Supporting Information

Single and Multi-Exciton Dynamics in Aqueous Protochlorophyllide Aggregates

Olga A. Sytina[1†], Ivo H. M. van Stokkum[1], Rienk van Grondelle[1] and Marie Louise Groot[1]

1 Department of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands,

† Current affiliation: FOM Institute AMOLF, P.O. Box 41883, 1009 DB Amsterdam, the Netherlands

sytina@amolf.nl , ivo@few.vu.nl , rienk@few.vu.nl , ML.Groot@few.vu.nl

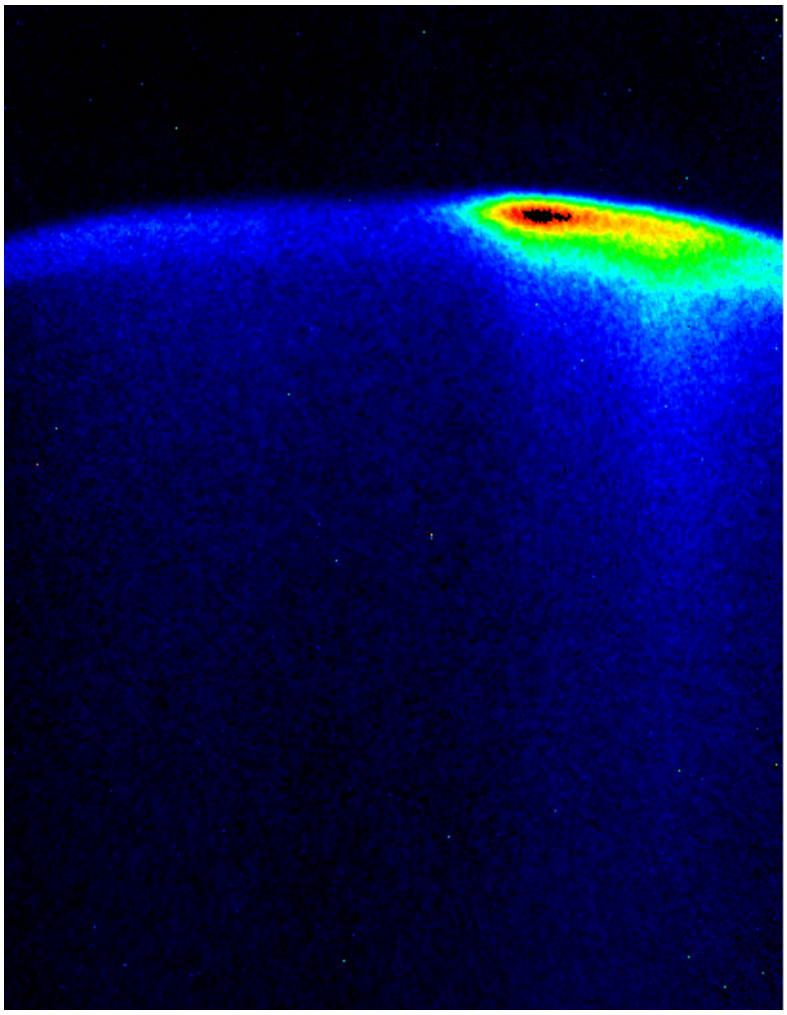

Figure S1. Image of the Pchlide fluorescence acquired with a streak camera. Full vertical scale is 200 ps, horizontal scale is 500-730 nm.

Table 1. Lifetimes obtained from the global analysis of the Pchlide in D_2O dataset recorded at different excitation energies per pulse, where applicable the rates in H_2O are reported as well.

