SUPPLEMENTARY INFORMATION

Identification of a mechanism of photoprotective energy dissipation in higher plants

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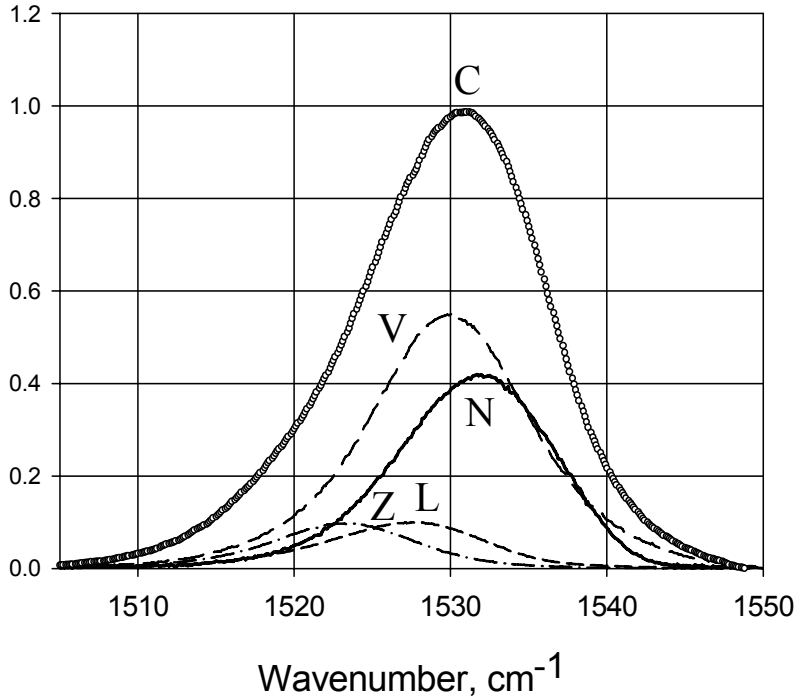
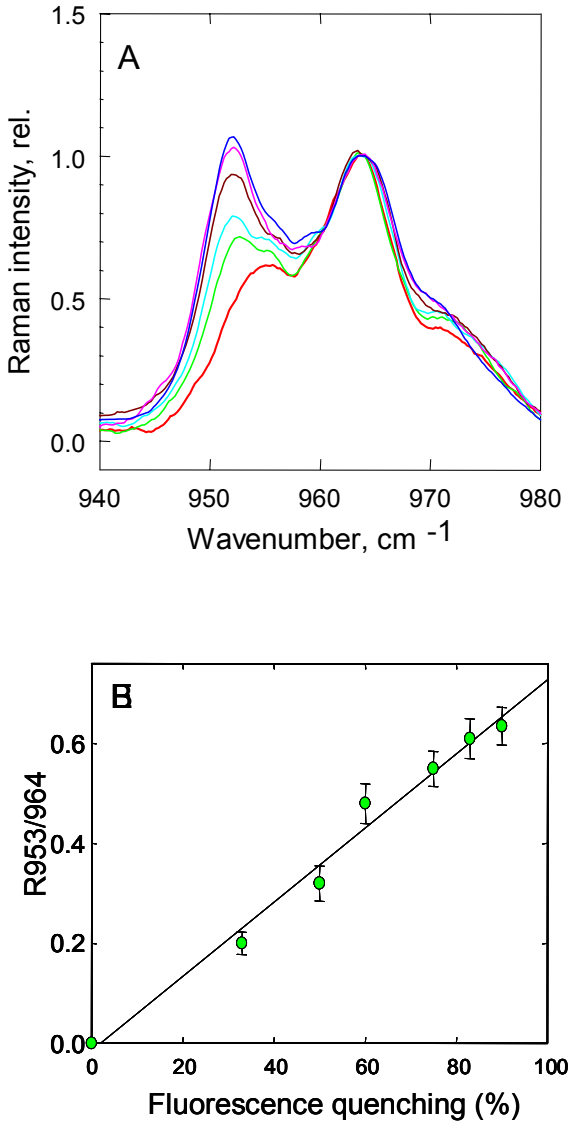
1. Calibration of in vivo resonance Raman spectra



