

SI: Single-molecule Identification of Quenched and Unquenched States of LHCII

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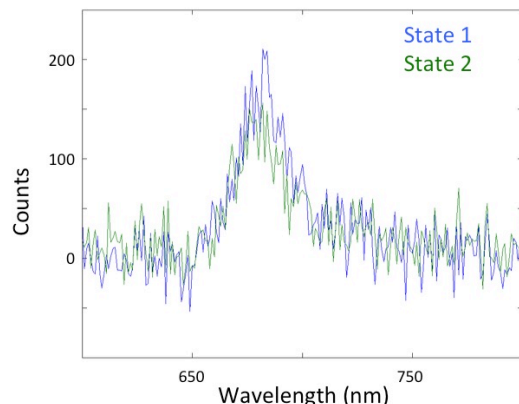


Figure S1. Average fluorescence spectra for states I and II. To compare states I and II, the fluorescence spectra for all occurrences of each state were summed and scaled for comparison purposes. Both states show very similar average fluorescence spectra.

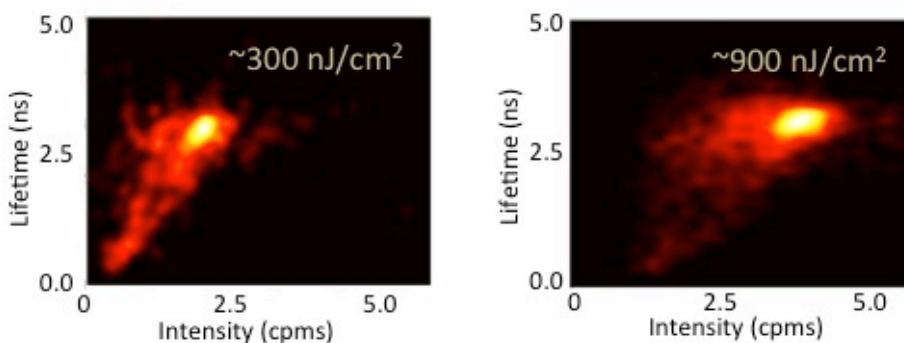


Figure S2. Fluorescence intensity-lifetime distributions as a function of excitation fluence. Fluorescence lifetime vs. intensity plots of the probability distribution determined from each period of constant intensity and its accompanying lifetime for two different excitation fluences: $\sim 300 \text{ nJ/cm}^2$ (A); and $\sim 900 \text{ nJ/cm}^2$ (B). The same general distribution and three states are observed under both conditions. Although the relative population of state III is lower in (B), the signal to background of state III is lower than the other states, leading to effects such as less robust trapping and insufficient photons to determine lifetimes before photobleaching. As a result, the population of state III is difficult to quantify reliably. The fluorescence intensity does not increase linearly with light intensity due to the advent of saturation effects, such as triplet shelving.

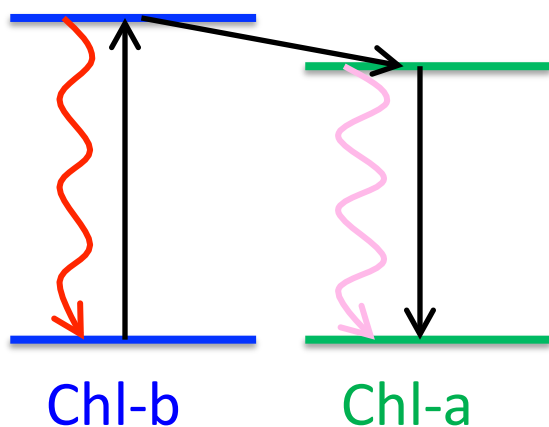


Figure S3. Simplified model of the photophysical pathways in LHCII upon excitation of the Chl-b. The system is excited in the Chl-b band. After excitation, energy rapidly transfers to the Chl-a and then fluorescence occurs out of the Chl-a band. Quenching can occur either on the Chl-b or the Chl-a. Both sites can prevent energy from transferring through the protein network to reach the reaction center.

