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Supplementary Information

A photosynthetic antenna complex foregoes unity carotenoid to bacteriochlorophyll energy transfer efficiency to ensure photoprotection

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Supplementary Results

Generation of a **ζ-carotene producing strain of Rba. sphaeroides**

A ζ-carotene producing strain of Rba. sphaeroides was generated by introduction of the gene encoding the Synechocystis 9,15,9′-tri-cis-ζ-carotene-forming 2-step phytoene desaturase (PDS) to a mutant lacking both the native all-trans-neurosporene-forming 3-step phytoene desaturase (CrtI) and hydroxy-neurosporene synthase (CrtC) encoding genes, as described in the Materials and Methods section in the main text. The crtC gene was deleted to prevent potential 1,2-hydration of any carotenoid species produced in the modified strain.

Figure S1 shows the native carotenoid biosynthesis pathways in Rba. sphaeroides (panel A) and oxygenic phototrophs (panel B), and the modified pathway in the ΔcrtI ΔcrtC PDS^+ strain described in the present study (panel C). In wild-type Rba. sphaeroides, 15-cis-phytoene (N = 3) is converted to all-trans-neurosporene (N = 9) by CrtI via all-trans-phytofluene (N = 5) and all-trans-ζ-carotene (N = 7) intermediates; all-trans-neurosporene is subsequently converted to spheroidene/spheroidenone (N = 10/N = 10+C=O) by the activities of three/four additional enzymes in the absence/presence of O_2 (1).

The pathway is different in oxygenic phototrophs, where four enzymes convert 15-cis-phytoene to all-trans-lycopene (N = 11) (2). First, 15-cis-phytoene is converted to 9,15,9′-tri-cis-ζ-carotene (N = 7) by PDS. Next, the 15-cis-bond in 9,15,9′-tri-cis-ζ-carotene is isomerized by ζ-carotene isomerase (Z-ISO) resulting in production of 9,9′-di-cis-ζ-carotene, the substrate for ζ-carotene desaturase (ZDS), which performs two-further desaturations generating 7,9,7′,9′-tetra-cis-lycopene (N = 11). Isomerization of the cis-double bonds at the 7,9 and 7′,9′ positions by a second carotenoid isomerase, CRT-ISO, yields all-trans-lycopene, the common precursor to all the mature carotenoids accumulated by Synechocystis. Notably the isomerizations can be catalyzed non-enzymatically by light (3, 4).

Deletion of crtI in Rba. sphaeroides results in accumulation of 15-cis-phytoene (1); this strain grows very slowly under phototrophic growth conditions as it cannot make LH2 complexes, which require visibly colored carotenoids for assembly (5). The Synechocystis pds gene was introduced to the ΔcrtI ΔcrtC mutant on a plasmid and incubation under phototrophic growth conditions resulted in a faster growing strain that contained ζ-carotene and LH2 (see Figure 1A in main paper for spectra of the isolated LH2 complex). Because LH2 binds all-trans-carotenoids (6, 7), and the cis bonds in 9,15,9′-tri-cis-ζ-carotene/7,9,7′,9′-tetra-cis-lycopene are photolabile (3, 4), incubation in the light appears to be sufficient to non-enzymatically photo-isomerize the product of PDS, 9,15,9′-tri-cis-ζ-carotene, to all-trans-ζ-carotene.

Isolation of all-trans-ζ-carotene

Geometric isomers of ζ-carotene were isolated from purified LH2 by HLPC as described in the Materials and Methods section of the main paper (Figure S2). The predominant species (peak 4) was all-trans-ζ-carotene, as expected because LH2 is known to bind all-trans-carotenoids (6, 7). Two smaller peaks that elute shortly before the all-trans isomer and have almost identical absorption spectra are most likely the 9,9′-di-cis (peak 2) and 9-cis or 9′-cis (eluting together; peak 3) isomers (see Figure S1c). The group of earlier eluting peaks collectively marked as (1) is associated with central cis-isomers of ζ-carotene that have isomerizations within the conjugated region of the molecule resulting in a characteristic ‘cis-peak’, which for ζ-carotene is observed at just below 300 nm (8).

Transient absorption of ζ-carotene in solvent at room temperature and at 77 K

Transient absorption (TA) measurements of all-trans-ζ-carotene in 2-methyltetrahydrofuran (2-MTHF) at room temperature and 77 K are shown in Figure S3. The carotenoid was excited at the (0-0) vibronic band. Figures S3A and D show exemplary TA spectra taken at various delay times after excitation. For comparative purposes steady-state absorption spectra are also provided (dash-dot, scaled to match). Global analysis of the TA performed according to the irreversible sequential decay of excitation is shown in Figures S3B and E. For both temperatures three kinetic components were necessary for satisfactory fitting; according to spectral and temporal characteristics these are associated with the decay of the S_2 state (EADS with lifetime ≤ 200 fs at room temperature and 260 fs at 77 K with characteristic ground state absorption bleaching and stimulated emission and $S_2 \rightarrow S_n$ excited state absorption in NIR), S_1 state vibrational equilibration (EADS with 1.34 – 3.5 ps lifetimes) and decay of the S_1 state (EADS with lifetime of 340 ps at RT/540 ps at 77 K). Panels C and F show dynamics extracted at the maximum excited state absorption band along with the fits obtained from global analysis.

