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**Supplemental information**

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LHCII in thylakoid membranes**

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## Supporting Information

### **Protein dynamics and lipid affinity of monomeric, zeaxanthin-binding LHCII in thylakoid membranes**

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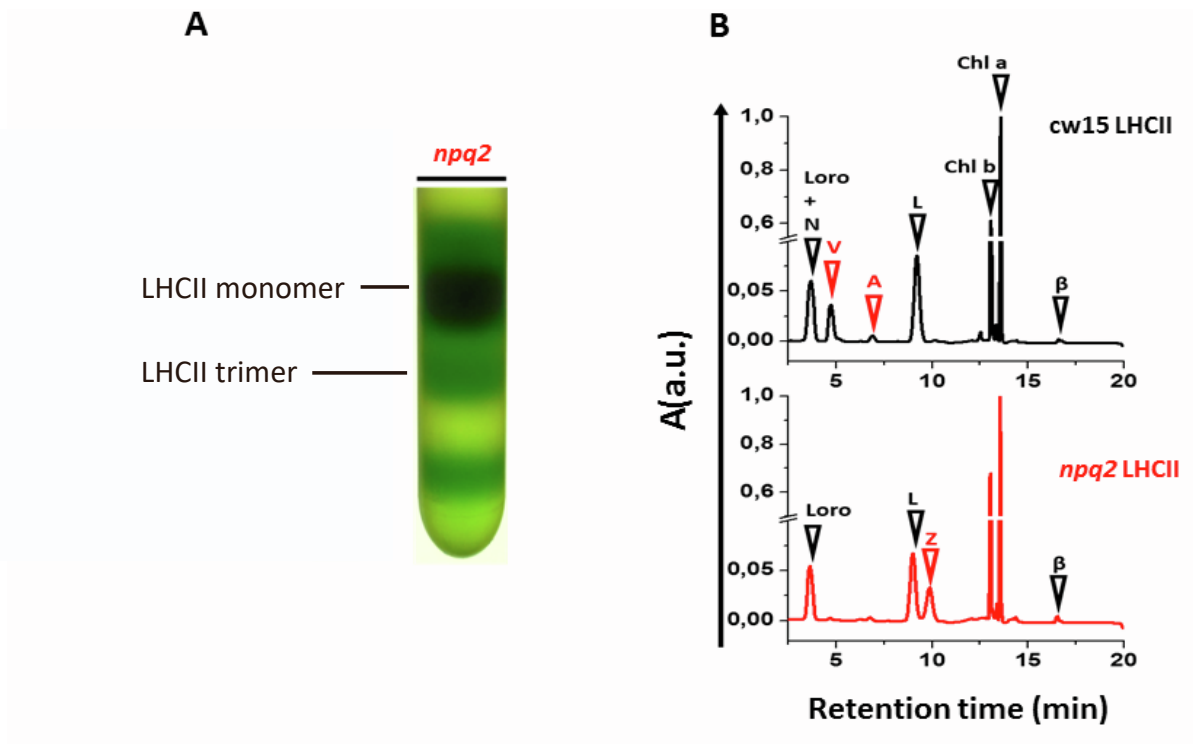
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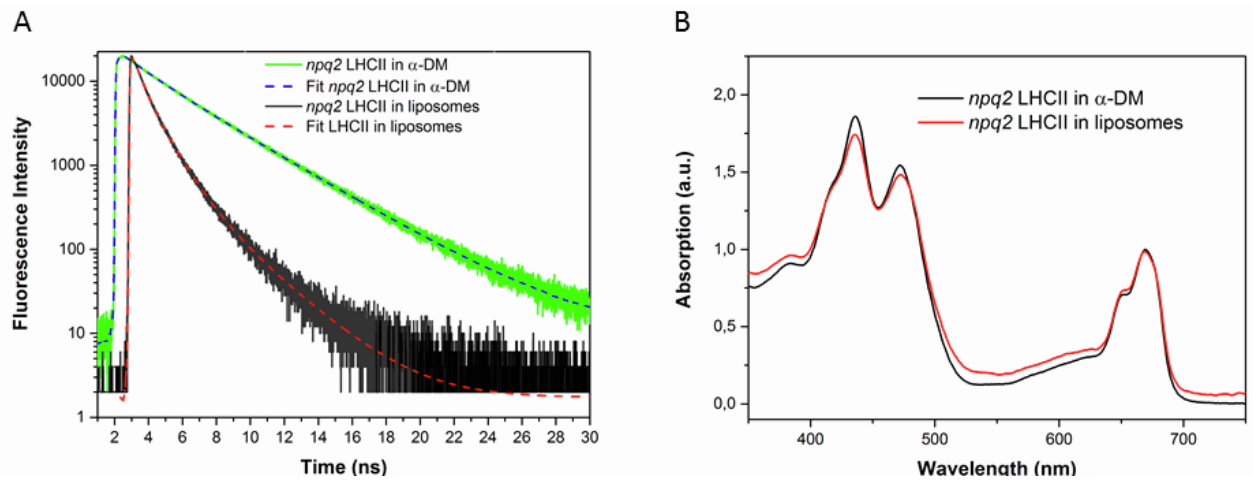
**Table S1**

