Supporting Information

Photoelectrochemical two-dimensional electronic spectroscopy (PEC2DES) of photosystem I to study charge separation dynamics in photosynthesis

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SI1. PSI-LHCI complexes purification:

Arabidopsis thaliana plants (Arabidopsis Col-0) were grown under white light at 120 μmol photons·m^{-2}·s^{-1}, 12 hr/12hr day/night cycle at 23 °C, for 5 weeks. Thylakoid membranes were isolated from Arabidopsis leaves according to s PSI-LHCI complexes were purified from thylakoid membranes with sucrose density gradients as previously described[4]. Chlorophyll concentration was adjusted to 0.5 mg Chl·ml^{-1} in 5 mM EDTA, 10 mM Hepes pH 7.5 and solubilized with an equal amount of detergent solution (1% a-decylmaltoside (DM) in 10 mM Hepes 7.5) for 10 min. After solubilization, the sample was centrifuged at 12,000 x g, for 10 min to eliminate the insolubilized material. The supernatant was loaded onto the sucrose gradients prepared by freezing and thawing a sucrose solution (500mM sucrose, 20 mM Hepes pH 7.5, 0.06% a-DM). The gradients were centrifuged for 16 hrat 4 °C at 160,000 x g. PSI-LHCI complexes were collected with a syringe. PSI-LHCI samples chlorophyll concentration is 0.28 μg·ul^{-1}.

SI2. Au-pIQA-cys-PSI-LHCI electrode preparation

Transparent gold electrodes Autr10 (Dropsens-Metrohm, Spain) were sonicated in ethanol before use. Electrodes were dried in nitrogen stream prior to incubation of linker peptide pIQA-cys 0.1 mM, in sodium acetate buffer 50 mM, pH 4.5, for 30 min at room temperature. After peptide incubation, electrodes were gently rinsed with Phosphate Buffer Saline (PBS) 50 mM, pH7.4. Droplets of 5 μl of PSI-LHCI sample (1.4 μg chlorophyll) were incubated in the electrode surface in the dark at 4ºC for 1h.

SI3. AFM characterization Au-pIQA-cys-PSI-LHCI biohybrid electrode

Atomic force microscopy (AFM) imaging. AFM scans were performed using an MFP-3D atomic force microscope (Asylum Research, Santa Barbara, CA) using V-shaped Si_3N_4cantilevers with sharp silicon tips and having a nominal spring constant of 0.12 N·m^{-1}(SNL, Bruker AFM Probes, Camarillo, CA). The AFM was operated in tapping mode in liquid, covering the sample with PBS 50 mM, pH 7.4.

SI4. Acquisition and setup details

Along t1 and t3, they are collected from 0 to 48 fs every 4 fs, while along t2 from 0 to 450fs every 15 fs, generating 3d matrices of 13x13x31 points. We select a rotating frame atIn figure 1, maps at t2=60fs are shown, the signal is completely decayed within 48 fs along t1 and t3, justifying our choice of stop at 48 the acquisition. The step of 4 fs along coherence time is suitable for a bandwidth of 4000 cm^{-1} respecting Nyquist limit. Therefore, using appropriate rotating frame (13342 cm^{-1}/0.4 fs^{-1}), it is possible to get all the responses within our laser spectrum (ranging from ~14000 to ~17000 cm-1).

The tuning of the laser fluence in the experiment was determined by several factors: the number of excitations per PSI-LHCI, the minimization of the Dazzler artifact, sample degradation, and the signal-to-noise ratio of the non-linear signal. The first three factors impose limitations that we could not exceed to ensure the validity of the experiment. Additionally, the energy per pulse had to be maintained above a certain threshold to prevent the generated current from falling below the noise level.

To cope with these constraints, we opted for a large beam (~2.5 mm in diameter), which allowed us to uniformly excite the PSI-LHCI sample across the electrode and maximize the current, while avoiding the pure linear regime. Through this careful optimization, we arrived at an energy of approximately 50 nJ per pulse.

