Supplemental Material

MOLECULAR ADAPTATION OF PHOTOPROTECTION: TRIPLET STATES IN LIGHT-HARVESTING PROTEINS

Andrew Gall*, Rudi Berera[†], Maxime T. A. Alexandre[†,‡], Andrew A. Pascal*, Luc Bordes*, Maria M. Mendes-Pinto*, Sandra Andrianambinintsoa*, Katerina V. Stoitchkova*[,§], Alessandro Marin[†], Leonas Valkunas[¶, ‖], Peter Horton**, John T. M. Kennis[†], Rienk van Grondelle[†], Alexander Ruban[††], and Bruno Robert[*, ‡‡].

Running title: Structure of Triplet States in Antennae

Power-induced resonance Raman spectroscopy. The carotenoid triplet state is populated by any excitation wavelength absorbed by light-harvesting complexes; however, we see it in resonance Raman (RR) only when the exciting light matches its T_1/T_n transition or one of the sublevels of this transition (1-6). Here we shall use the LH2 complex from *Rbl. acidophilus* (7) as an example to illustrate the usefulness of power-induced resonance Raman spectroscopy. Fig. S1 shows that except for the excitation wavelength at 514.5 nm there is no evidence of the appearance, and power-induced relative increase, of a Raman vibrational mode at 1493 cm^{-1} which is indicative of rhodopin glucoside in its triplet state (1). It is noteworthy that, although the absolute intensity of ^{G}Car (1517 cm^{-1}) increases with laser power, the ^{T}Car/$^{G+T}$Car ratio tends towards a saturated steady state sub-population of ^{T}Car. The intensity of the RR modes attributed to ^{T}Car is fully reversible upon reducing the level of incident radiation (Fig. S2, closed circles). In order to fit the data points, we need a model for the behavior of the system under this power-saturation scheme. We consider an ensemble of *N* pigments; where light is incident at a frequency close to a particular transition energy of the pigment. If an individual pigment molecule is described as a two-level system (ground state and excited state) then the absorption process can be expressed using the kinetic equation:

$$\frac{dn}{dt} = \sigma_0 I P_0 - \sigma_{se} I n - \frac{n}{\tau}, \qquad (1)$$

where n is the population of the excited states in the ensemble, σ_0 and σ_{se} are cross sections of the ground state absorption and stimulated emission, respectively, I is the excitation intensity (photons/sec), and P_0 is the probability of finding the system in the ground state. P_0 can be defined as:

$$P_0 = 1 - n. \qquad (2)$$

For steady-state illumination we have to consider stationary conditions; thus, we get:

$$\sigma_0 I P_0 - \sigma_{se} I n - \frac{n}{\tau} = 0, \qquad (3)$$

giving a saturation-type description:

$$n = \frac{\tau \sigma_0 I}{1 + \tau(\sigma_0 + \sigma_{se}) I} \qquad (4)$$

Although this description is for the absorption cross-section of one particular transition, it allows us to examine the evolution of the Raman transitions of the ground and excited carotenoid states. As excitation energy increases, the relative intensities of the finger-print ν_1 Raman modes attributed to ^{T}Car and ^{G}Car should increase and decrease respectively, in direct relation to their electronic absorption transitions. Applying [equation 4] to the experimental data produces the solid line in Fig. S2.

References.

1. Angerhofer, A., F. Bornhaeuser, A. Gall, and R. J. Cogdell. 1995. Optical and optically detected magnetic resonance investigation on purple photosynthetic bacterial antenna complexes. Chem. Phys. 194:259-274.
2. Rondonuwu, F. S., T. Taguchi, R. Fujii, K. Yokoyama, Y. Koyama, and Y. Watanabe. 2004. The energies and kinetics of triplet carotenoids in the LH2 antenna complexes as determined by phosphorescence spectroscopy. Chem. Phys. Lett. 384:364-371.
3. Hashimoto, H., Y. Koyama, Y. Hirata, and N. Mataga. 1991. S1 and T1 species of ß-carotene generated by direct photoexcitation from the *all-trans*, 9-*cis*, 13-*cis*, and 15-*cis* isomers as revealed by picosecond Transient absorption and transient Raman spectroscopies. J. Phys. Chem. 95:3072-3076.
4. Ohashi, N., N. KoChi, M. Kuki, T. Shimamura, R. J. Cogdell, and Y. Koyama. 1996. The structures of S-0 spheroidene in the light-harvesting (LH2) complex and S-0 and T-1 spheroidene in the reaction center of Rhodobacter sphaeroides 2.4.1 as revealed by Raman spectroscopy. Biospectroscopy 2:59-69.
5. Hashimoto, H., and Y. Koyama. 1988. Time-resolved resonance Raman-spectroscopy of triplet ß-carotene produced from all-*trans*, 7-*cis*, 9-*cis*, 13-*cis*, and 15-*cis* isomers and high-pressure liquid-chromatography analyses of photoisomerization via the triplet-state. J. Phys. Chem. 92:2101-2108.
6. Mukai-Kuroda, Y., R. Fujii, N. Ko-chi, T. Sashima, and Y. Koyama. 2002. Changes in molecular structure upon triplet excitation of all-*trans*-spheroidene in *n*-hexane solution and 15-*cis*-spheroidene bound to the photo-reaction center from Rhodobacter sphaeroides as revealed by resonance-Raman spectroscopy and normal-coordinate analysis. J. Phys. Chem. A 106:3566-3579.
7. Cogdell, R. J., I. Durant, J. Valentine, J. G. Lindsay, and K. Schmidt. 1983. The isolation and partial characterization of the light-harvesting pigment-protein complement of Rhodopseudomonas acidophila. Biochim. Biophys. Acta 722:427-435.

