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

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Abstract

Carotenoids serve a dual purpose in photosynthesis, providing light-harvesting and photoprotective energy dissipation functions within pigment–protein complexes. The carbon–carbon double bond (C=C) conjugation length of carotenoids (N), generally between 9 and 15, determines the carotenoid-to-(bacterio)chlorophyll ((B)Chl) energy transfer efficiency. Here, we purified and spectroscopically characterized light harvesting complex 2 (LH2) from Rhodobacter sphaeroides containing the N = 7 carotenoid zeta (ζ)-carotene, not previously incorporated within an antenna complex. Transient absorption and time-resolved fluorescence show that, relative to the lifetime of the S_1 state of ζ-carotene in solvent, the lifetime decreases ~250-fold when ζ-carotene is incorporated within LH2, due to transfer of excitation energy to the B800 and B850 BChls a. These measurements show that energy transfer proceeds with an efficiency of ~100%, primarily via the $S_1 \rightarrow Q_x$ route because the $S_1 \rightarrow S_0$ fluorescence emission of ζ-carotene overlaps almost perfectly the Q_x absorption band of the BChls. However, transient absorption measurements performed on microsecond timescales reveal that, unlike the native $N \geq 9$ carotenoids normally utilized in light-harvesting complexes, ζ-carotene does not quench excited triplet states of BChl a, likely due to elevation of the ζ-carotene triplet energy state above that of BChl a. These findings provide insights into the co-evolution of photosynthetic pigments and pigment-protein complexes. We propose that the $N \geq 9$ carotenoids found in light-harvesting antenna complexes represent a vital compromise that retains an acceptable level of energy transfer from carotenoids to (B)Chls while allowing acquisition of a new, essential function, namely photoprotective quenching of harmful (B)Chl triplets.

Significance statement

Photosynthesis uses carotenoids as light-harvesting pigments and for photoprotective energy dissipation. The carbon–carbon double bond conjugation length of carotenoids (N) affects the carotenoid-to-(bacterio)chlorophyll energy transfer efficiency but the photoprotective capability was considered to be independent of N. Using light-harvesting complex 2 from the model photosynthetic bacterium Rhodobacter sphaeroides containing ζ-carotene (N = 7) or neurosporene (N = 9), we demonstrate that decreasing the conjugation length increases the carotenoid-to-bacteriochlorophyll energy transfer efficiency, in the case of ζ-carotene to approximately 100%. However, unity quantum efficiency comes at the cost of photoprotection, suggesting that naturally evolved photosynthesis tolerates some energetic loss to allow essential energy dissipation, explaining why longer conjugation length carotenoids are utilized in native pigment-protein complexes.

Author contributions

D.M.N. and A.H. designed research; D.M.N., D.J.K.S., D.P.C. and A.H. performed research. All authors analyzed data and wrote the paper.

Conflict of interest statement

The authors declare no competing interest.

Introduction

Carotenoids (Cars) are natural pigments found in plants, algae and photosynthetic (cyano)bacteria where they function in light harvesting, stabilization of pigment-protein complex structure and photoprotection (1, 2). In antenna complexes and photosystems Cars act as accessory light-harvesting pigments and transfer excitation energy to (bacterio)chlorophylls ((B)Chls). The absorption band of Cars typically spans the 400-550 nm spectral range and is associated with the $S_0 \rightarrow S_2$ electronic transition of the linear carbon-carbon double bond (C=C) conjugation (N), a characteristic structural motif (3, 4). Cars also have an important photoprotective functions in light-harvesting complexes, typically quenching harmful excited triplet states of (B)Chls to inhibit formation of reactive oxygen species such as singlet oxygen (5). The photophysical properties of Cars and their roles in numerous light harvesting antennae have been investigated (6). The LH2 complex from purple photosynthetic bacteria has been a model antenna for such studies (7-16) as crystal structures are available (17, 18), the Car content can be manipulated (14, 19-22), and its relatively simple pigment composition reduces the number of features that must be interpreted in spectroscopic analyses.

