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 Δ*crtI* Δ*crtC* 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 Δ*crtI* Δ*crtC* 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

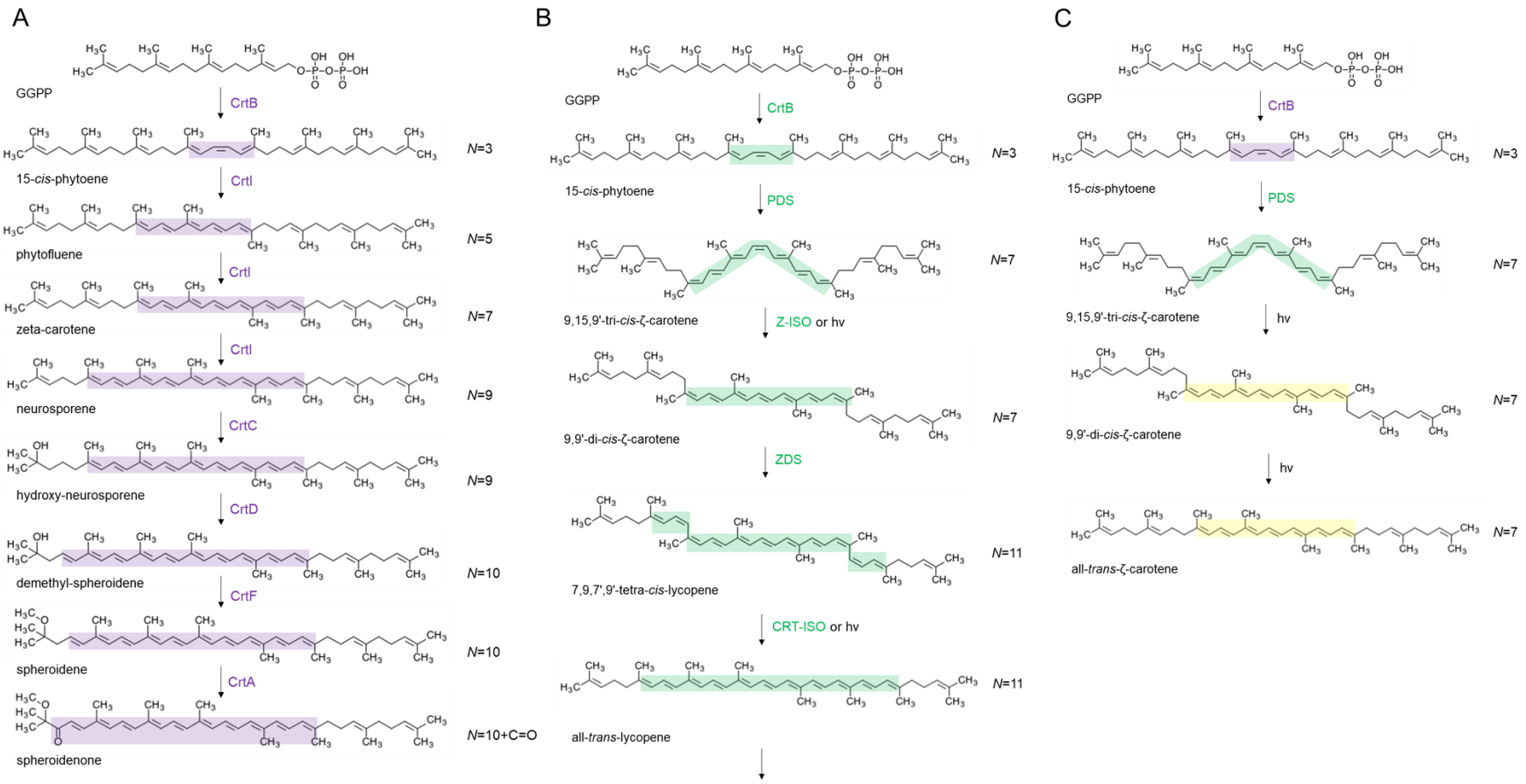

Figure S1. Carotenoid biosynthesis in wild-type *Rba. sphaeroides* (A), oxygenic phototrophs (B) and the Δ*crtI* Δ*crtC* PDS^{+} strain of *Rba. sphaeroides* (C). The carbon-carbon double bond conjugation (*N*) is indicated with shaded boxes. In (C) we predict that 9,15,9′-tri-*cis*-ζ-carotene generated by introduction of the *Synechocystis* 2-step PDS to the Δ*crtI* Δ*crtC* 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).

