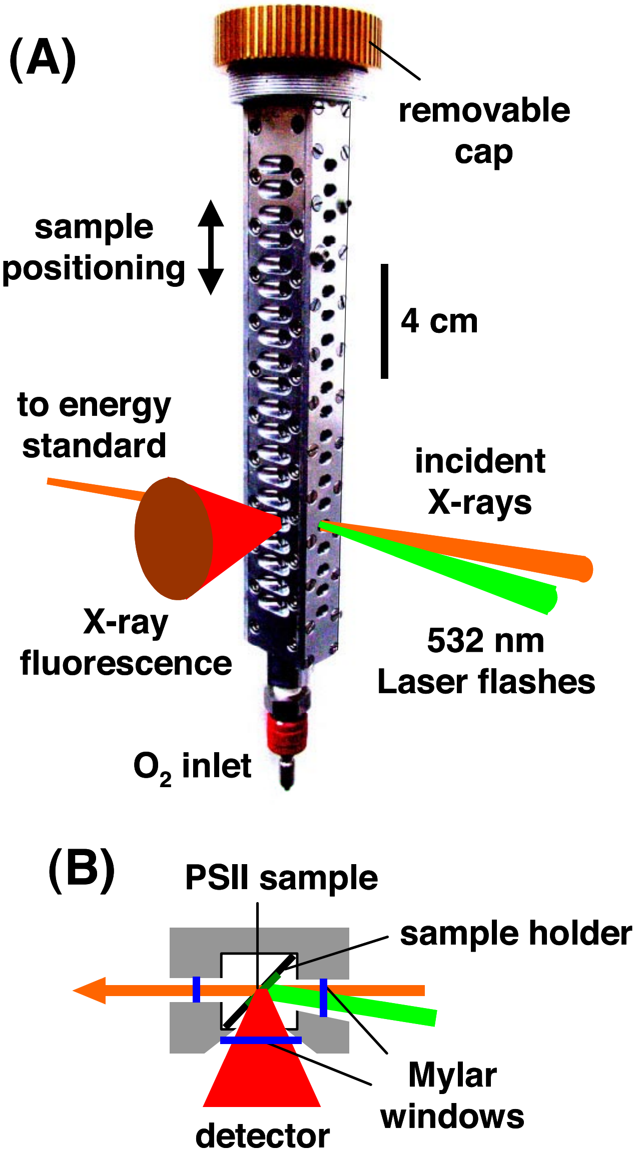

Fig. S1. Pressure cell for X-ray experiments. (*A*) Photograph of the cell; the paths of the X-ray beam and the laser flashes are indicated. The cell was mounted on a linear stage for sample positioning in the X-ray beam with micrometer precision. The removable cap allows for rapid change of the sample holders (Teflon strips with 20 PSII samples). (*B*) Schematic drawing of the sample in the pressure cell and of the beam alignment. The windows of the cell consisted of transparent Mylar foil (100 μm, except for the entrance window, which was 175 μm thick).

