Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

SWITCHING LIGHT HARVESTING COMPLEX II INTO PHOTOPROTECTIVE STATE INVOLVES THE LUMEN-FACING APOPROTEIN LOOP

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Running title: photoprotective switch in light harvesting antenna protein

Supporting Material

Belgio et al. Supplemental Table 1

Sample	%/MR	Neo	Vio	Ant	Zea	L	∑ Car.	Car/Chl	Chl *a/b*	Chl *a*	Chl *b*
WT	%	29%±1	4.5±1.5	0	0	66.6±0.7					
	MR	0.85	0.15	0	0	2	3	0.26±0.05	1.3±0.1	6.5	5
D111V	%	28±1	4.6±0.2	0	0	65±1					
	MR	0.85	0.14	0	0	2	2.99	0.27±0.05	1.4±0.1	6.5	4.5
E94G	%	32±2	2±0.1	0	0	66±1					
	MR	0.95	0.05	0	0	2	3	0.26±0.05	1.3±0.2	6.5	5

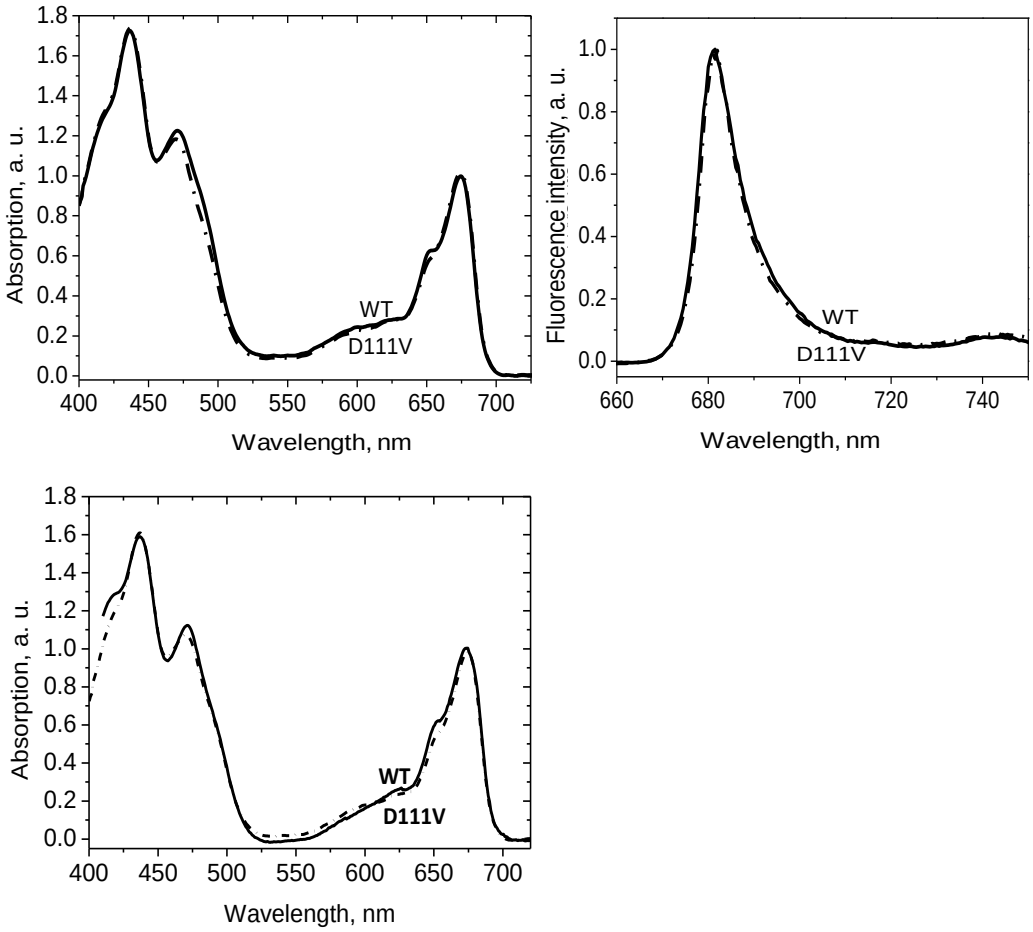
Table S1. Pigment composition of WT, D111V, E94G recombinant LHCII complexes. %, carotenoid content as percentage of total; MR, estimated molar ratio; neo, neoxanthin, vio, violaxanthin, ant, antheraxanthin, zea, zeaxanthin, lut, lutein; Car/Chl, molar ratio of total carotenoid to total chlorophyll, Chl *a/b*, molar ratio of chlorophyll *a/b*. Data are the averages ± S.D. of at least three replicate assays of two or three separate preparations.