Numerous studies have shown that the Car-to-BChl a energy transfer efficiency ($\Phi_{Car \rightarrow BChl}$) varies in LH2s from different purple bacterial species and appears to correlate with the Car conjugation length. The yields for Cars such as neurosporene (N = 9) or spheroidene (N = 10) are 85-95% (11, 19, 23-25). The yields decrease to 55–65% for Cars where N = 11 (e.g. rhodopin, rhodopin glucoside or lycopene) (11, 24-26) and reduce further to ~30% when N = 13 (spirilloxanthin) (14). As longer C=C conjugation length (larger N) lowers the energies of both the S_2 and S_1 excited states (2, 6), the broadly accepted explanation for the variation in $\Phi_{Car \rightarrow BChl}$

in LH2 is linked with the inherent excited-state properties of the particular Car. Typically, both S_2 and S_1 states are involved in energy transfer to BChls and if the S_1 state falls below the Q_y bands of the B800 and B850 spectral forms of BChl a, reducing spectral overlap of the hypothetical S_1 emission and BChl a absorption, then the $\Phi_{Car \rightarrow BChl}$ declines sharply. At first glance some Cars do not appear to fit into this correlation, for example okenone with N = 13 + C=O, where nominally there are 13 conjugated carbon-carbon bonds and a terminal C=O group that could extend the conjugation length. Okenone bound within the LH2 complex from the bacterium Merichromatium purpuratum (formerly Chromatium purpuratum) shows $\Phi_{Car \rightarrow BChl}$ = 85.6% (27), substantially above the expected value of ~30% observed for other N = 13 Cars. However, previous investigations have demonstrated that when conjugation is extended into the cyclic ends of Cars the double bonds in the rings act as only ~0.3 of a double bond in the linear backbone, and in case of Cars with aryl rings such as okenone the contribution may be even smaller (28, 29). Indeed, it has been shown that the energies of the excited states of okenone are only marginally smaller than their counterparts for the open chain Car spheroidenone (N = 10 + C=O) (30), which when bound to LH2 shows a $\Phi_{Car \rightarrow BChl}$ of 92-94% (11, 20), similar to that of okenone.

Unlike $\Phi_{Car \rightarrow BChl}$ efficiency, the ability to provide photoprotective quenching of BChl a excited states (triplets) is more or less the same for all of the above mentioned Cars incorporated into LH2 (7, 31, 32). It could therefore be perceived that the ability to photoprotect (B)Chls in photosynthetic antennas is a universal characteristic of all Cars, however this is not the case. Energetic requirements between (B)Chl and Car triplet states must also be considered and, like the S_2 and S_1 states, the triplet states of Cars have well-defined energies that also depend on N,

although this is impossible to directly confirm due to undetectable phosphorescence. Interestingly, photosynthetic organisms have a strong preference for Cars with N ≥ 9 for incorporation into light-harvesting proteins (1), suggesting that Cars with N ≥ 9 have triplet state energies in a "sweet spot" corresponding to the triplet state energies of specific (B)Chls to ensure the most efficient sensitization ((B)Chls triplet quenching) (33). In support of this idea, experiments that tested photoprotective abilities of open chain and cyclic Cars of different N lengths in Car-Chl a model mixtures demonstrated that photoprotective capability appear to falls sharply for Cars with N ≤ 8 (34-36).

Here we set out to better understand the relationship between Car conjugation length, Car-to-BChl energy transfer efficiency, and Car photoprotective ability using LH2 complexes from the model purple phototrophic bacterium Rba. sphaeroides containing either neurosporene (N = 9) or zeta (ζ)-carotene (N = 7). Using this model antenna we provide direct evidence that Cars with a conjugation length of only 7 are fully efficient in Car-to-BChl a energy transfer, but are unable to quench excited states of BChl a, a conflict that could have influenced the evolution of a compromise between energy transfer and photoprotection in photosynthetic light-harvesting complexes from other organisms.

Results

ζ-carotene to BChl a energy transfer in LH2 proceeds with unity quantum efficiency

We performed comparative spectroscopic studies of ζ-carotene bound to LH2 (see Materials and Methods, SI Appendix, figure S1 for details of the ζ-carotene producing strain of Rba.