Energy per pulse (nJ)	t1, ps (black)	t2, ps (red)	t3, ps (blue)	t4, ps (green)
10	0.5	2.3	48	1500
20	0.69	6.2	89	1900
40	0.49	5.5	83	1840
50 in H_2O	0.74	5.5	76	1500
60	0.49	4.3	46	1350
80	0.5	5.9	70	1630
100	0.56	7.2	96	3060
100 in H_2O	0.69	9.4	116	1580
130	0.62	9	126	2880
200	0.54	8.9	149	4100
200 in H_2O	0.62	11.2	178	1990
300 in H_2O	0.62	8.4	131	2430
430	0.54	8.1	150	2760

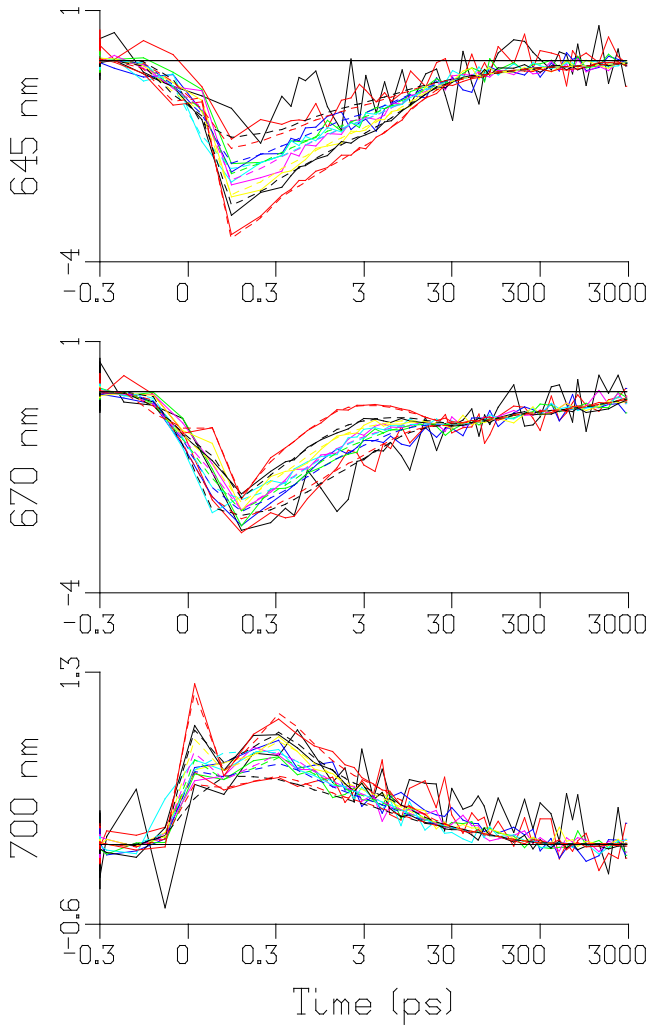

Figure S2. Target fit (dashed lines) of the power dependent TA traces (solid lines) at three selected wavelengths indicated as ordinate label. Different colors indicate experiments at different powers. At 645 nm the magnitude of the signal increases with power, and the color order is black, red, blue, green, magenta, cyan, yellow, black, red. At 670 nm the signal becomes less negative with increasing power. The black noisy traces were measured at 10 nJ per pulse. The other powers used are 20, 40, 60, 80, 100, 130, 200, 430 nJ. For comparison they have been divided by scaling numbers, 1.67, 2.89, 3.70, 4.54 ,4.92, 5.64, 7.02, 10.13 respectively.

