Supplementary Information

2D Data analysis procedures

Global and target analysis techniques described previously[1-3] were applied for the parameterization and extraction of population dynamics measured in 2DES experiments. In short, an analysis procedure is based on the postulated population connectivity scheme, described by a number compartments *n*, each featuring a characteristic 2DES spectrum $\sigma_i(\omega_1,\omega_3)$ and time-dependent population $c_i(t_2)$. Usually (but not necessarily) the population flow between these compartments is described by a set of linear kinetic equations. With the characteristic transfer times denoted $\tau_{i\to j}$ (where $i\to j$ signifies the population transfer from the i^{th} to the j^{th} component, and $i\to i$ signifies the decay of the i^{th} component to the ground state), the populations can be obtained by solving the following set of equations:

$$\frac{dc_i}{dt} = A_i I_{pump}(t) + \sum_{\substack{j=1,\\ j\neq i}}^{n}\left(\frac{c_j(t)}{\tau_{j\to i}}\right) - \sum_{j=1}^{n}\left(\frac{c_i(t)}{\tau_{i\to j}}\right), \text{where } i = 1,2,\text{K}\ ,n. \qquad (1)$$

Here A_i a signifies the initial excited state population distribution among one ($A_i=\delta_{i0}$) or several compartments; $I_{pump}(t)$ and is the instrument response to the pump pulse; Each of the components is attributed a unique species associated 2DES spectrum $\sigma_i(\omega_1,\omega_3)$, and the product of the time-dependent concentrations and frequency-dependent is used to construct the global approximation of the time-resolved data:

$$S(t_2,\omega_1,\omega_3) = \sum_{j=1}^{n} c_j(t)\sigma_j(\omega_1,\omega_3). \qquad (2)$$

The population transfer rates $\tau_{i\to j}$, and component spectra $\sigma_i(\omega_1,\omega_2)$, are estimated by the optimization algorithm and come out as free parameters of the fit.

The simplest case of population dynamics (1) is the sequential or evolutionary connectivity scheme, where the initially excited compartment No. 1 transfers the population exclusively to compartment No. 2, and so on, and the last compartment in the series decays to the ground state:

$$\begin{aligned}
\frac{dc_1}{dt_2} &= A_i I_{pump}(t_2) - \frac{c_1(t_2)}{\tau_{1\to 2}}, \\
\frac{dc_i}{dt_2} &= \frac{c_{i-1}(t_2)}{\tau_{i-1\to i}} - \frac{c_i(t_2)}{\tau_{i\to i+1}}, i = 2,...,n-1, \\
\frac{dc_n}{dt_2} &= \frac{c_{n-1}(t_2)}{\tau_{n-1\to n}} - \frac{c_n(t_2)}{\tau_{n\to n}}
\end{aligned} \qquad (3)$$

In this case the entire 2D dataset is viewed as a set of 2D spectra evolving into each other. The advantage of such technique is the ability to concisely describe the exponential-like population dynamics (leaving out the oscillatory features) as a set of 2D spectra, each of which is emerges at a specific population time t_2 in the experiment. Such scheme does not necessarily imply physical meaning of specific compartments; it merely serves as a tool to reduce a complex population dynamics into a small number of 2DES carpets, each with its characteristic temporal features.

When the connectivity scheme is constructed with physical states in mind for each of the compartments used, the global analysis technique is called target analysis; the models are then tested and selected based on the obtained component spectra and their correspondence with physical intuition. Such analysis has been very successful for time-resolved fluorescence and transient absorption experiments[2, 3]. Here we employ a simple sequential model to

characterize the dominant kinetic processes. The resulting 2D-EADS are shown in Figure 3 for the parallel data and here below in Figure S1 for the perpendicular data.