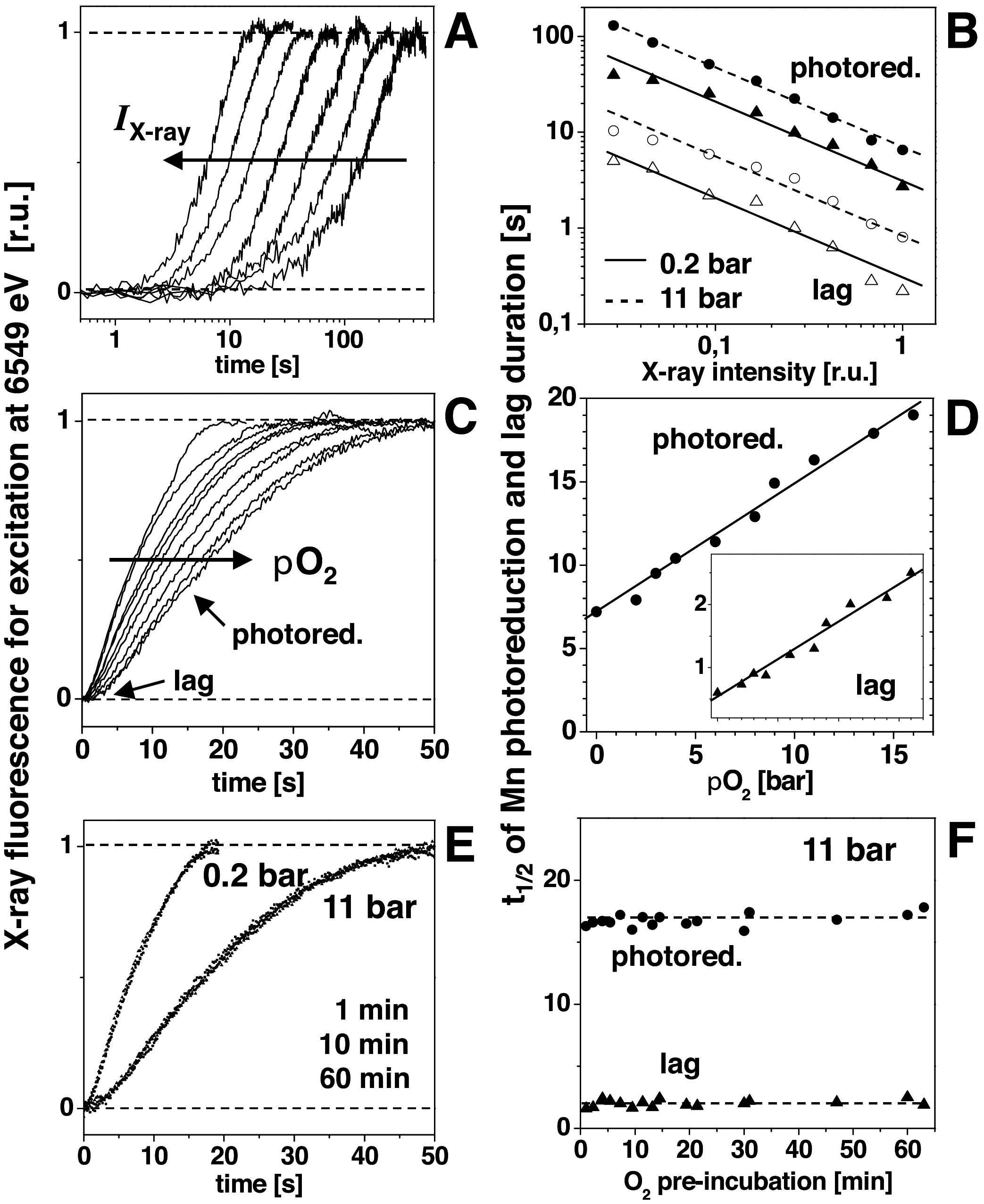

Fig. S2. Experiments addressing the velocity of X-ray photoreduction of Mn in PSII in the pressure cell. X-ray fluorescence time scans were measured at an excitation energy of 6549 eV (0.1–1 s per data point); 3–5 transients from fresh samples were averaged; final amplitudes were normalized to unity. (*A*) Transients at 11 bar O_2 at decreasing X-ray intensity on the samples. (*B*) Duration of the lag phase (d_{lag}, open symbols) and the half-time of the monophasic Mn reduction (t_{red}, filled symbols) at 0.2 bar (dashed lines) and 11 bar (solid lines) O_2 from data in *A*. (*C*) Time scans at pO_2 values between 0.2 and 16 bar O_2. (*D*) Magnitudes of d_{lag} and t_{red} derived from the data in *C*; lines represent linear regressions. (*E*) Mn photoreduction at the indicated pO_2 and incubation times of samples before the time scans. (*F*) Magnitudes of d_{lag} and t_{red} vs. the incubation time from data as in *E*.

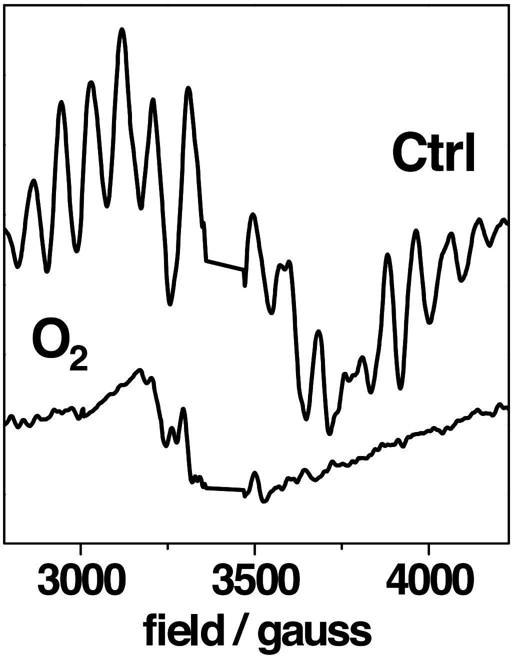

Fig. S3. EPR signals at X-band. Ctrl, multiline signal in PSII samples illuminated at 200 K for 2 min (6) (traces from 2 samples averaged). O_2, dark-adapted PSII samples incubated at 15 bar O_2 for 30 min at room temperature (traces from 5 samples averaged). Signals were measured on a Bruker ESP300E spectrometer equipped with a rectangular microwave cavity in the laboratory of F. Lendzian (Technical University Berlin); microwave power, 40 mW; modulation amplitude, 10 G; modulation frequency, 12.5 kHz. Samples were kept in an Oxford ESR900 helium cryostat at 8 K. From the raw traces, signals of dark-adapted PSII samples at ambient pO_2 (S_1 state), which showed no multiline signal, were subtracted after scaling to the rhombic iron signal at $g \approx 4$ (data not shown) to largely remove background contributions; the center region ($g = 2$), containing the $Y_D^{\cdot}$ radical signal, has been deleted for clarity.