SI5. Phase modulation

The phase modulation using Dazzler has already been described in previous work[1].
The modulation is based on evolving individual phases of the 4 pulses at every laser repetition with a different step. Different combinations of modulation frequencies (f_n) are linked to different combination of phases of the response of the system, that in torn is connected to the response frequencies combinations : $\pm f_1 \pm f_2 \pm f_3 \pm f_4 \rightarrow \pm\varphi_1 \pm \varphi_2 \pm \varphi_3 \pm \varphi_4 \rightarrow \pm\omega_1 \pm \omega_2 \pm \omega_3 \pm \omega_4$.
The table below summarize the different combination and frequencies.

component	divisors	Frequency (Hz)
linear		
f_{21}	6-0	250
f_{31}	8-0	333.33
f_{41}	9-0	375
f_{32}	8-6	83.33
f_{42}	9-6	125
f_{43}	9-8	41.66
4th order		
f_{reph}	-0+6+8-9	208.33
$f_{non\text{-}reph}$	-0+6-8+9	291.66
f_{2Q}	-0-6+8+9	458.33

Table SI-1

SO, rephaseing and non rephasing signals are extracted at 208.33 and 291.66 Hz respectively and the linear signal shown in the main text at 250 Hz.

SI6. Dazzler intensity artifact

It has been reported that Dazzler generates artifacts in population detected 2DES especially in the rephasing signal[2]. This artifact affects the shape of the rephasing spectrum maps in time domain displaying a constant signal on the diagonal (t_1=t_3) and has shown to be constant also along the delay time t_2. Potential artifacts have been limited comparing PEC2DES with a control signal measuring excitation intensity simultaneously acquired with a linear detector (see below). Uneven excitation is not responsible for the overall PEC2DES signal as photo-diode signal has a different shape with respect to photo-current response.

The Dazzler has limited capabilities in keeping costant intensity while changing phases of a quartet of pulse. This give rise to nonlinearities that are not dependent on the response of the sample but on the fluctuant intensity of excitation. This effect, mainly present in the rephasing signal, has already been reported in the PhD thesis of Roeding S. from the group of Brixner[2]. They correct this effect by iteratively searching for intensity correction factor at any collection point, until a linear response sample gives no non-linear effect.

We adopted instead a aposteriori approach where we detect the linear response of a photodiode simultaneously with the experiment and making a properly normalized subtraction of the photodiode generated feature.