Belgio et al. Supplemental Table 2

	A_1	τ_1	A_2	τ_2	A_3	τ_3	τ_{Av}
uWT	**72%**	**3.6**	**26%**	**1.8**	**3%**	**0.2**	**2.9±0.1**
uD111V	**68%**	**3.5**	**29%**	**1.7**	**1%**	**0.1**	**3±0.1**
qWT	**38%**	**2.2**	**37%**	**0.5**	**25%**	**0.05**	**1.13±0.05**
qD111V	**19%**	**2.1**	**46%**	**0.35**	**38%**	**0.05**	**0.58±0.05**

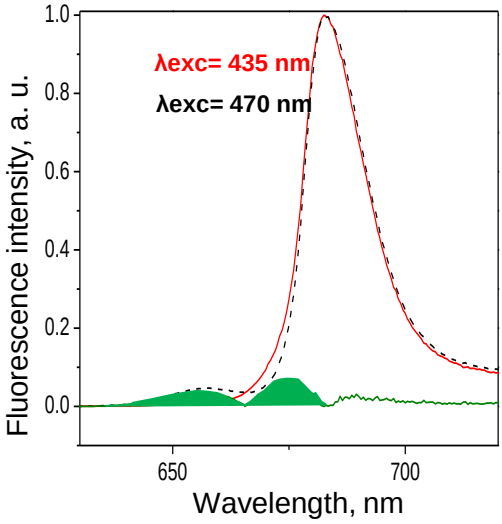
Table S2. Time-resolved fluorescence analysis of WT and D111V mutant in unquenched and quenched state. Shown are fitting results of fluorescence decay traces (emission detected at 682 nm) measured on recombinant LHCIIs. Samples were reconstituted WT and D111V in unquenched (u) and quenched (q) states. Different complexes were diluted in the presence of 0.03% β-DM and 20 mM HEPES (pH 7.8) or 0.003% β-DM and 20 mM citrate (pH 5), in order to induce unquenched or quenched conditions, respectively. $A_{1–3}$, amplitude of the exponential components 1–3; $\tau_{1–3}$, decay time constants (ns) of the exponential curves 1–3 used to fit the fluorescence decay curves; τ_{Av}, average fluorescence decay lifetime (ns) means ±SEM from three replicates.

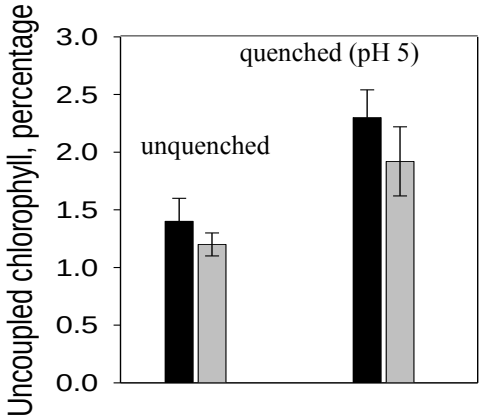
Belgio et al. Supplemental Figure 1



Supplemental Figure 1. Comparison between WT (solid line) and D111V (dash-dotted line). Top: absorption (left) and fluorescence (right) spectra of unquenched samples; bottom: absorption spectra of quenched samples (pH 5). Samples were prepared as described in the Methods section.