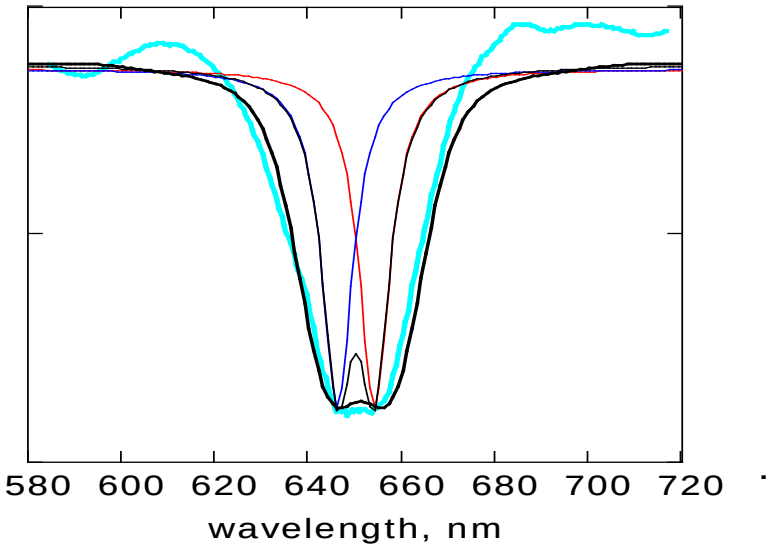

Figure S3. Simulation of double-peaked spectrum (black line) with Voigt convolution of sum of two Lorentz lines at 645.5 nm (blue line) and 653.5 nm (red line) with broader Gaussian profile. Original spectrum from target analysis is represented by cyan line.

Analysis of the saturation curves.

The excitation energy was recalculated into the number of absorbed photons per molecule per pulse in each experiment, using the following expressions for number of absorbed photons $N_{photons}$ and number of molecules $N_{molecules}$:

$$N_{photons} = \frac{E - E \cdot 10^{-ODex}}{E_{photon} \cdot S_f} \qquad (1)$$

$$N_{molecules} = \frac{N_A \cdot C(OD, \varepsilon, path) \cdot V}{S_f \cdot (1 - T)} \qquad (2)$$

where E is excitation energy in Joules; OD_{ex} is optical density at the excitation wavelength; E_{photon} is energy of one photon at the excitation wavelength; N_A is Avogadro number; V is the volume of the sample illuminated by each laser shot.

$$S_f \cdot (1-T) = S_f \cdot (1-10^{-ODex}) \qquad (3)$$

is the factor accounting for area covered by absorbing pigments, S_f was 160 μm, and cell path length was 200 μm.

The concentration of the sample

$$C(OD, \varepsilon, path) = \frac{OD}{\varepsilon \cdot path} \qquad (4)$$

is a function of path length of the sample cell, the optical density OD and extinction coefficient ε at the detection wavelength, which was either 650 nm for Pchlide in water, or 630 nm for Pchlide in organic solvents. For the extinction coefficient of Pchlide in organic solvents we used $\varepsilon = 30.4 \cdot (10^{-3} M \cdot cm)^{-1}$ at 630-640 nm [53,54]. Since we could find no reports of the extinction coefficient of Pchlide in aqueous media, we determined the concentration in water using the assumption that the total oscillator strength F does not change in different media, which is related to the extinction coefficient through the following expression:

$$F \cong 4.3 \cdot 10^{-9} \int \varepsilon \cdot d\lambda \cdot$$

Thus, the integral of the extinction coefficient in THF should be identical to the one in water:

$\int \varepsilon \cdot d\lambda$ (in THF) $\cong \int \varepsilon \cdot d\lambda$ (in water).

Remembering that the integral of the extinction coefficient is linked to the integral optical density of the sample via formula (4) we can determine the concentration of Pchlide in water relative to the concentration in THF. In this way we converted the excitation energy per pulse into excitation density, i.e. number of photons per molecule for both aggregates and monomers. The final corrected curves are shown in the figure 5.B where a clear difference between aggregate and monomer absorption saturation is seen. The different saturation measurements in THF and methanol now nicely coincide on one line,

whereas the five measurements in water still show some scattering behavior. Possibly, this again reflects small differences in concentrations and aggregation sizes.

References

(53) Brouers, M.; Michelwolwertz, M. R. *Photosynthesis Research* 1983, *4*, 265.

(54) Klement, H.; Helfrich, M.; Oster, U.; Schoch, S.; Rudiger, W. *European Journal of Biochemistry* 1999, *265*, 862.