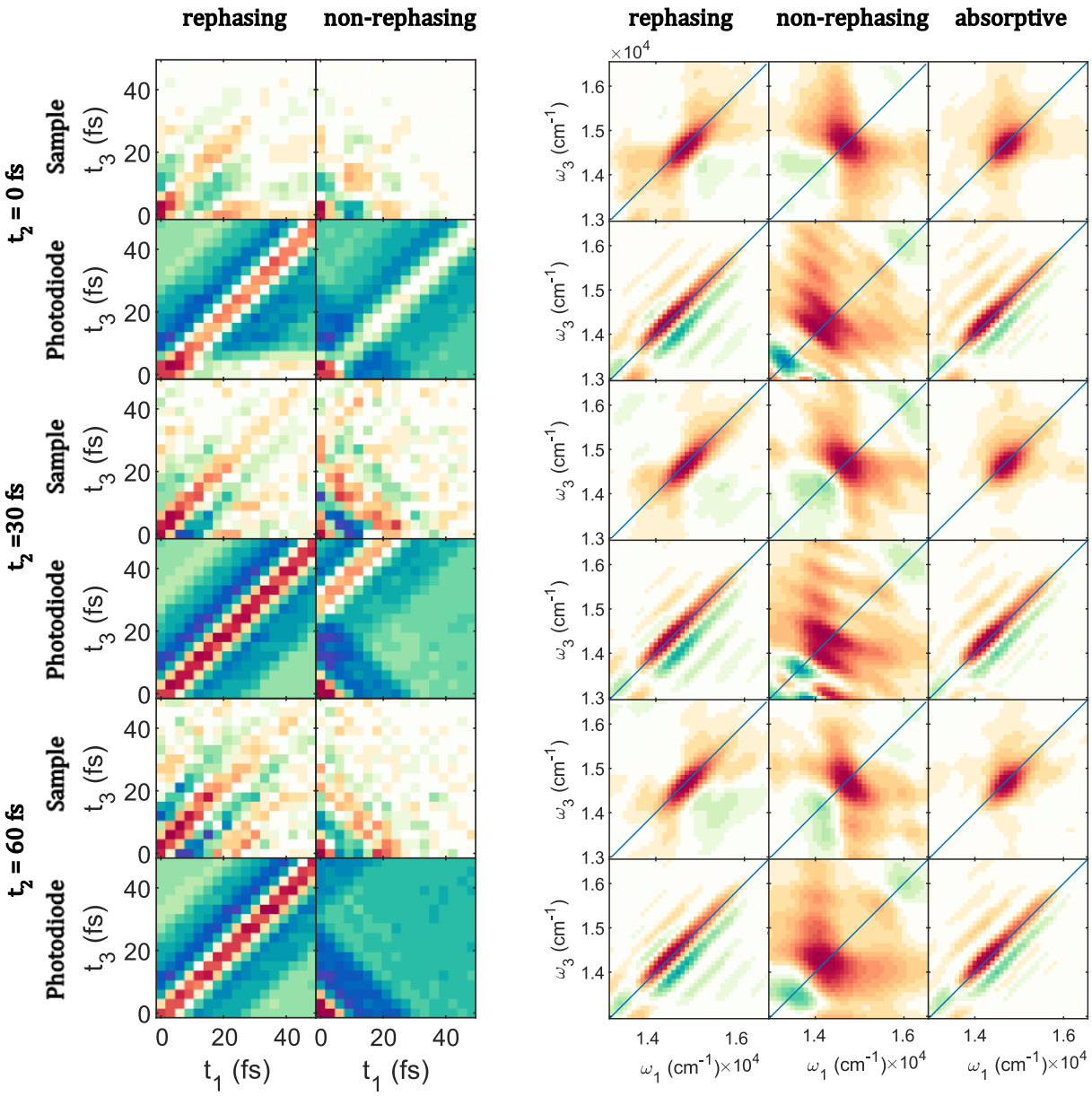

Figure SI-F1 Signal coming from sample and photodiode at different t_2. The time-dependent photodiode rephasing signal shows a costant feature along the diagonal that does not decrease increasing coherence times. Ths lead to a frequency dependent signal distributed along the diagonal that will depend on the coherence time range used. In the sample signal the photodiode collected artifact is removed, and the remaining signal decays showing the typical shape in the frequency domain. Moreover, note that in the time dependent non-rephasing the artifact disappears when t_2 is larger than coherence time range. In the frequency dependent non-rephasing the peak is shift with respect of the sample confirming that the signal from the sample is not due to dazzler artifact.

SI7. Incoherent mixing, linear reconstruction

Incoherent mixing is an effect that takes place when the detected signal is not proportional to the population generated by the quartet of pulses which is the first principle of population detected 2DES. This proportionality will not hold if excitons, or eventually charges, interact and annihilate between each other before being detected. In the latter case, part of the linear signal falls into the modulated detection frequencies of rephasing and non-rephasing signals. This contribution is constant along t2 and thus would fit well our situation. The reconstructed linear signal into the 2D maps (see Supplementary below) has similar shape to rephasing and non-rephasing maps but is expected to have opposite sign. Moreover, the presence of the vibrational coherence and the different intensity of rephasing and non-rephasing signals suggest that, at least in part, non-linear signal is present in the detected frequencies. A way to evaluate the amount of coherent mixing is to compare the non-linear spectrum with the reconstructed linear on the t_1-t_3 map using equations 28 and 29 found in Gregoire et al. paper[3]