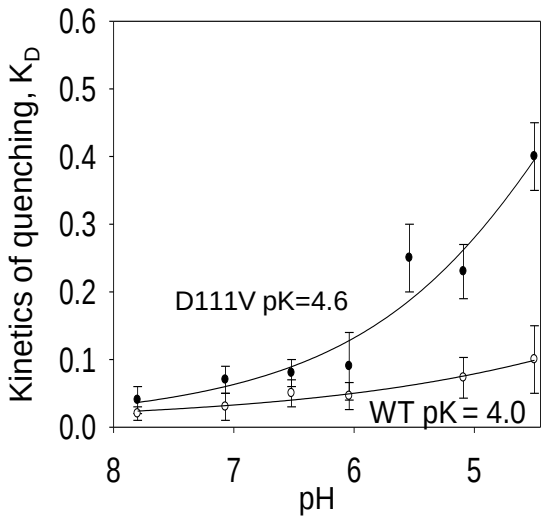
Belgio et al. Supplemental Figure 2





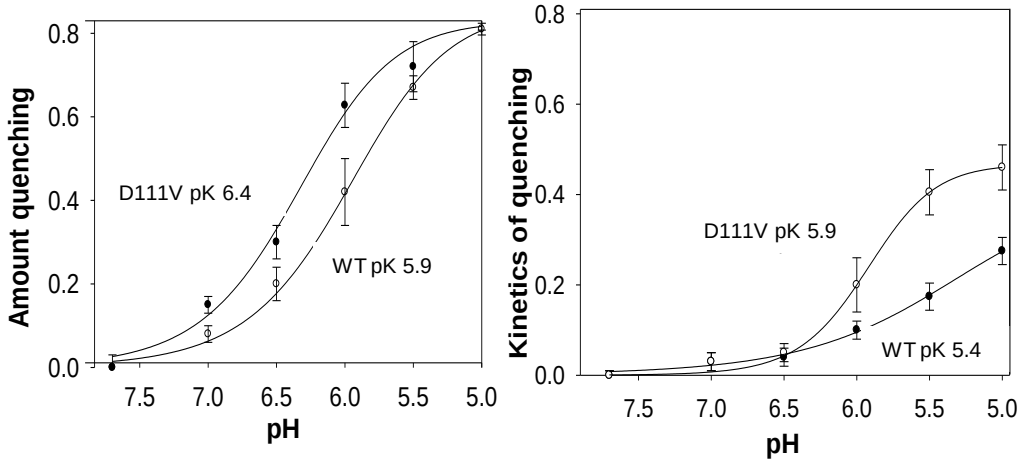
Supplemental Figure 2. Determination of uncoupled chlorophyll. Top: example of the method shown for the unquenched WT sample. 77 K fluorescence was excited at 435 nm (red line) and 470 nm (black dashed line), and the area under the absolute difference spectrum (green) calculated and divided by the total fluorescence area in order to estimate percentage of uncoupled chlorophyll. Bottom: uncoupled chlorophyll in the unquenched and quenched WT (solid bars) and mutant (grey bars) samples.

Belgio et al. Supplemental Figure 3



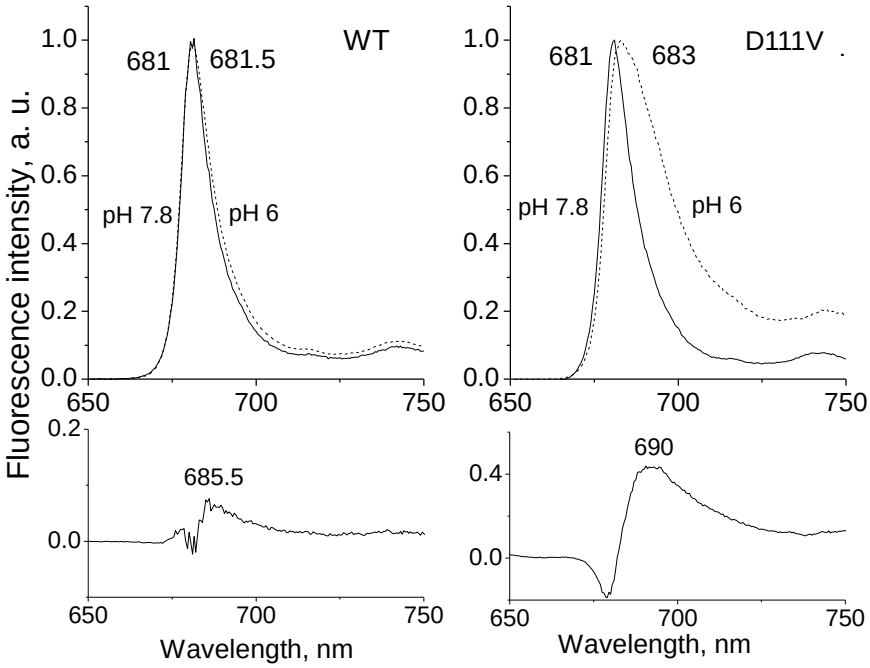
Supplemental Figure 3. pH titration curves for quenching kinetics of WT and D111V mutant. The relationship between quenching kinetics and pH were obtained from experiments performed as shown in Fig. 3. K_D were calculated at each pH point by fitting each trace with the 3-parameter hyperbolic decay function: $y= y_0+ ab/[b+x]$ where 1/b represents the K_D of the process. pK values were then obtained by fitting the data points to the sigmoidal Hill equation $y = [ax^b]/[c^b + x^b]$ where c represents the pK of the process. Data presented as means ±SEM from three replicates.