Fluorescence lifetime analysis of  $U\text{-}^{13}\text{C}\text{-}^{15}\text{N}$  *Cr npq2* LHCII in  $\alpha$ -DM and in proteoliposomes.

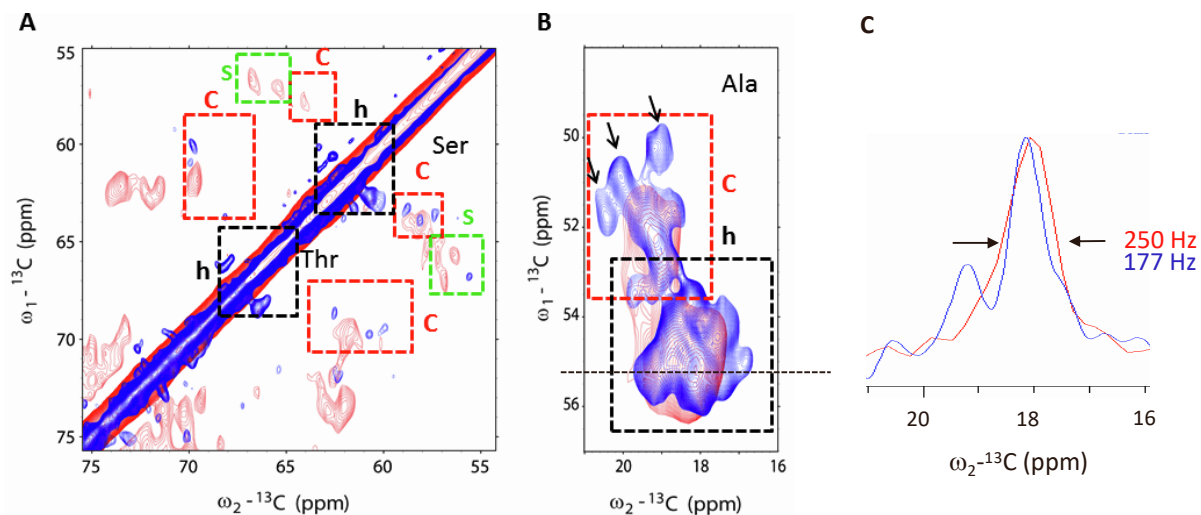
<i>Sample</i>	<i>A1</i>	$\tau_1$ (ns)	<i>A2</i>	$\tau_2$ (ns)	<i>A3</i>	$\tau_3$ (ns)	$\tau_{av}$ (ns)
<i>npq2</i> LHCII in $\alpha$ -DM	62%	3.6	25%	1.7	13%	0.2	3.3
<i>npq2</i> LHCII in liposomes	50%	1.1	44%	0.4	6%	2.5	1.1



