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Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols

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Abstract

We present a methodology, called fast repetition rate (FRR) fluorescence, that measures the functional absorption cross-section ($Ïf_{PS\ II}$) of Photosystem II (PS II), energy transfer between PS II units (p), photochemical and nonphotochemical quenching of chlorophyll fluorescence, and the kinetics of electron transfer on the acceptor side of PS II. The FRR fluorescence technique applies a sequence of subsaturating excitation pulses (â€˜flashletsâ€™) at microsecond intervals to induce fluorescence transients. This approach is extremely flexible and allows the generation of both single-turnover (ST) and multiple-turnover (MT) flashes. Using a combination of ST and MT flashes, we investigated the effect of excitation protocols on the measured fluorescence parameters. The maximum fluorescence yield induced by an ST flash applied shortly (10 Î¼s to 5 ms) following an MT flash increased to a level comparable to that of an MT flash, while the functional absorption cross-section decreased by about 40%. We interpret this phenomenon as evidence that an MT flash induces an increase in the fluorescence-rate constant, concomitant with a decrease in the photosynthetic-rate constant in PS II reaction centers. The simultaneous measurements of *Ïƒ*$_{PS\ II}$, *p*, and the kinetics of Q$^{âˆ’}$$_A$ reoxidation, which can be derived only from a combination of ST and MT flash fluorescence transients, permits robust characterization of the processes of photosynthetic energy-conversion.

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Abbreviations

$a_{PS\ II}$, optical cross section of PS II; Chl, chlorophyll; *C(t)*, fraction of closed PS II reaction centers at time *t* during FRR excitation protocol; *f(t)*, fluorescence yield at time *t* during FRR protocol; FRR, fast repetition rate; F_o, minimal fluorescence yield; F_m, maximal fluorescence yield; *g(t)*, function describing the kinetics of Q$^{âˆ’}$$_A$ reoxidation; *g(t)*=*Î±*$_1$exp(âˆ’*t*/*Ï„*$_1$)+*Î±*$_2$exp(âˆ’*t*/*Ï„*$_2$)+*Î±*$_3$exp(âˆ’*t*/*Ï„*$_3$); HF1, F_m induced by first ST excitation in dark-adapted cells; HF2, F_m induced by ST flash applied following MT flash; HFM, F_m induced by MT excitation; *i(t)*, excitation intensity at time *t* in FRR protocol; *I(t)*, cumulative excitation energy in FRR protocol; LED, light-emitting diode; LF, F_m induced by ST excitation; MT, multiple turnover; *p*, extent of energy transfer between PS II reaction centers; PAM, pulse amplitude modulation fluorometry; P&P, pump-and-probe fluorometry; PQ, plastoquinone pool; PS II, Photosystem II; $q_p(t)$, photochemical quenching at time *t* during the FRR protocol; Q_A, the primary quinone electron acceptor in PS II; Q_B, the secondary quinone electron acceptor in PS II; *Ïƒ*$_{PS\ II}$, functional (i.e., the photochemically effective) cross section of PS II; RC II, reaction center of PS II; ST, single turnover; ST1, ST2, ST flashes applied before and after the MT flash, respectively

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