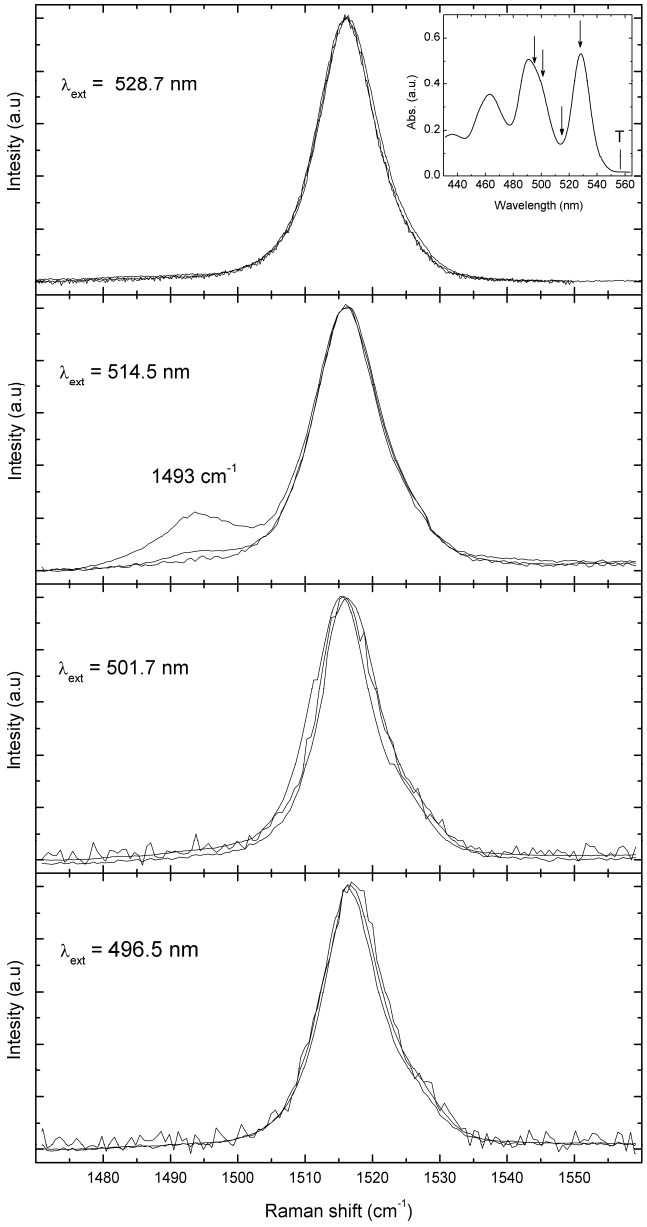

Fig. S1. 77K Resonance Raman spectra of the ν_1 Raman vibrational mode of LH2 as a function of incident laser power for a series of excitation wavelengths: 528.7 nm (0.5, 5.4 and 21.5 μW), 514.5 nm (0.1, 0.5 and 21.5 μW), 501.7 nm (0.5, 5.4 and 21.5 μW) and 496.5 nm (0.5, 5.4 and 21.5 μW). For clarity, the RR spectra are normalized to the maximum peak intensity at 1517 cm^{-1} that is ascribed to the vibrational mode of the conjugated C=C bonds of rhodopin-glucoside in its ground state. Only short accumulation times were recorded at the lowest laser power for the 501.7 and 496.7 nm excitation wavelengths, which accounts for their relatively poor signal-to-noise ratio. Insert: Ground state 77K absorption spectrum of the carotenoid region in LH2. The position of the excitation wavelengths (see arrows) show their relative position to the main Carotenoid triplet-triplet absorption band (T) which is located at 557 nm (see Ref. 1). Only the excitation wavelength at 514.5 nm is in resonance with a satellite of the T_1/T_n transition.

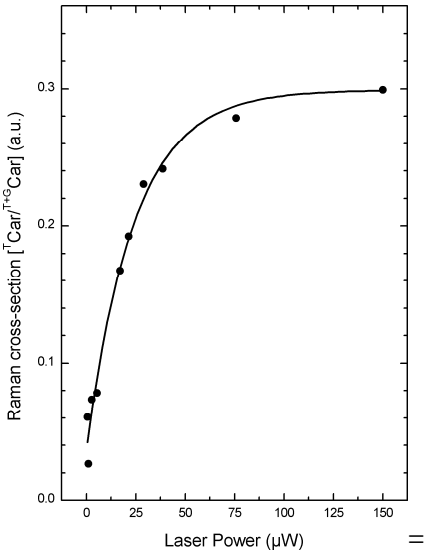

Fig. S2. Evolution of triplet carotenoid (^{T}Car) as a function of incident radiation on LH2 displayed as the relative ratio of ^{T}Car (1493 cm^{-1}) *vs* $^{G+T}$Car (closed circles). Excitation wavelength = 514.5 nm, T=77K.