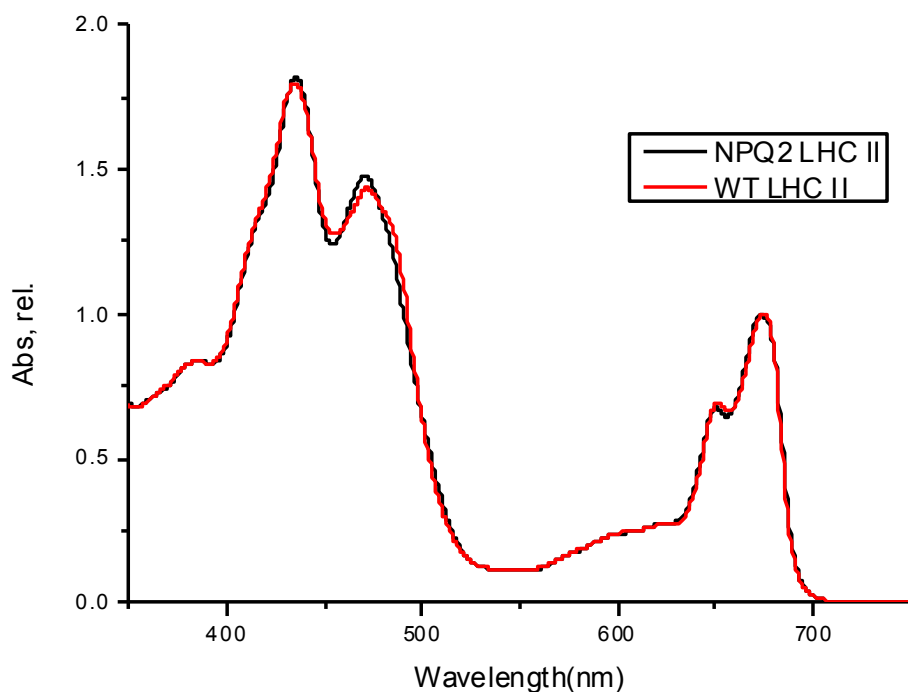


Figure S4. Linear absorption of WT and NPQ2 LHCII.

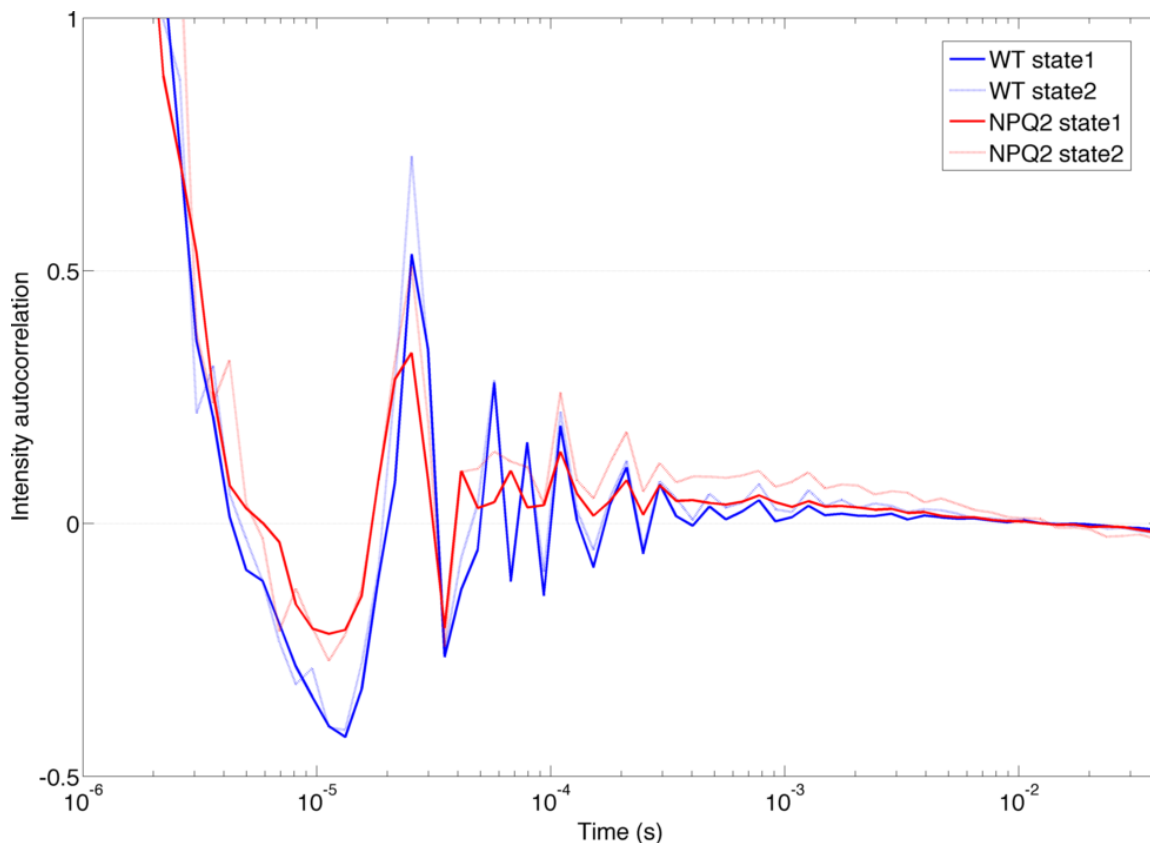


Figure S5. Photon correlation function of state I and state II for WT-LHCII and NPQ2-LHCII. On the microsecond timescale, the oscillatory behavior is due to the Knight's tour scan pattern. For a given state, correlation appears on the timescale determined by the sum of the rate into and rate out of the state. For the Chl triplet at this intensity, the timescales are $\sim 70 \mu\text{s}$ into the triplet state and $\sim 1 \text{ ms}$ out of the triplet state. Thus, there should be correlation on the tens to hundreds of microseconds timescale, with a tail into the millisecond region. Increased correlation in this region is not observed for state II for WT-LHCII.

	a/b	Chl/car	Chl-b	Chl-a	Total Chl	Total car
NPQ2 LHCII	1.37 (+/- 0.01)	4.05 (+/- 0.02)	5.92 (+/- 0.02)	8.08 (+/- 0.02)	14	3.46 (+/- 0.01)
WT LHCII	1.35 (+/- 0.00)	3.64 (+/- 0.02)	5.97 (+/- 0.01)	8.03 (+/- 0.01)	14	3.85 (+/- 0.02)

Table S1. Pigment composition per LHCII monomer for the NPQ2 mutant and the WT. The columns (from left to right) show the ratio of Chl-*a* to Chl-*b*, the ratio of total Chl to carotenoids, and the numbers of Chl-*b*, Chl-*a*, total chlorophyll, and total carotenoids.