Supplementary Figure 1: Curve fitting of the ν1 band of the Resonance Raman spectrum of chloroplasts (C) excited in the neoxanthin region (488.0 nm). Individual contours correspond to the spectra of isolated xanthophylls, V, violaxanthin, N, neoxanthin, L, lutein, Z, zeaxanthin. The neoxanthin contribution to ν1 is 45%. However, the contribution of this xanthophyll to ν4 is different since all xanthophylls have different ν4/ν1 ratios. In order to find the percentage of neoxanthin contribution to the ν4 band we have corrected each xanthophyll area by the ν4/ν1 ratios, therefore the N % in the ν4 band is $((\nu4_{neo}/\nu1_{neo}) * k_{neo} / \sum (\nu4_i/\nu1_i) * k_i) * 100\%$, and is close to 65%, where k_i is the fraction of the area of each xanthophyll in the ν1 band of the Resonance Raman spectrum of chloroplasts.

2. Raman spectroscopy of LHCII aggregates

Isolated LHCII was obtained in a range of oligomerisation states, which cover a 10-fold difference in fluorescence yield. The Raman spectra reveal a progressive increase of the intensity of the 953 cm^{-1} band upon LHCII oligomerisation (Fig. 2A) and there was a linear relationship between the percentage change of fluorescence and the intensity of this Raman band (Fig. 2B).



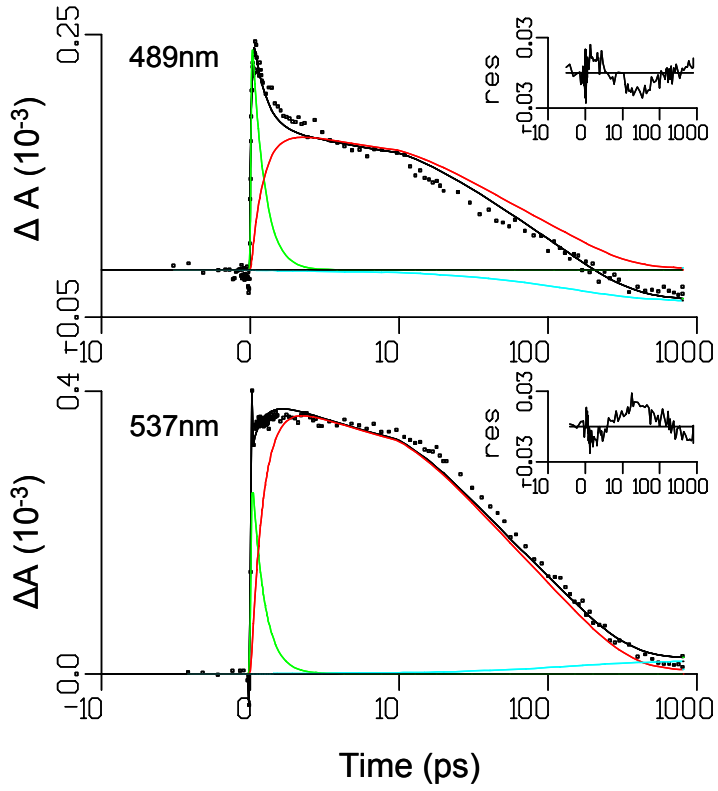
Supplementary Figure 2 Raman spectroscopy of LHCII

A. ν4 region of the Resonance Raman spectrum for neoxanthin excitation at 488.0 nm, for trimeric LHCII (red) and LHCII in increasing states of oligomerisation to a maximum value (blue), normalised to the amplitude of the band at 964 cm^{-1}.