sphaeroides and purification of LH2) and isolated ζ-carotene in solvent; steady-state absorption in both environments and fluorescence emission spectrum of ζ-carotene in solvent and in LH2 are shown in Figure 1. The absorption spectrum of LH2-bound ζ-carotene shows two clearly pronounced vibronic bands at 441 and 413 nm. The near-perfect overlap of the absorptance (1-transmittance) and the fluorescence excitation spectrum when monitoring emission exclusively from BChl a shows that ζ-carotene is a perfect excitation energy donor to BChl a with $\Phi_{Car \rightarrow BChl}$ ~100% (Figure 1A). This ideal Φ value suggests that there are no additional processes competing with energy transfer (ET), such as triplet formation via singlet fission or formation of a Car cation, which reduces $\Phi_{Car \rightarrow BChl}$ by 5–15% for Cars with longer N values (11, 21, 37). Examination of the spectra reveals that the S_2 excited state is too far from the Q_x absorption resulting in insufficient overlap to permit $S_2 \rightarrow Q_x$ energy transfer. Transfer from $S_2 \rightarrow Q_x$ is typical for Cars with longer conjugations (i.e. S_2 energies red shifted relative to that of ζ-carotene) (10), therefore it is possible that the majority of the ET occurs from the S_1 excited state. It is broadly accepted that LH2 complexes bind Cars in all-trans geometry (17) and for comparative studies with isolated Car in solvent we purified the all-trans geometric isomer of ζ-carotene (the purification procedure is described in SI Appendix, Figure S2). To examine the intrinsic properties of the all-trans-ζ-carotene S_1 state, we combined steady-state and time-resolved fluorescence measurements with transient absorption spectroscopy. As demonstrated in Figure 1B, the Car dissolved in solvent has detectable fluorescence. We assign the main band between 550 and 700 nm as emission from the $S_1 \rightarrow S_0$ transition and the smaller band at ~480 nm as emission from $S_2 \rightarrow S_0$. Generally, Cars are known to have very low fluorescence quantum yields, therefore the fluorescence spectrum could easily be overwhelmed by sample

contaminants. To ensure that the emission is associated with the Car fluorescence the excitation spectrum was measured (Figure 1B red dash-dot line). The very good agreement with the absorptance (1-transmittance) spectrum of all-trans-ζ-carotene confirms that the observed emission is that of the $S_1 \rightarrow S_0$ fluorescence emission of the Car.

ζ-carotene to BChl a energy transfer proceeds from the S_1 excited singlet state

The fluorescence spectra show that emission from the S_1 and S_2 transitions partially overlap and it is uncertain if the vibronic band at ~550 nm originates from the S_1 excited state (0-0 vibronic band) or if an additional peak at shorter wavelengths is obscured by the tail of the S_2 state emission. Using time-resolved fluorescence (TRF) of the Car, as shown by the pseudo-color 2D fluorescence decay contour map in Figure 2A, we could unambiguously assign these features. It is clear that both emissive transitions are temporally resolved. What is not apparent from the contour is that there is a weaker, longer-lived band with a maximum at ~550 nm that is likely associated with emission from sample contaminants, which may form and accumulate during laser exposure. Global analysis of the TRF data was used to temporally and spectrally separate emission bands; Figure 2B shows these results along with the kinetic scheme used for fitting. The fitting results (SAS – species associated spectra) reflect emission from the excited states of Car and sample contaminants. Fitting shows that the intrinsic lifetime of the S_1 state is 340 ps. The quality of the fit is shown in Figure 2C, which contains the fluorescence decay profiles for the S_1 and S_2 states together with fits from global analysis. In order to obtain the S_1 state energy from the fluorescence emission, the 340 ps SAS was subjected to a simulation of the vibronic progression with Franck-Condon (FC) factors according to a simplified formula that assumed

only one average vibronic mode $<\nu>$ contributing to the spectrum with an average displacement $<\Delta>$ between the state coordinates (Figure 2D) (see Materials and Methods for more details):

$$FC(\nu) = C\nu \sum_{\upsilon} \frac{\langle\Delta\rangle^{2\upsilon}}{2^{\upsilon}\upsilon!} \exp\left(-\frac{\langle\Delta\rangle^{2}}{2}\right) \frac{1}{2\pi\sigma} \exp\left(-\frac{[\nu-(\nu_{00}-\upsilon\langle\nu\rangle)]^{2}}{2\sigma^{2}}\right) \quad (1)$$

The simulated spectrum (red line) was obtained based on the following parameters: υ = 6 (number of vibronic bands), $<\Delta>$ = 2.21, $<\nu>$ = 1,120 cm^{-1}, ν_{00} = 17,980 cm^{-1} (energy of the S_1 state), σ = 540 cm^{-1} (width of the vibronic band in cm^{-1}), C = 72 (arbitrary scaling constant).