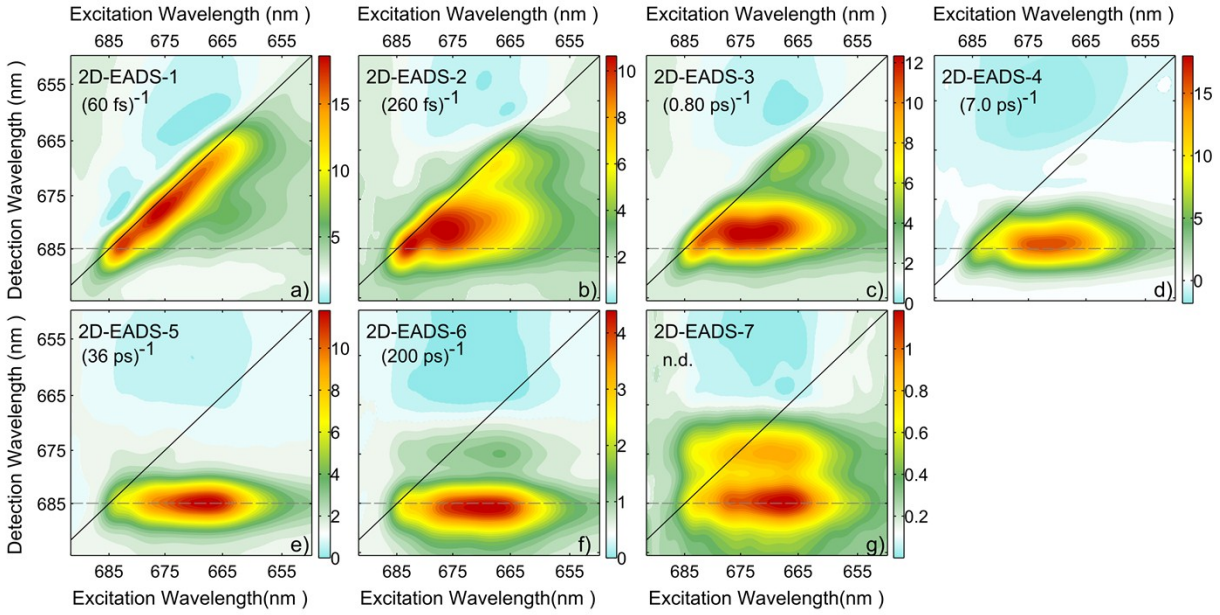

Figure S1: Two-Dimensional Evolution-Associated Difference Spectra (2D-EADS) for the PSII CC at 77K resulting from a global data analysis with a sequential model to the perpendicularly-polarized 2D spectral dataset. Six exponential decay components, 2D-EADS-1 ~ 2D-EADS-6 (a-f) and one non-decaying component, n.d., 2D-EADS-7 (g), are required for an adequate fit. Each component decays with a rate constant as indicated in the legend The polarization of two pump pulses was perpendicular to that of the probe pulse. The amplitude of each panel is shown in its respective color bar. The solid black line denotes the diagonal of excitation and detection wavelength axes, while the dashed grey line indicates a detection wavelength of 685 nm.