Supplementary figures

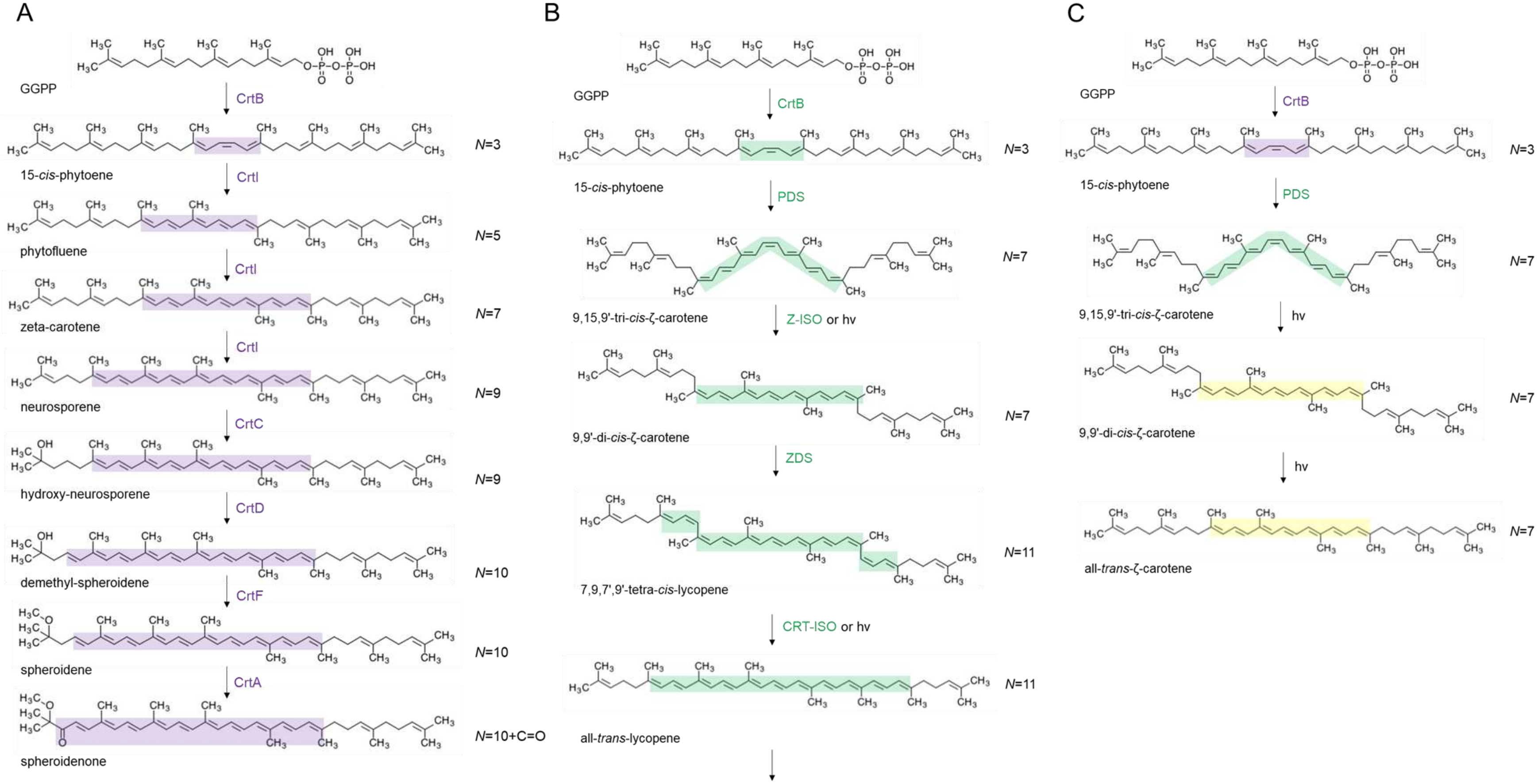

Figure S1. Carotenoid biosynthesis in wild-type Rba. sphaeroides (A), oxygenic phototrophs (B) and the ΔcrtI ΔcrtC PDS^{+} strain of Rba. sphaeroides (C). The carbon-carbon double bond conjugation (N) is indicated with shaded boxes. In (C) we predict that 9,15,9′-tri-cis-ζ-carotene generated by introduction of the Synechocystis 2-step PDS to the ΔcrtI ΔcrtC mutant of Rba. sphaeroides is photo-isomerized to 9,9′-di-cis-ζ-carotene and all-trans-ζ-carotene. Carotenoid structures are taken from the KEGG database (https://www.genome.jp/kegg-bin/show_pathway?map00906).

