Supporting Text

Experimental Methods and Data Analysis. Transient absorption measurements have been performed in two spectral ranges corresponding to the Q_x and Q_y ranges of the chlorins and in two time ranges of 50 and 500 ps, and all these data sets were combined in a global analysis. The pump and white-light probe pulses were polarized at magic angle relative to each other. The transient absorption kinetics were measured by using a sensitive diode-array camera as described in refs. 1–3. The typical rms noise in a single pixel of the detection system was about $\pm 2 \times 10^{-5}$ OD units. Before analysis, four to six adjacent pixels (0.5-nm interval) were averaged, thus decreasing the noise further to $\leq \pm 1 \times 10^{-5}$ OD units. For excitation of the isolated RCs, pulses of 120-fs width (6-nm spectral width) and for photosystem (PS) II, core pulses of 60-fs width were used.

The original kinetic $\Delta A(t, \lambda)$ surfaces were transformed into the lifetime space as described in refs. 2 and 4. A basis set with 100 exponentials with fixed lifetimes τ_i ranging from 100 fs to 10 ns was used (Eq. **1**). The preexponential factors $\Delta A_i(\lambda_{det})$ were calculated by transformation of the $\Delta A_i(\lambda_{det}, t)$ data surface (Figs. 5 and 7) from time space to lifetime space by an inverse Laplace transform method [lifetime density analysis (LFD)] and are presented as lifetime density maps (LFD) (for details of this transformation and a discussion of the potential of this analysis, see ref. 2).

$$\Delta A(\lambda_{\mathrm{det}}, t) = \sum_{i=1}^{N} \Delta A_i(\lambda_{\mathrm{det}}) \cdot e^{(-\frac{t}{\tau_i})} \quad . \qquad \textbf{[1]}$$

Note that the fixed lifetimes τ_i have no physical but only a mathematical meaning. The resulting $\Delta A_i(\lambda_{det})$ values of the LFD maps fulfill the condition that an ideal description of the original 3D kinetic data surface $\Delta A_i(\lambda_{det}, t)$ results, without any systematic deviation of the experimental and modeled curves. The method has been tested extensively on

simulated data and was found suitable to reliably resolve close-lying discrete exponential lifetimes and to distinguish them from continuous lifetime distributions (2, 4).

Compartment Modeling. We applied global compartment modeling (1, 2, 5) to the kinetic 3D hypersurfaces (ΔA vs. time vs. wavelength), which can be obtained directly from the LFD maps (see Eq. **1**). This method is on the one hand fully equivalent to performing global modeling on the original data 3D surfaces (the lifetime hypersurfaces are in essence simple transformations of the original data) but can be performed using dramatically less computing time, because the deconvolution with the excitation pulse does not have to be performed in every cycle, which is important in view of the huge number of fitting cycles that have to performed for the complex models. Furthermore, this method allows a simple visual check of the resulting model lifetimes and of the original data. Lifetimes in the range >1 ps were taken into account in the modeling. This excludes the ultrafast intra-reaction center (RC) equilibration processes and focuses on the electron transfer and slow energy transfer processes. Various compartment models were tested by varying all rate constants over a very large range, using a grid search method to find regions of possible good fits. This method prevents the search from falling into local minima on the fitting surface. The "good" regions then were submitted to further optimization. The only minimal compartment models leading to a very good fit are shown in Fig. 3 (six-compartment model for intact cores and five-compartment model for isolated RCs). For the isolated RC, the model comprises one compartment for the excited external Chl_z pigments, an excited RC compartment (RC^*) representing the equilibrated excited state of the six RC pigments, and three radical pairs (RPs) in a sequential fashion. For the intact PSII core, two excited antenna compartments, the excited RC^* compartment, and three RP compartments are required. The rate constants shown in Fig. 3 *A* and *C* represent the optimal sets of rate constants for the two systems that simultaneously give a good fit (as judged by mathematical fitness criteria like, e.g., χ^2-values and residual plots) and at the same time provide spectroscopically "reasonable" species-associated difference spectra (SADS) (see *Discussion*). This is of fundamental importance, because usually only the kinetic model and the rate constants are shown, which is insufficient for deciding on a good model. According to our experience for

models of the complexity shown here, there usually exist several largely different sets of good (in terms of the mathematical fitting criteria) solutions for the rate constants. If the SADS are not examined at the same time it is not possible to select from this set of "mathematically" satisfactory solutions the "correct" physical model (5, 6).