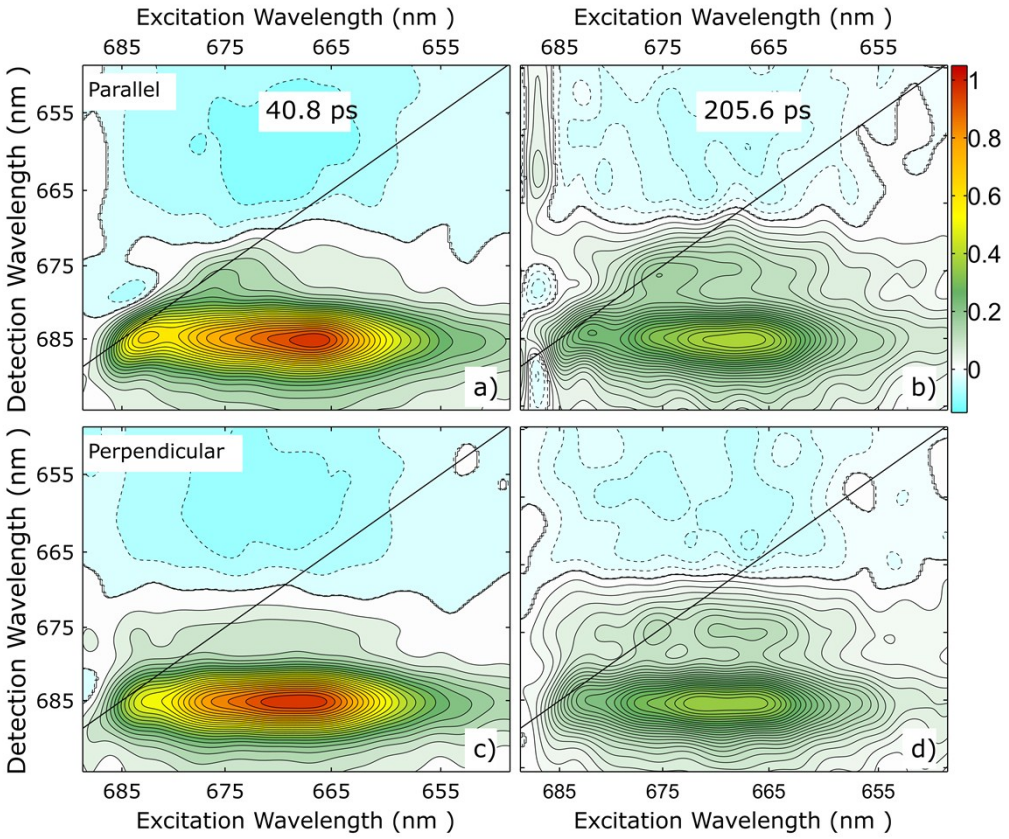

Figure S2: Absorptive 2D electronic spectra of the PSII CC at 77K recorded at different t_2 waiting times as labeled. Upper panels, all pump and probe pulses are parallel (a-b); Bottom panels, the polarization of two pump pulses was perpendicular to that of the probe pulse (c-d). In both polarization conditions, spectral amplitudes are normalized to the maximum of the earlier waiting time, i.e. 40.8 ps. Positive amplitude is shown in solid contour lines, and negative amplitude in dashed lines. Contours are evenly spaced at 4% of the maximum. The solid black line indicates the diagonal of excitation and detection wavelength axes.

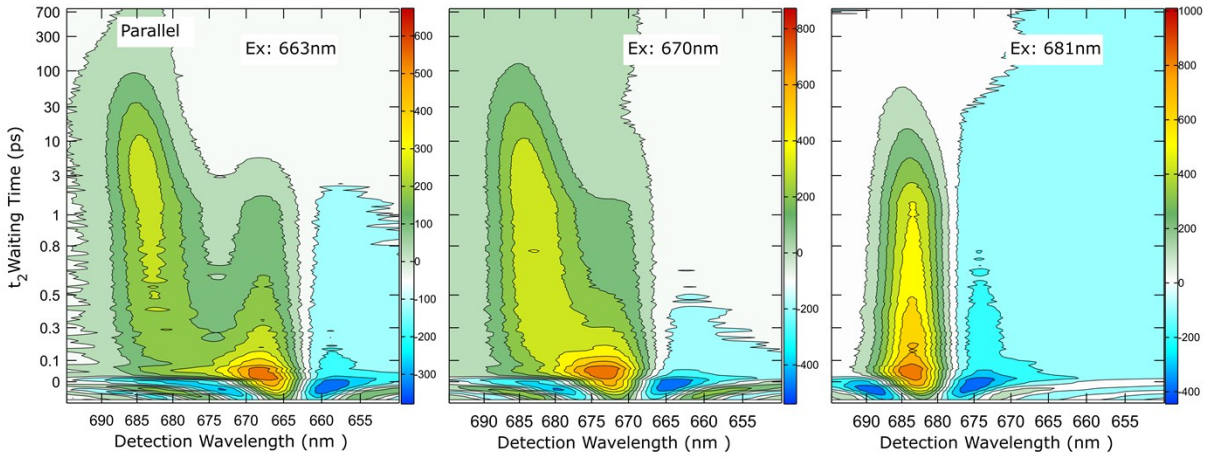

Figure S3: Pump-Probe-like absorption electronic spectra of the PSII CC at 77K derived from 2DES spectra at three different excitation wavelengths of 663, 670 and 681 nm, respectively. The signals were obtained by integrating the 2DES data along the excitation frequency axis over a 6nm excitation bandwidth centered at each selected excitation wavelength. All pump and probe pulses were set at parallel polarization. 2D spectra were collected from t_2= -100 fs to 1 ps, with linear sampling every 10 fs, followed by logarithmic intervals to a maximum waiting time of 750 ps.