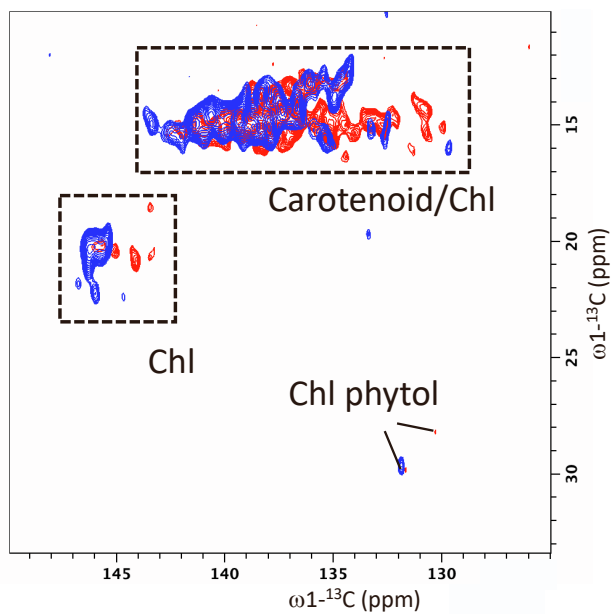
**Figure S1** A: LHCII purification from sucrose gradient of thylakoid membranes from *npq2* *Cr* cells. B: HPLC analysis of wt (*cw15*) and *npq2* LHCII fractions. Traces were normalized on the peak of chlorophyll a (Chl a). Identification of lettered peaks is as follows: Loro, loroxanthin; N, neoxanthin; V, violaxanthin; A, antheraxanthin; L, lutein; Z, zeaxanthin;  $\beta$ ,  $\beta$ -carotene.



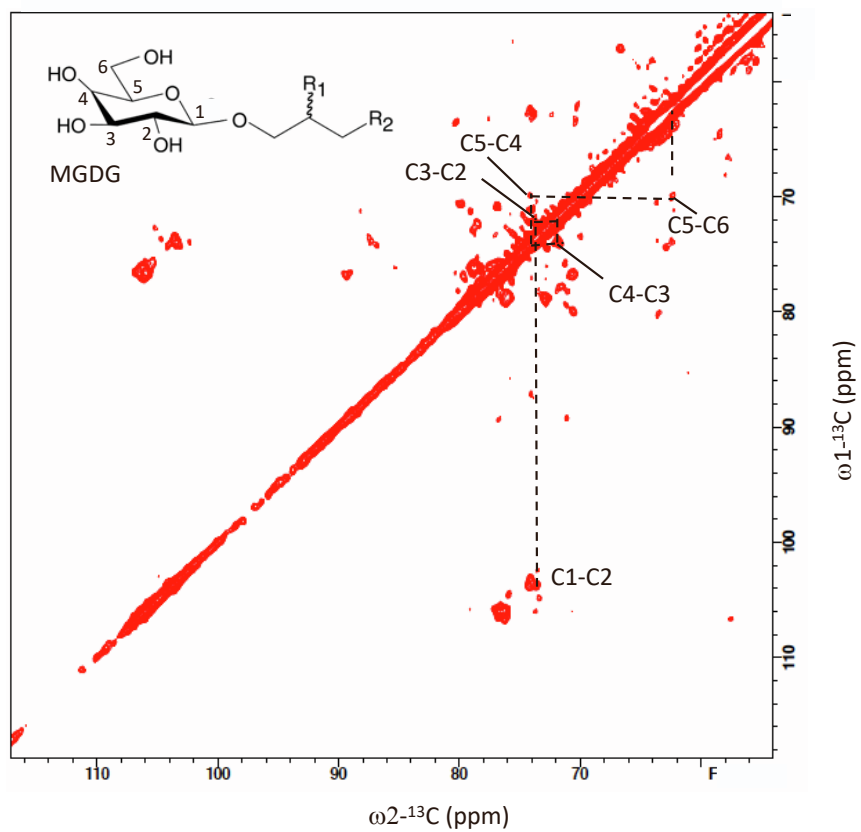
**Figure S2.** A. Time resolved fluorescence (excitation 440 nm, detection 680 nm) of *npq2* LHCII in  $\alpha$ -DM detergent (green) and *npq2* LHCII in liposomes (black). B. Absorption spectra of *npq2* LHCII in  $\alpha$ -DM detergent (black) and after inserting into liposomes (red).