	Neo	Vio	Lut	Ant	Zea	β -caro
NPQ2 LHCII	0.00 (+/- 0.00)	0.00 (+/- 0.00)	2.29 (+/- 0.01)	0.04 (+/- 0.00)	1.07 (+/- 0.01)	0.06 (+/- 0.00)
WT LHCII	0.94 (+/- 0.01)	0.22 (+/- 0.01)	2.69 (+/- 0.04)			

Table S2. Carotenoid composition per LHCII monomer. The numbers of each type of carotenoid per LHCII monomer are shown with the standard deviation in parenthesis. These are neoxanthin (Neo), violaxanthin (Vio), lutein (Lut), antheraxanthin (Ant), zeaxanthin (Zea), and beta-carotene (β -caro). The NPQ2 mutant contains Zea, whereas the WT contains Neo and Vio.

Materials and Methods:

To simultaneously record fluorescence spectra, a 70:30 beamsplitter is inserted into the detection path and the reflected component (30%) is spectrally dispersed by a volume-phase holographic grating (600 lines per millimeter; Wasatch) and focused onto an Si EMCCD camera (Ixon Ultra 897; Andor). The spectra are recorded at a 10-Hz frame rate, and the photon record of the APD is synced with the spectra through the timing module. Detected photons are summed along the direction perpendicular to the dispersion axis to yield a spectrum of the detected emission. The spectrometer was calibrated by recording the bulk fluorescence spectrum of LHCII and comparing it to an emission spectrum measured with a fluorimeter. The resolution of the spectrometer was determined to be 1 nm by measuring the standard deviation of the apparent spectral width of the Ti:sapphire laser beam in continuous wave mode.

While the apparatus has ~ 5 ms time resolution for determining intensity level, dynamics on a faster timescales are accessible because each photon arrival time is time

tagged with 8 ps accuracy. Thus, from the photon correlation function, dynamics on microsecond and millisecond timescales can also be explored. Specifically, as discussed below, a significant population of Chl triplets in state II would appear as an increase in the correlation function on the timescale of the Chl triplet lifetime, which is \sim ms. Figure S5 exhibits the correlation function for state I and state II for both WT-LHCII and NPQ2-LHCII, and neither state reflects a significant population of Chl triplets.

Molecular Mechanism of state II:

A second potential mechanism for state II is a conformational change that increases the triplet lifetime either by stabilizing the triplet state or trapping the triplet on the Chl. A longer triplet lifetime would reduce the fluorescence intensity from the excited singlet state through singlet-triplet annihilation. This occurs on a timescale shorter than the instrument response, and thus the measured excited state lifetime would remain the same. The correlation with Zea substitution suggests the mechanism must involve Zea, and an increased Chl triplet population has been observed previously in the Zea-enriched LHCII¹. To investigate this possibility, we look at the correlation function of the photon detection times shown in Figure S5. From the correlation curves, changes in intensity on microsecond to millisecond timescales can be extracted, including triplet state dynamics. States I and II for WT LHCII and state I for Zea-enriched LHCII all have a similar Chl triplet population, whereas state II of Zea-enriched LHCII has a larger Chl triplet population. Thus, while Chl triplets are present, they cannot be entirely responsible for states I and II. Notably, here we have determined that the majority of the Chl triplet increase previously observed in Zea-enriched LHCII comes from state II.

A third mechanism for state II is a conformational change that alters both the intrinsic radiative rate and the amount of quenching at the emissive state, which could produce the observed behavior. However, a conformational change that alters the intrinsic radiative rate would most likely have a spectral signature as well, and the average emission spectra of states I and II are very similar, as shown in Figure S1. Additionally, this eliminates the possibility that state II is an intensity decrease correlated with previously observed red-shifts².

References

- (1) Mozzo, M.; Dall'Osto, L.; Hienerwadel, R.; Bassi, R.; Croce, R. Photoprotection in the Antenna Complexes of Photosystem II: ROLE OF INDIVIDUAL XANTHOPHYLLS IN CHLOROPHYLL TRIPLET QUENCHING. *Journal of Biological Chemistry* **2008**, *283*, 6184-6192.
- (2) Krüger, T. P.; Novoderezhkin, V. I.; Iliaia, C.; Van Grondelle, R. Fluorescence Spectral Dynamics of Single LHCII Trimers. *Biophys. J.* **2010**, *98*, 3093-3101.