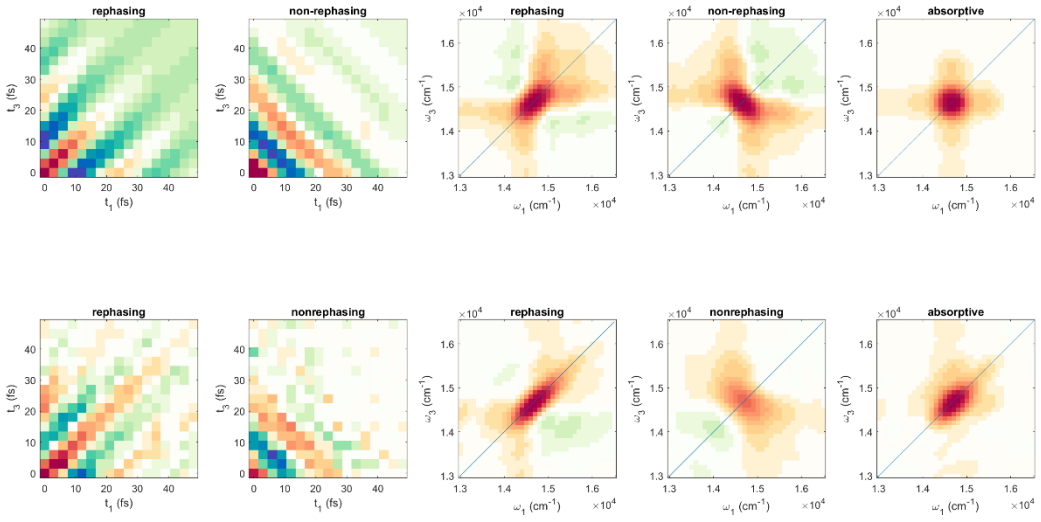

Figure SI-F2 Linear signal reconstruction (top row) and actual acquired signal at t2 = 60 fs (bottom row).

The comparison between the reconstructed linear signal and the acquired ones. The shapes of rephasing and non-rephasing are very similar in between each other. However, the intensity ratio between rephasing and non-rephasing is different (as expected for a non-linear response, because of the slower decay along coherence time of the rephasing signal) giving rise to different absorptive maps. The non-linear is indeed elongated along the diagonal.

SI8. Incoherent mixing, evolution along t_2.

As seen in figure 3 in the main document, the dynamics along the population time are almost flat. We performed anyway the global fit using an oscillation component that outputs the CAS at 750 cm^{-1}. That's the only CAS obtainable from the fitting that has a distribution of amplitudes (shape) that resemble the 2d signal. We show in figure SI-F3 that the Fourier analysis performed on the residuals of a global exponential fitting (only real) in different positions are very noisy, but all of them contain the 750 cm^{-1} component.