**Figure S3**  $^{13}\text{C}$  -  $^{13}\text{C}$  CP-PARIS NMR spectra of wt (blue) and npq2 (red) LHCII. Spectra were collected at  $-18\text{ }^{\circ}\text{C}$  and with 17 kHz MAS frequency. A. Thr and Ser region. Helix, coil and  $\beta$  strands contribution are presented with black, red and green boxes. B. The Ala region. Arrows indicate resonance signals that are only observed in the spectrum of WT LHCII. C. Linewidth comparison for Ala peak (1D slice  $\omega_1\text{-}^{13}\text{C} = 55.2\text{ ppm}$ ); red, npq2 LHCII, blue, wt LHCII. Data of the wt LHCII proteoliposomes has been presented in previous work. <sup>1</sup>

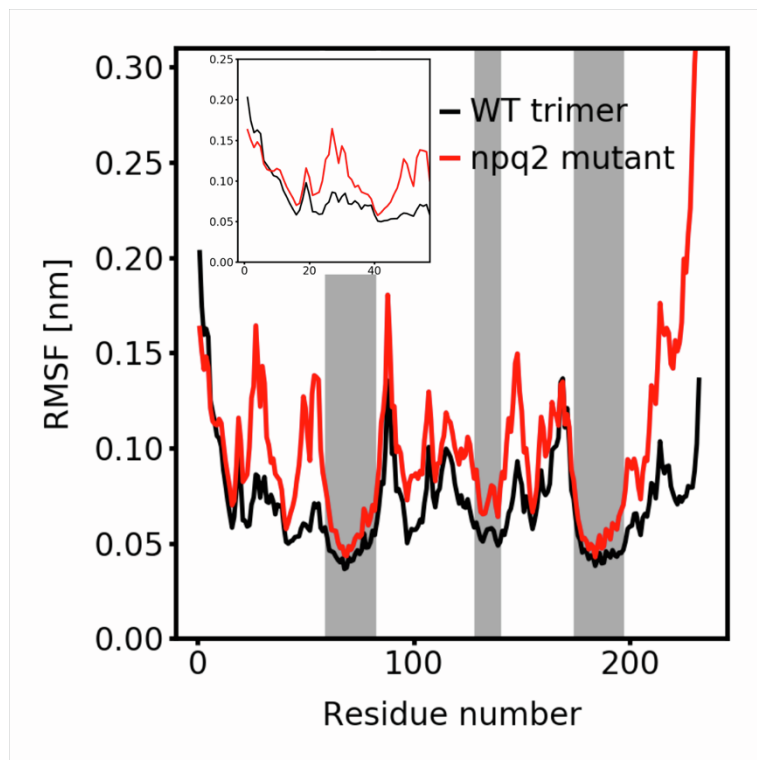


**Figure S4**  $^{13}\text{C}$  -  $^{13}\text{C}$  CP-PARIS NMR spectra of wt (blue) and npq2 (red) LHCII. Spectra were collected at  $-18\text{ }^{\circ}\text{C}$  and with 17 kHz MAS, pigment region. Data of the wt LHCII proteoliposomes has been presented in previous work. <sup>1</sup>



**Figure S5.**  $^{13}\text{C}$ - $^{13}\text{C}$  PARIS NMR spectrum of npq2 LHCII, collected at  $-3\text{ }^\circ\text{C}$  and with 14 kHz MAS frequency, galactosyl region with MGDG NMR chemical shift correlation signals.





**Figure S6** RMSF of the protein backbone of the wt LHCII trimer and the monomeric npq2 LHCII evaluated for the aggregation simulations containing 4 LHCII trimers and 12 npq2 LHCII monomers, respectively, embedded in thylakoid membrane. The inlay shows a zoom of the N-terminus.

## References

- [1] Azadi-Chegeni, F., Ward, M. E., Perin, G., Simionato, D., Morosinotto, T., Baldus, M., and Pandit, A. (2021) Conformational Dynamics of Light-Harvesting Complex II in a Native Membrane Environment, *Biophys J* 120, 270-283.