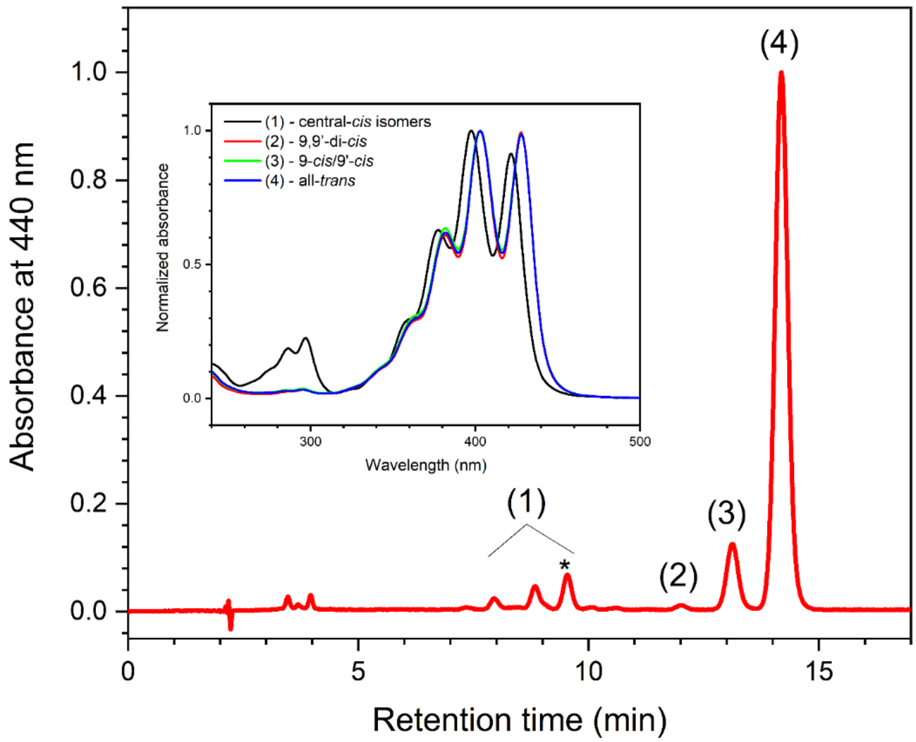

Figure S2. Isolation of the all-trans-ζ-carotene from purified LH2. Elution of carotenoid species was monitored at 440 nm. The peaks associated with various carotenoid isomers are numbered 1-4 and their normalized absorption spectra are plotted in the inset panel. The all-trans isomer (peak 4) is expected to be the most dominant species as LH2 is known to bind carotenoids in the all-trans configuration. Peaks 2 and 3 are predicted to be the 9,9′-di-cis (peak 2) and a mixture of 9-cis and 9′-cis isomers (peak 3). The small peaks collectively labeled as 1 are cis-isomers with central (in respect to conjugation) isomerizations, identified by the prominent 'cis-peak' at ~300 nm. The representative absorption spectrum of a central-cis isomer shown in the inset panel corresponds to peak marked with an asterisk (*). For further details see the text.