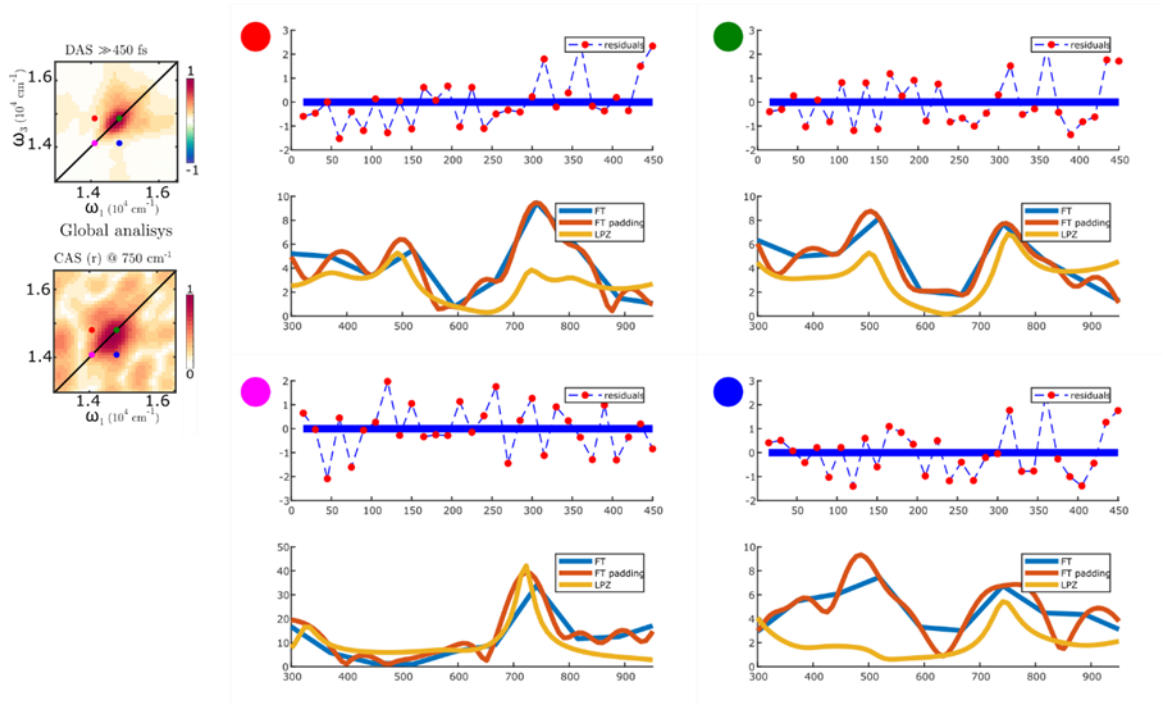

Figure SI-F3 Fourier analysis of the residuals of an only real exponential global fitting. The 750 component is present with every technique in all the positions.

SI9. Custom electrochemical cell design

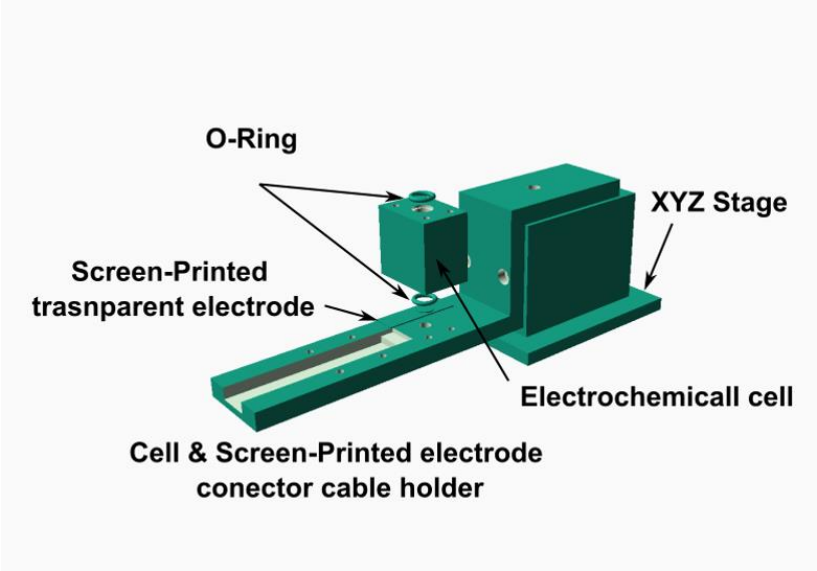

Figure SI-F3 Blueprint of electrochemical cell and screen-printed electrode holder adapted to spectro-electrochemical set-up.

SI10. Pulse compression

Pulse duration (~10 fs) was measured by exploiting standard sum frequency signal (FROG) generated on a 10 μm BBO crystal.

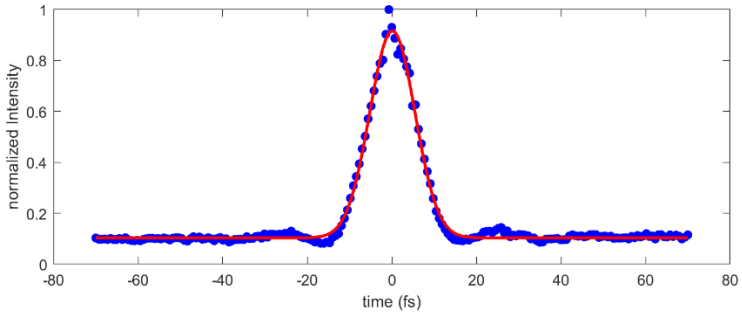

Figure SI-F4 Frog trace from -70 to +70 fs.

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