OA2.3 - PSII crystals reveal new processes in photosynthetic water oxidation: Discovery of multiple flash periods in the catalytic cycle

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Crystallized samples of Photosystem II (PSII) contain the most uniform copies, by far, of nature's water splitting reaction center where O_2 is evolved. Despite their widespread use in crystallographic structural studies, few functional studies of PSII operation in crystals have been carried out. Their extreme homogeneity allows the observation of processes within PSII previously obscured by sample environment and intrinsic biological polydispersity. Time-resolved oximetric studies of PSII crystals demonstrate several previously uncharacterized phenomena: 1) Sustained oscillations in O_2 yield are seen over 200 flashes, approaching the upper natural limit of the efficiency of PSII-dependent water oxidation; 2) the highest O_2 quantum yield yet observed in any PSII *in vivo or in vitro* (peak photochemical efficiency of 61.6%); 3) photoreduction of O_2 and elimination by various exogenous electron acceptors; 4) observation of PSII turnover probability monitored in realtime as the pool of quinone acceptors is filled with electrons (regulation by quinol formation); 5) observation of backward transitions in which light reverses the catalytic cycle of the WOC; 6) the efficiency of electron removal from the native quinone acceptors, Q_A and Q_B, by exogenous acceptors controls the O_2 quantum yield (and by inference, the size and redox poise of the native plastoquinone/plastoquinol pool *in vivo*); 7) the lifetimes of the S2 and S3 states in the dark are greatly extended in PSII microcrystals, by over an order of magnitude compared to native culture, greatly confounding studies of individual S states. Unique discoveries from PSII crystals: O_2 evolution flash yields can exhibit multiple alternate flash periods other than the dominant classic period-4 arising from the four-step water oxidation process. A period-2 cycle in O_2 yield is observable attributed to two overlapping processes: primarily the acceptor-side gating of electron flux through Q_AQ_B, predicted by Shinkarev, but also a second mechanism postulated to stem from peroxide generation at the donor side. Additional cycles with period ~2.3, 4.7, and ~22 are observed, under specific electron acceptor conditions, which reflect new coherences in the WOC catalytic cycle yet to be deciphered. (Supported by the DOE BES, Photosynthetic Systems Program)