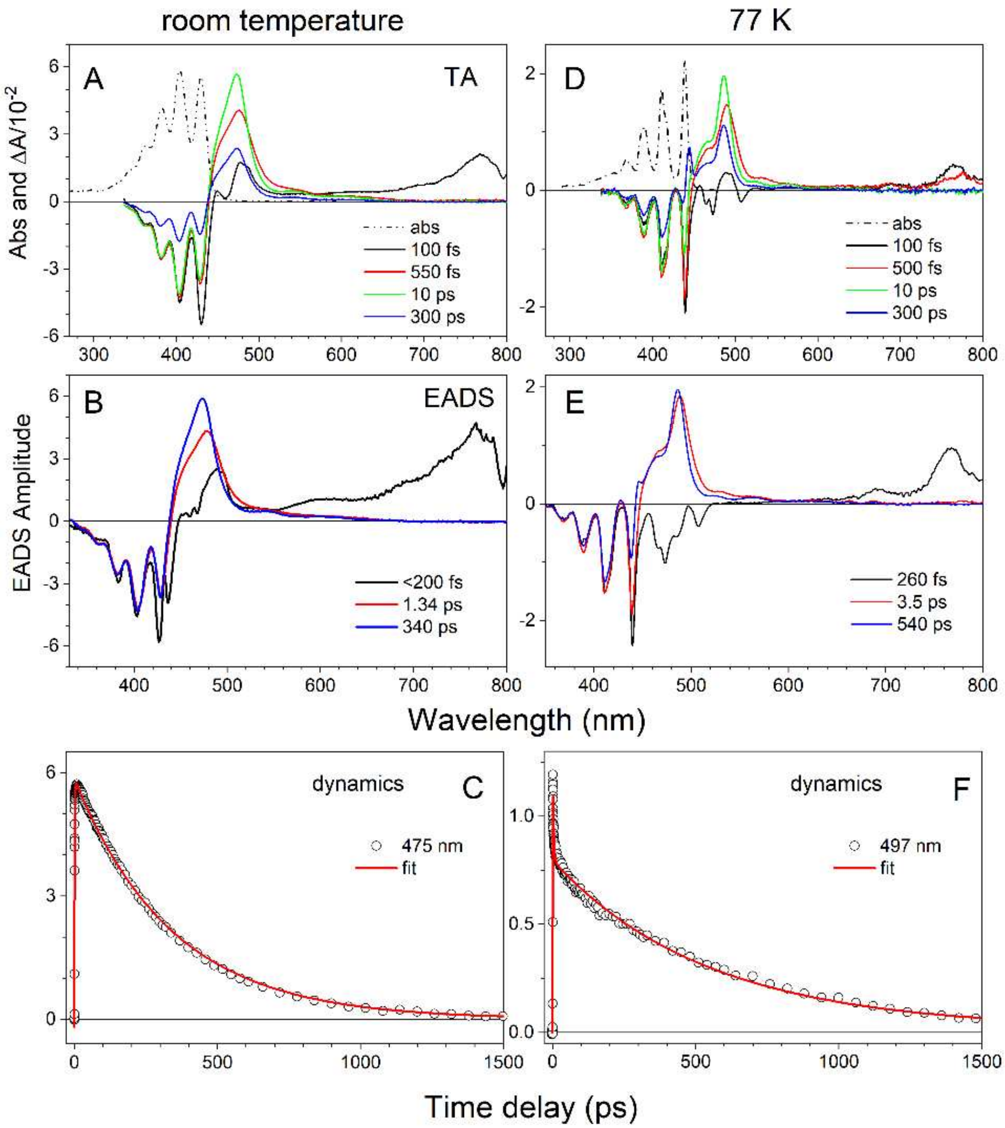

Figure S3. Transient absorption of all-trans-ζ-carotene in 2-MTHF at room temperature (left panels, A-C) and at 77 K (right panels, D-F). (A, D) TA spectra taken at various delay times after excitation at the (0-0) vibronic band of the $S_0 \rightarrow S_2$ absorption (429 nm at RT and 438 nm at 77 K). Scaled steady-state absorption spectra (dash-dot, black) are also provided for comparative purposes. (B, E) Global analysis results, EADS – evolution associated decay spectra, resulting from sequential fitting model. (C, F) Exemplary TA decays extracted from the ESA band (475 nm at RT and 497 nm at 77 K) accompanied with the fits obtained from global analysis.

Supplementary references

1. S. C. Chi et al., Assembly of functional photosystem complexes in Rhodobacter sphaeroides incorporating carotenoids from the spirilloxanthin pathway. BBA-Bioenergetics **1847**, 189–201 (2014).

2. S. Takaichi, Carotenoids in Algae: Distributions, Biosyntheses and Functions. Mar Drugs **9**, 1101-1118 (2011).

3. F. Q. Li, C. Murillo, E. T. Wurtzel, Maize Y9 encodes a product essential for 15-cis-ζ-carotene isomerization. Plant Physiol. **144**, 1181-1189 (2007).

4. K. Masamoto, H. Wada, T. Kaneko, S. Takaichi, Identification of a gene required for cis-to-trans carotene isomerization in carotenogenesis of the cyanobacterium Synechocystis sp PCC 6803. Plant and Cell Physiology **42**, 1398-1402 (2001).

5. H. P. Lang, C. N. Hunter, The relationship between carotenoid biosynthesis and the assembly of the light-harvesting LH2 complex in Rhodobacter sphaeroides. Biochem. J. **298**, 197–205 (1994).

6. J. Koepke, X. C. Hu, C. Muenke, K. Schulten, H. Michel, The crystal structure of the light-harvesting complex II (B800-850) from Rhodospirillum molischianum. Structure **4**, 581-597 (1996).

7. R. J. Cogdell et al., The structure and function of the LH2 (B800-850) complex from the purple photosynthetic bacterium Rhodopseudomonas acidophila strain 10050. Prog Biophys Mol Bio **68**, 1-27 (1997).

8. Y. Chen, F. Li, E.T. Wurtzel, Isolation and characterization of the Z-ISO gene encoding a missing component of carotenoid biosynthesis in plants. Plant Physiol **153**, 66-79 (2010).