To permit comparison with the LH2-bound Car, the excited state lifetime of all-trans-ζ-carotene in solvent was also determined by transient absorption (TA) spectroscopy. The TA results obtained at room temperature and 77 K, along with an expanded description, are provided in Figure S3. Fitting of the TA data gives an S_1 state lifetime of 340 ps, in excellent agreement with TRF measurements despite the vastly different temporal resolutions of the two methods. The extensive overlap of the ζ-carotene emission spectrum with the 600 nm BChl a Q_x absorption, combined with its strong fluorescence emission (i.e. high quantum yield) and long S_1 excited state lifetime, should make ζ-carotene an ideal accessory light-harvesting pigment. We predict that ζ-carotene will perform better than its counterparts with longer conjugations such as neurosporene (N = 9) or spheroidene (N = 10), which show much weaker, almost undetectable $S_1 \rightarrow S_0$ fluorescence emission and have no spectral overlap with the BChl a Q_x band (38).

Transfer dynamics of the Car $S_1 \rightarrow$ BChl a Q_x excitation energy transfer

To determine the excited state properties of ζ-carotene bound within LH2, transient absorption spectroscopy was applied to the entire complex allowing simultaneous measurement of ζ-

carotene and the energy-accepting BChls (Figure 3). Figure 3A shows a pseudo-color 2D contour map measured between 330 and 910 nm over 1300 ps plotted on a semi-logarithmic time axis for better visibility of the transient bands. A subset of representative TA spectra taken at various times after excitation are shown in Figure 3B and dynamics at chosen wavelengths are shown in Figure 3C. It is evident that the excited state absorption (ESA) band associated with the S_1 state (positive band at 489 nm) decays almost entirely within 4 ps. Given that the S_1 state lifetime in solvent is 340 ps, this dramatic reduction shows the high efficiency of the $S_1 \rightarrow Q_x$ route in the energy transfer process.

More details of the ET dynamics were revealed with global analysis of the TA data provided in Figure 3D. Fitting was performed according to a sequential pathway of excitation decay and migration (irreversible steps from the fastest to the slowest lifetimes) to generate evolution associated decay spectra (EADS). This analysis does not necessarily reproduce the transient spectra of each species in the pathway but it is satisfactory for obtaining macroscopic rates (observed lifetimes) of each distinct spectral component. These EADS can be interpreted by comparison with previous studies of other LH2 complexes. The EADS with lifetime of 220 fs can be assigned to decay of the initially excited ζ-carotene S_2 state. The lifetime is similar to the S_2 lifetime observed in solvent at room temperature (SI Appendix, Figure S3), which suggests that there is negligible ET to BChl a, and the excitation internally converts to the S_1 state. The S_1 state has a lifetime of 1.37 ps, ~250-fold less than the 340 ps lifetime in solvent, confirming that it participates in extremely efficient ET to the Q_x bands of both the B800 and B850 spectral forms of BChl a as demonstrated by simultaneous bleaching of the B800 and B850 bands associated with depletion of BChls in the ground state. Two EADS are associated with BChls,

the B800*→B850 energy transfer at 4.9 ps (following excitation of the B800 BChl a from the Car S_1 state) and the decay of the excited B850 BChl a at 850 ps, for which the excited state lifetime is known to be ~1000 ps (22, 31, 32, 37).

ζ-carotene is not a quencher of BChl a triplets

Figure 3D shows a fifth EADS with an infinite (inf) lifetime, meaning it belongs to a process that is far slower than the 7 ns measurement time window. The long lifetime and spectral shape suggest that it is associated with the B850 BChl a triplet, formed by inter-system crossing (ISC) at an efficiency of ~30% (32). The presence of this component is interesting because it is well established that B850 BChl a triplets are rapidly quenched by sensitization of Car triplets, at most within a few tens of nanoseconds of formation, preventing their accumulation (31, 32). The accumulation of BChl a triplets is potentially damaging as they may sensitize harmful singlet oxygen, whereas Car triplets are harmless as the triplet energy safely dissipates as heat. Therefore, triplet quenching by Cars provides an essential photoprotective function. Here, the presence of a kinetic component corresponding to long-lived B850 BChl a triplets suggests that this essential photoprotective mechanism is absent in LH2 complexes containing ζ-carotene. To test this hypothesis the LH2 complexes with ζ-carotene and a second control LH2 complex containing neurosporene (N = 9) were studied by TA in the microsecond time domain (Figure 4). The control LH2 was isolated from a ΔcrtC mutant of Rba. sphaeroides, described previously (19). Figure 4A, B shows 2D-pseudocolor TA contours with transient bands marked with arrows. These are accompanied by selected TA spectra (Figure 4C and D) at various delay times after excitation, with inset panels showing the spectral range of the Car triplet transient band. It is apparent that for the ζ-carotene-containing LH2 the Car triplet spectrum is absent, whereas it can

be clearly seen for the control complex with neurosporene. Conversely the triplet state of B850 is long-lived in the ζ-carotene-containing complex, whereas it completely decays within ~70 ns in the neurosporene-containing LH2. Taken together these data show that in the ζ-carotene-containing LH2 the B850 BChl a triplet state is not quenched by formation of a Car triplet, whereas Car triplet formation is observed in the control neurosporene-containing LH2 sample (compare Figure 4E and F).