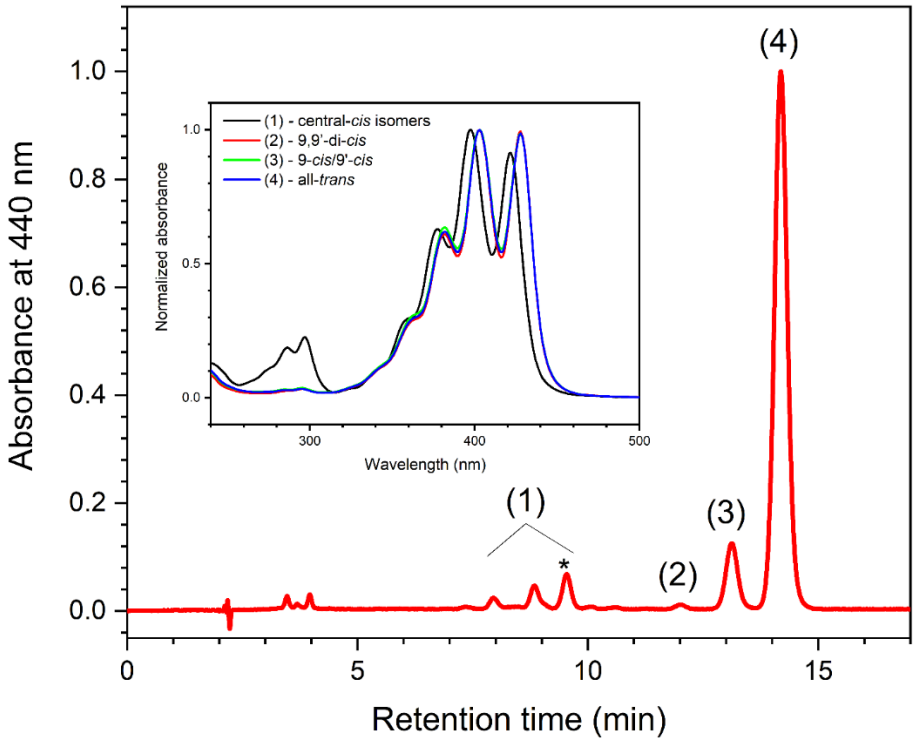

Figure S2. Isolation of the all-*trans*-ζ-carotene from purified LH2. Elution of carotenoid species was monitored at 440 nm. The peaks associated with various carotenoid isomers are numbered 1-4 and their normalized absorption spectra are plotted in the inset panel. The all-*trans* isomer (peak 4) is 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.

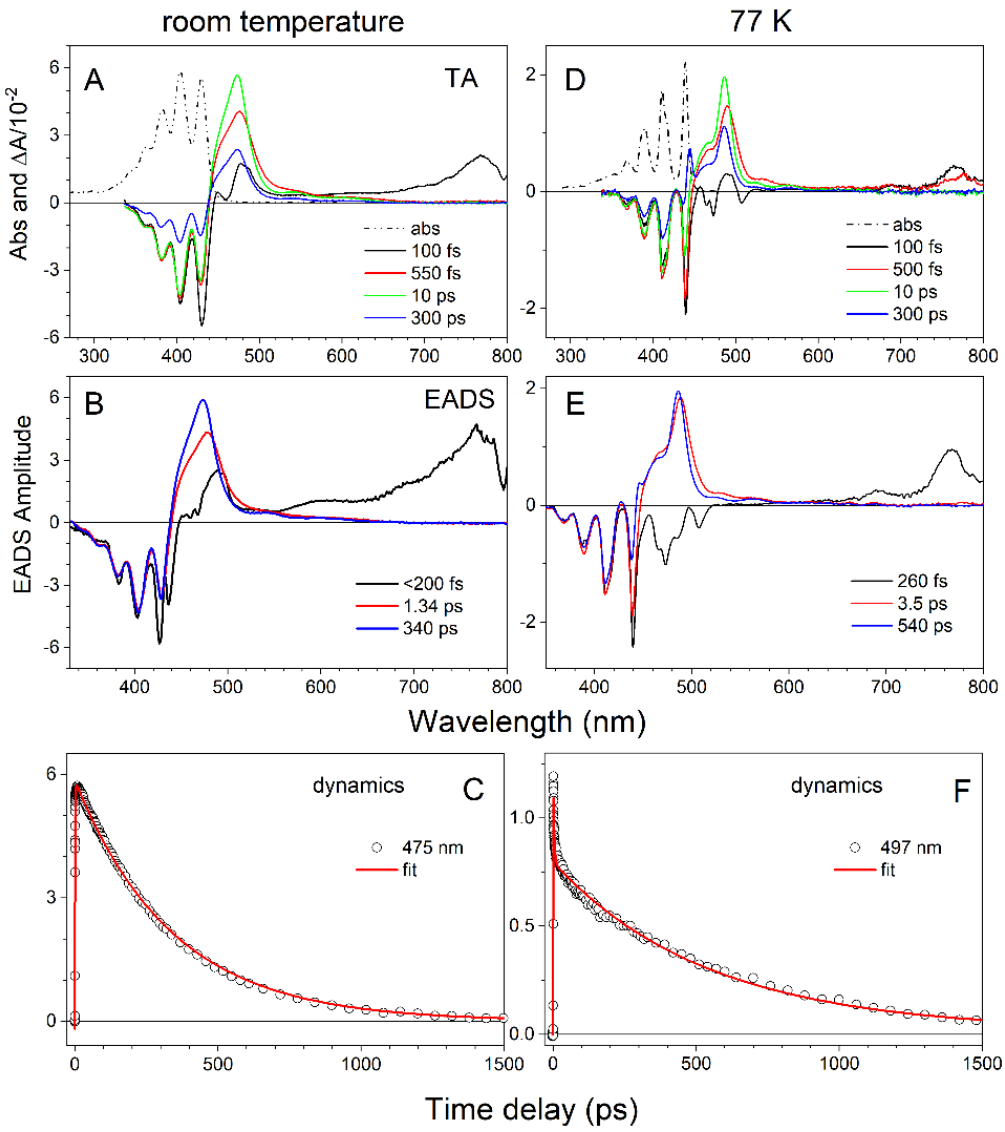

Figure S3. Transient absorption of all-*trans*-ζ-carotene in 2-MTHF at room temperature (left panels, A-C) and at 77 K (right panels, D-F). (A, D) TA spectra taken at various delay times after excitation at the (0-0) vibronic band of the $S_0 \rightarrow S_2$ absorption (429 nm at RT and 438 nm at 77 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

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