Pigment transition dipole moments

The parameters for all our calculations were taken from Shibata et al.[4] Only the transition dipole moments were not listed. Those we have calculated from PDB file 3WU2[5] by assuming that transition dipole moments point from NB to ND atoms in both chlorophylls and pheophytins. The chlorophyll transition dipole moments were normalized to 4.4 Debye, while those of pheophytins – to 3.4 Debye. For convenience, the dipole moments are listed in Table S1.

Table S1. The transition dipole moments for PSII CC pigments. Pigment notation is the same as in Shibata et al.[4]

Pigment	x	y	z
PD1	-3.31	1.60	2.43
PD2	4.19	0.38	1.30
AccD1	4.27	-0.30	1.02
AccD2	-3.39	2.29	1.62
PheD1	-1.65	-2.63	-1.40
PheD2	-0.02	-0.25	-3.39
ChlZD1	0.84	3.78	2.10
ChlZD2	1.24	0.07	4.22
Chl11	1.92	-0.29	3.95
Chl12	-4.18	-0.86	-1.06
Chl13	0.15	4.36	-0.56
Chl14	3.13	-3.02	0.67
Chl15	-3.76	-0.15	2.28
Chl16	3.22	2.98	-0.31
Chl17	-1.46	4.13	-0.42
Chl21	3.29	0.78	-2.82
Chl22	1.62	-3.82	-1.46
Chl23	0.14	2.47	-3.64
Chl24	-3.88	-1.82	0.98
Chl25	2.84	-3.33	0.45
Chl26	-2.79	2.87	-1.82
Chl27	-4.12	-1.04	-1.15
Chl28	-4.29	-0.91	0.38
Chl29	0.40	-2.03	-3.88
Chl33	0.94	-2.81	3.25
Chl34	-3.11	3.11	0.07
Chl35	3.72	1.46	-1.84
Chl37	2.46	-2.88	2.23
Chl41	-3.61	-2.51	0.16
Chl42	-3.92	1.80	-0.86
Chl43	-0.87	-4.24	0.82
Chl44	2.77	0.79	-3.33
Chl45	-3.42	2.66	-0.75
Chl46	2.85	-3.35	-0.23
Chl47	1.34	-1.38	-3.96
Chl48	3.99	-0.03	-1.85
Chl49	0.68	-4.27	0.83

1. Holzwarth, A.R., ed. *Data Analysis in time-resolved measurements*. Biophysical Techniques in Photosynthesis, ed. J. Amesz and A.J. Hoff. 1996, Kluwer: Dordrecht, The Netherlands.
2. van Stokkum, I.H.M., D.S. Larsen, and R. van Grondelle, *Global and target analysis of time-resolved spectra (vol 1658, pg 82, 2004)*. Biochimica Et Biophysica Acta-Bioenergetics, 2004. **1658**(3): p. 262-262.
3. van Stokkum, I.H.M., D.S. Larsen, and R. van Grondelle, *Global and target analysis of time-resolved spectra.* Biochimica Et Biophysica Acta-Bioenergetics, 2004. **1657**(2-3): p. 82-104.
4. Shibata, Y., et al., *Photosystem II Does Not Possess a Simple Excitation Energy Funnel: Time-Resolved Fluorescence Spectroscopy Meets Theory.* Journal of the American Chemical Society, 2013. **135**(18): p. 6903-6914.
5. Umena, Y., et al., *Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 angstrom.* Nature, 2011. **473**(7345): p. 55-61.