Discussion

This study provides direct evidence that Cars with shorter conjugation lengths (here N = 7) are unable to participate in the essential function of photoprotection in a model bacterial light harvesting complex. Triplet state energies of monomeric (B)Chls are already well defined and depending on the (B)Chl species span an energetic range of 8,000–10,750 cm^{-1} (930–1250 nm). All these triplet states lie above the 7,870 cm^{-1} (1,270 nm) energy level of singlet oxygen (33), making them efficient sensitizers of this harmful product. Although the triplet energies of Cars cannot be measured directly due to a lack of spectral features, they can be inferred by their interactions with observable triplets of other molecules. Specifically, the triplet state of BChl a is ~8,200 cm^{-1} (1,220 nm), marginally above singlet oxygen (33). Because Cars with $N \geq 9$ efficiently quench BChl a, this strongly suggests that they also have triplet energies below that of singlet oxygen, allowing efficient and harmless BChl a triplet quenching, as observed here in LH2 containing neurosporene. However, Cars with $N \leq 7$ are likely to have triplet energies above BChl a rendering triplet exchange to the Car energetically unfavorable, as observed here for LH2

containing ζ-carotene. In the absence of quenching, long-lived BChl a triplets will sensitize singlet oxygen leading to oxidative damage of the cell.

So what is the situation with Cars and N in other photosynthetic antenna complexes that use different species of B(Chl) for light-harvesting? As discussed above, the triplet state energy levels of the various (B)Chls that exist in nature span a broad energetic range, thus the "cut-off" conjugation length at which Cars lose their photoprotective function in respect to a specific (B)Chl species may not be strictly limited to N = 7 in other light-harvesting complexes. However, because the triplet state of BChl a is energetically only marginally higher than that of singlet oxygen, the lack of quenching of BChl a triplets by ζ-carotene strongly suggests that the energy of the triplet state of ζ-carotene is higher than the energy level of singlet oxygen. Consequently, employing a Car with N = 7 (or less) in any LH complex is unlikely to be beneficial as even if the Car can quench a specific (B)Chl triplet, it will in turn become a singlet oxygen sensitizer itself.

In summary, our study provides an evolutionary insight into the coevolution of photosynthetic pigment-protein complexes and BChl a and Car biosynthesis. Despite Cars with shorter conjugation length being simpler for the organism to produce and able to transfer energy to (B)Chls with increased quantum efficiency, below a certain N value the inability to provide photoprotection of harmful B(Chl) excited states suggests it is not coincidental that all antenna complexes of photosynthetic organisms utilize Cars with $N \geq 9$.

Materials and Methods

Growth of Rba. sphaeroides and generation of a ζ-carotene producing strain

Liquid cultures of Rba. sphaeroides were grown in M22+ medium (39) supplemented with 0.1% (w/v) casamino acids. For microoxic growth cultures were shaken at 175 rpm at 30 °C. Phototrophic growth was performed in completely full culture vessels agitated with magnetic stir bars under ~50 μmol photons m^{-2} s^{-1} illumination provided by OSRAM classic 116 W halogen bulbs. Medium was supplemented with 30 μg ml^{-1} kanamycin and/or 10% (w/v) sucrose as appropriate.

The neurosporene-accumulating ΔcrtC and phytoene-accumulating ΔcrtI strains of Rba. sphaeroides 2.4.1 have been described previously (19); the crtC gene (rsp_0267) was deleted from the ΔcrtI mutant using the pK18mobsacB allelic exchange construct described in the same study. The plasmid was conjugated into the ΔcrtI strain from Escherichia coli S17-1 with selection on M22+ agar containing kanamycin. Subsequent plating on M22+ agar containing sucrose isolated colonies where the plasmid was excised from the genome and ΔcrtC mutants were identified by PCR and verified by automated DNA sequencing.