Comparison with Other Kinetic Models for Isolated D1-D2 RCs. The formal kinetic model as shown in Fig. 4*A* (identical with the formal kinetic model used in our analysis) (Fig. 3*A*) has been proposed by Groot and coworkers (7) for explaining the fluorescence kinetics of isolated D1-D2 RCs. After our submission of the present work, Groot *et al.* (8) reported on a mid-IR transient absorption study on isolated D1-D2 RCs for an excitation wavelength of 681 nm where again the same formal kinetic model has been applied. The authors concluded from their analysis that Pheo should be reduced in the first RP (RP1) by electron donation from the Chl_{acc}^{D1} and that the secondary RP was assigned to $P_{D1}^{+}Pheo^{-}$. These are the same conclusions as drawn in the present work. We note, however, that their formally identical kinetic model results in very different rate constants. Thus, the set of rate constants of Groot *et al.* results in subpicosecond primary charge separation with a lifetime of 0.85 ps as compared with a lifetime of 3.2 ps in our model (Fig. 3*A*). The secondary electron transfer step is associated with an apparent lifetime of 5.6 ps in the model of Groot *et al.*, whereas this lifetime is 11 ps in our analysis. Furthermore, the rate constants for primary charge separation and charge recombination are equal in the model of Groot *et al.*, whereas these rate constants show a ratio of 2.5 in our model. The rate constant of primary charge separation is 500 ns^{-1} in the model of Groot *et al.*, whereas it is 180 ns^{-1} in our analysis (Fig. 3*A*). Despite the fact that the formal kinetic models are the same, it would be highly surprising if such vastly different sets of rate constants would provide equally good fits and at the same time also physically reasonable descriptions of the kinetics, consistent with the same electron transfer mechanism.

We have analyzed our present transient absorption data in the visible detection range (Figs. 1 and 5) for isolated D1-D2 RCs recorded at the same excitation wavelength (681 nm) in terms of the rate equation model of Groot *et al.* (7, 8). The resulting SADS for this

analysis are shown in Fig. 4*B*. Of particular interest is the region of 543 nm, i.e., in the Pheo Q_x bleaching band. The SADS in that region show that the active Pheo is fully bleached only in RP2, whereas it is only partially bleached in the first and the third RP, i.e., in RP1 and RP3. Furthermore Pheo is also partially bleached in the excited RC state (denoted as PC^*). The state PC^* also shows a small absorption increase above 700, a region where in fact a small absorption decrease is expected for an excited state due to stimulated emission. It appears to us that based on our transient absorption data in the visible range, the rate model of Groot *et al.* is in contradiction with the interpretation of the mechanism of Groot *et al.* Based on these data, one would have to conclude that Pheo is actually reduced in the secondary electron transfer step, rather than in the first step. We have shown, among other things, that the Pheo Q_x band is fully bleached already in the first RP and remains bleached to exactly the same amount in all three RPs, as is expected for a mechanism as proposed both by us and by Groot *et al.* We thus cannot rationalize the set of rate constants given in the model of Groot *et al.* with their conclusions regarding the mechanism. We appreciate that Groot *et al.* conclude from the SADS of the first RP that Pheo is reduced and that a Chl is oxidized. However, the rates provided do not agree with our data in the visible range. In addition to the problems related to the Pheo bleaching, also the apparent lifetime of the primary charge separation (0.85 ps) is not consistent with our data, because we do not observe any stimulated emission decay in that time range (see Fig. 1), which is a necessary phenomenon accompanying charge separation. We assume that the possible reasons for these discrepancies are the high excitation probabilities used in their measurements (8). From the data provided, we can estimate the number of absorbed photons per particle per pulse to be in the range of 1.3 for the low-intensity measurement to 4 in the higher-intensity measurement. Such high excitation density leads to annihilation in the RC, which will shorten the apparent charge separation lifetime, as shown in a detailed annihilation study (9). The latter study indicated the onset of annihilation already at an excitation intensity >0.4 absorbed photons per particle per pulse. Finally, the fact that we obtain the same rate constants for the electron transfer steps in isolated RCs and in intact cores provides strong support for our model and conclusions.

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