Belgio et al. Supplemental Figure 4



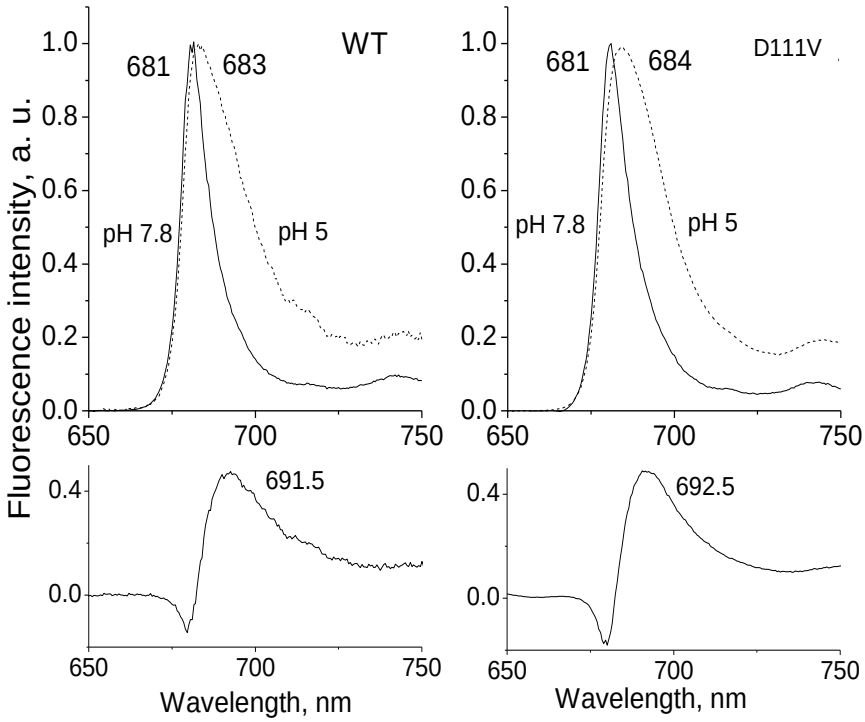
Supplemental Figure 4. pH titration curves for fluorescence quenching and kinetics of WT and D111V mutant in trimeric state**.** The relationship between quenching and pH were obtained from experiments as shown in Fig. 3. Amount of quenching were calculated at each pH point as the difference in fluorescence after 30s divided by the amplitude of the unquenched fluorescence recorded for a sample diluted into 0.03% DM, normalized to 1. K_d were calculated at each pH point by fitting each trace with the 3-parameter hyperbolic decay function: $y= y_0+ ab/[b+x]$ where 1/b represents the K_d of the process. pK values were then obtained by fitting the data points to the sigmoidal Hill equation $y = [ax^b]/[c^b + x^b]$ where c represents the pK of the process. Data presented as means ±SEM from three replicates.

Belgio et al. Supplemental Figure 5



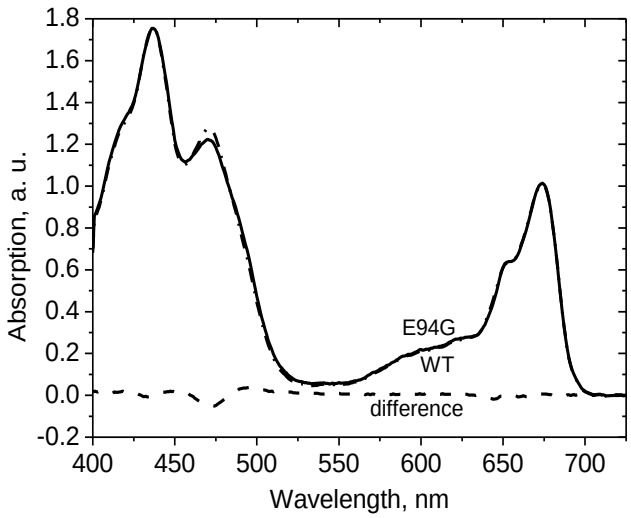
Supplemental Figure 5. Low temperature steady state fluorescence of WT and mutant samples taken from in column quenching experiment. Top: 77 K fluorescence spectrum of WT (left) and D111V mutant (right) immobilised to the Ni resin in the unquenched (solid line) and quenched (dotted line, pH 6) states. Bottom: Calculated difference spectrum of quenched *minus* unquenched WT (left) and mutant (right).

Belgio et al. Supplemental Figure 6



Supplemental Figure 6. Low temperature steady state fluorescence of WT and mutant samples taken from in column quenching experiment. Top: 77 K fluorescence spectrum of WT (left) and D111V mutant (right) immobilised to the Ni resin in the unquenched (solid line) and quenched (dotted line, pH 5) states. Bottom: Calculated difference spectrum of quenched *minus* unquenched WT (left) and mutant (right).

Belgio et al. Supplemental Figure 7



Supplemental Figure 7.

Comparison between WT (solid line) and E94G (dashed line) absorption spectra and diffecence spectrum WT *minus* D111V (dashed line). Samples were prepared as described in Methods section.