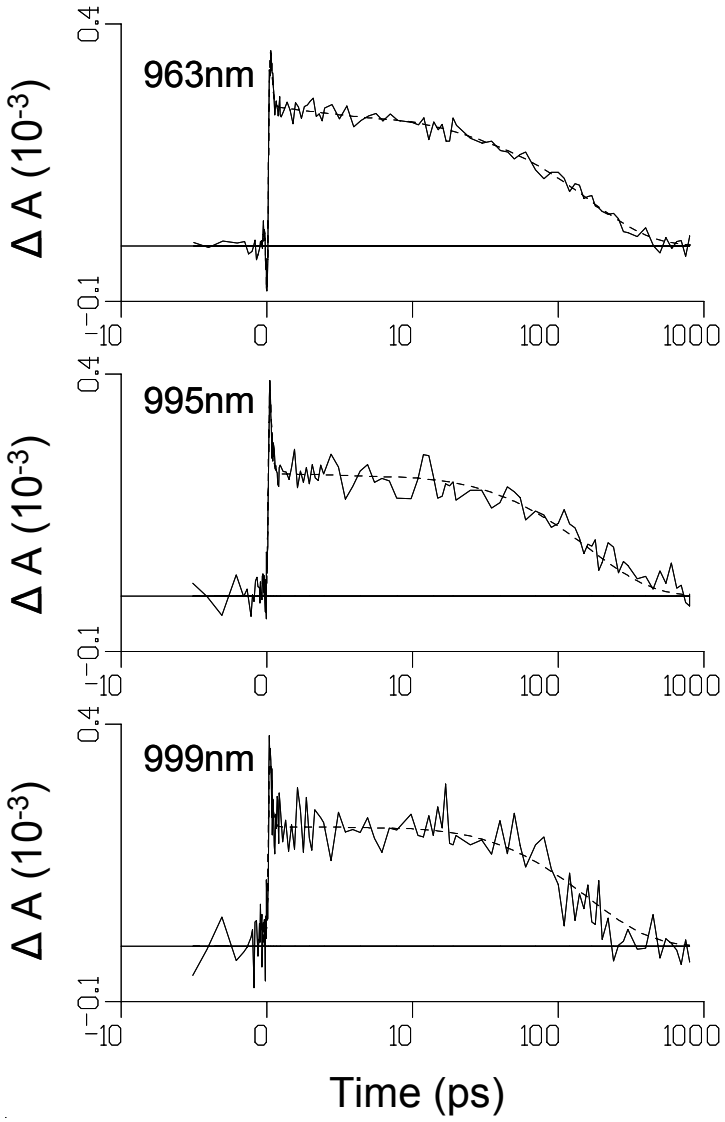
B. Relationship between the change in Raman intensity at 953 cm^{-1} relative to the intensity of the 964 cm^{-1} band (obtained from A) and the fluorescence intensity, expressed as a percentage of the unquenched trimeric LHCII. The error bars are the calculated amplitude of noise on the spectrum, obtained using Datamax GRAMS32 Galactic software (Instruments SA, Inc).

3. Time-Resolved Measurements

Target analysis describes the data by a kinetic model (figure 5) and estimates the rate constants (indicated in figure 5 and tabulated in Table 1) and spectra of the molecular states involved in the various processes. The estimated species associated difference spectra (SADS) have been shown in figure 2d in the text.

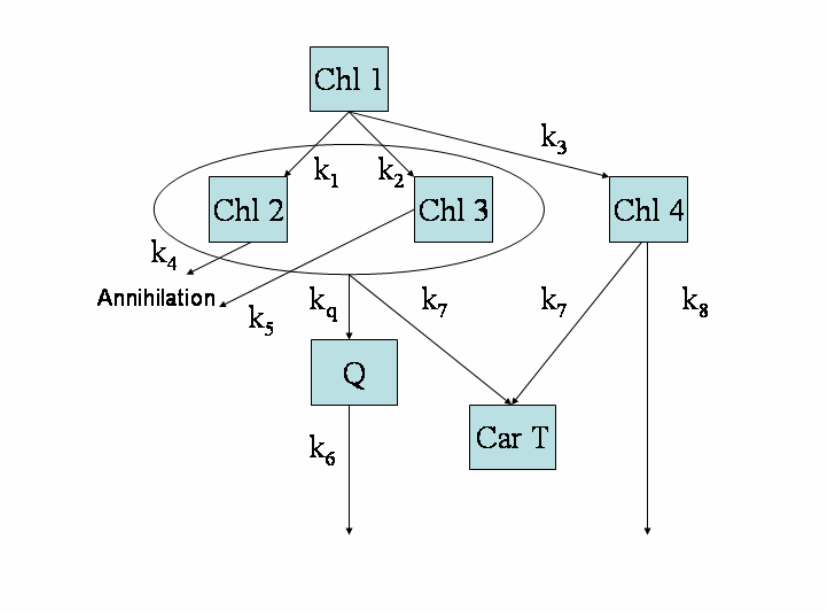


Supplementary Figure 3: Selected kinetic traces at 489 nm, corresponding to the carotenoid ground state absorption region and 537 nm taken as representative of the carotenoid $S_1 \rightarrow S_n$ excited state absorption for the sample in the quenched state. The corresponding fit was obtained by applying a kinetic model without quenching via a carotenoid excited state. The lack of fit is clear as can be seen by comparing it with figure 2a,b in the text. The bad quality of the fit is also evidenced by the insets where the residuals show clear trends. The red and cyan curves depict the contributions from Chl ESA and Car Triplet.

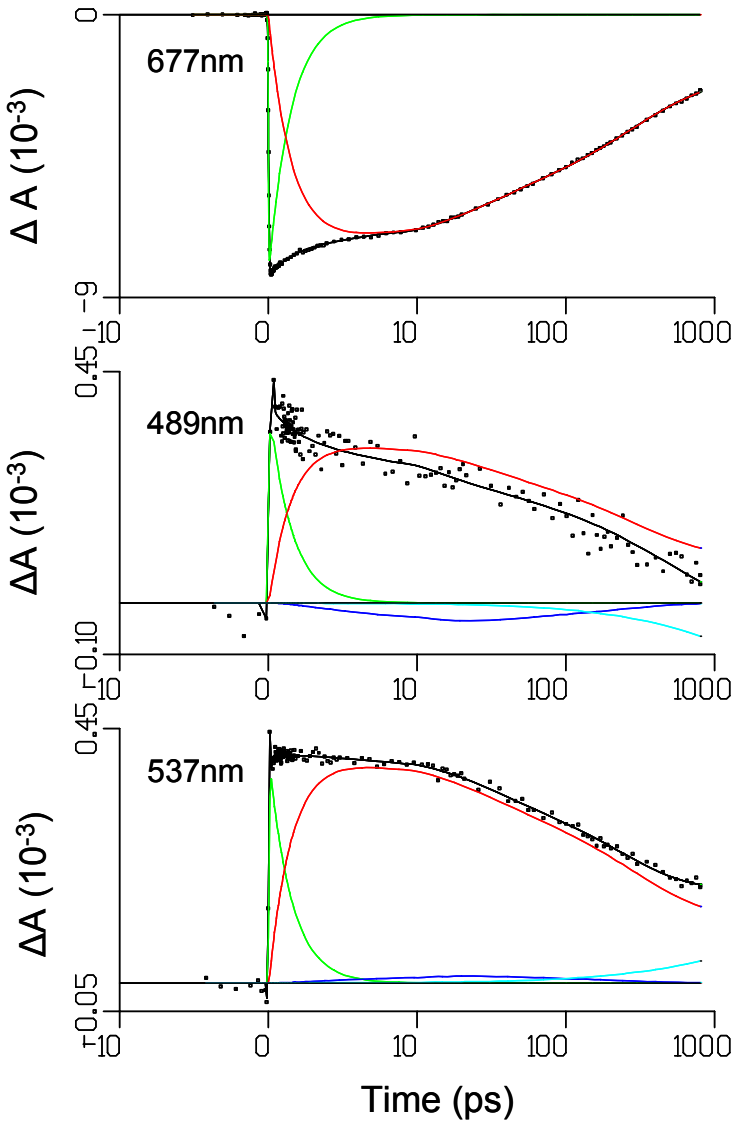


Supplementary Figure 4:

Selected kinetic traces in the near-IR region for the quenched sample. The fit was obtained from a sequential model consisting of three lifetimes: a ~100 fs component to account for ultrafast relaxation, a ~12 ps component to account for singlet-singlet annihilation of chlorophyll excited states and a 160 ps component which corresponds to the lifetime of the chlorophyll excited state. Furthermore, the maximum amplitude of the signal was about 0.3 mOD over the wavelength range 900-1020 nm. The traces thus reflect the decay of the chlorophyll excited-state absorption and show no evidence for the formation of any other species during the quenching process.



Supplementary Figure 5:
The kinetic model applied for a simultaneous analysis of three samples in different quenching states. The excitation at time zero sits on the Chl1 compartment, and is distributed to the three compartments Chl2, Chl3 and Chl4 (in about 1 ps) with rate constants k_1, k_2 and k_3 respectively. In the fit k_1, k_2 and k_3 are allowed to vary to reflect the change in quenching and annihilation in the various samples upon aggregation. Chl2 and Chl3 are quenched via the quenching state with a rate constant k_q. Both Chl2 (fast) and Chl3 (slow) contain an annihilation channel to account for the multi-exponential character of singlet-singlet annihilation (rate constants k_4 and k_5 respectively). Both compartments also populate the long-living triplet state with a rate constant k_7 corresponding to a very small yield. Chl 4 represents the unquenched chlorophyll, which is present in the unquenched and mildly quenched samples. It populates the long-living triplet state with a rate constant k_7 and decays to the ground state with rate constant k_8. The rate constant k_8 which is also present with Chl2 and Chl3 has been omitted for clarity. The rate constants that were estimated by fitting the observed kinetics are reported in Table 1. Transient absorption kinetics for the intermediately-quenched sample plus their fit to this model are shown in Fig. 6.



Supplementary Figure 6:
Selected kinetic traces for a sample with an intermediate degree of quenching and corresponding fit obtained from the kinetic model in figure 5. Note that the blue curves which represent the absorption changes due to the quencher Q are inbetween those of the unquenched and strongly quenched samples depicted in, respectively, figure 2a and 2b in the text.

Supplementary Table 1:
Rate constants obtained from the kinetic model in figure 5 for three samples: unquenched(1), intermediately quenched (2) and strongly quenched (3) with relative fluorescence yields (Kd) of 0, 2 and 9. Note that for the unquenched sample most of the excitations end up in Chl4, the pool of chlorophyll where the excited states decay slowly. Estimated error in the rate constants k_1, k_2, k_3, and k_q is about 10%, the other rate constants were fixed.

	1	2	3
k_1	$(15.87ps)^{-1}$	$(8.54ps)^{-1}$	$(2.39ps)^{-1}$
k_2	$(17.85ps)^{-1}$	$(3.74ps)^{-1}$	$(1.3ps)^{-1}$
k_3	$(2.87ps)^{-1}$	$(4.38ps)^{-1}$	$(51.8ps)^{-1}$
k_4	$(25ps)^{-1}$	$(25ps)^{-1}$	$(25ps)^{-1}$
k_5	$(400ps)^{-1}$	$(400ps)^{-1}$	$(400ps)^{-1}$
kq	$(770ps)^{-1}$	$(670ps)^{-1}$	$(217ps)^{-1}$
k_6	$(8ps)^{-1}$	$(8ps)^{-1}$	$(8ps)^{-1}$
k_7	$(10ns)^{-1}$	$(10ns)^{-1}$	$(10ns)^{-1}$
k_8	$(10ns)^{-1}$	$(10ns)^{-1}$	$(10ns)^{-1}$