To produce ζ-carotene in Rba. sphaeroides, the Synechocystis sp. PCC 6803 phytoene desaturase (PDS) encoding gene (pds, slr1254) was introduced to the ΔcrtI ΔcrtC background. The pds gene was amplified from genomic DNA with primers slr1254_F (5'-ACTGAGATCTATGCGCGTTGTGATCGCC-3'; BglII site underlined in italics) and slr1254_R (5'-TCGACTCGAGTTAACCCACGGTGACTATTTCCCTG-3'; XhoI site underlined in italics) using Q5 DNA polymerase (NEB). The resulting 1439 bp PCR product was digested with BglII/XhoI and ligated into the same sites of the replicative pBBRBB-Ppuf843–1200 plasmid such that expression of pds is under control of the truncated puf promoter (40) The sequence

verified plasmid was conjugated into the ΔcrtI ΔcrtC strain of Rba. sphaeroides from E. coli S17-1 with selection on M22+ agar containing 30 μg ml^{-1} kanamycin.

ζ-carotene extraction and purification

Pigments (Cars and BChl a) were extracted with acetone and methanol at a 50:50 (v:v) ratio in approximately 10 mL volume. Water (~0.5 mL) was added, followed by approximately 10 mL of petroleum ether. The mixture was agitated and saponified by adding few small pellets of NaOH. Water was added until ζ-carotene was fully partitioned into the ether layer, which was carefully removed. The ether was dried down using a stream of nitrogen gas in darkness and the Cars were resuspended in acetone. The mixture of ζ-carotene isomers was purified using an Agilent 1100 HPLC system consisting a quaternary pump, in line diode array UV-Vis detector and 4.6 × 250 mm Zorbax Eclipse XBD-C18 column. The flow rate was 1 mL/min, the sample injection volume was 100 μL (in acetone) and the mobile phase was 40:30:30 (v/v/v) acetonitrile:methanol:tetrahydrofuran. All-trans-ζ-carotene was isolated using a YMC Carotenoid C30 column with the same HPLC system, mobile phase and flow rate (see SI Appendix for more details). All solvents were HPLC grade (Millipore Sigma).

Purification of ζ-carotene or neurosporene containing LH2

LH2 from the ζ-carotene or neurosporene producing strains was purified by ion-exchange and size-exclusion chromatography as described previously (41).

Spectroscopic methods

Room temperature and 77 K steady-state absorption spectra of the LH2 complexes and isolated ζ-carotene were recorded using a UV-1800 spectrophotometer (Shimadzu). Fluorescence emission spectra of ζ-carotene and the LH2 samples were measured using an RF-6000

spectrofluorometer (Shimadzu) following excitation of the (0-0) vibronic peak of the $S_0 \rightarrow S_2$ absorption band of the Car dissolved in HPLC eluent (solvent mix; see above) and the Soret band of BChl a (370 nm), respectively. Excitation and emission bandwidths were set to 5 nm and a 455 nm (650 nm) long pass glass filter was used for emission measurements in solvent mix (LH2) at the detector entrance. Fluorescence excitation spectra were collected monitoring emission at 855 nm for LH2 and at 630 nm for the Car in solvent, with excitation and emission bandwidths of 5 nm. To minimize scattering effects, long-pass glass filters were placed at the detector entrance (610 nm for ζ-carotene in solvent mix and 780 nm for LH2). Cryogenic measurements at 77 K were carried out using a VNF-100 liquid nitrogen cryostat (Janis). ζ-carotene was dissolved in 2-methyltetrahydrofuran (2-MTHF) in a 1 cm cryogenic quartz cuvette (NSG Precision Cells) and slowly frozen in nitrogen vapor to form a transparent glass. For fluorescence studies all samples were adjusted to an absorbance of 0.1 at either the maximum of the $S_0 \rightarrow S_2$ absorption band (ζ-carotene) or the B850 band (LH2).

Transient absorption (TA) experiments were carried out using Helios-EOS, a tandem femtosecond/nanosecond time-resolved pump-probe absorption spectrometer (UltrafastSystems LLC) coupled to a Spectra-Physics femtosecond laser system described in detail previously (42). The LH2 complex was excited at 442 nm corresponding to the (0-0) vibronic peak of the ζ-carotene absorption band. ζ-carotene in 2-MTHF was excited at 428 nm (RT) or at 439 nm (77 K). The energy of the excitation beam, focused on the sample in a spot of approximately 1 mm diameter, was 200-400 nJ, corresponding to $6\text{-}1\times10^{13}$ photons cm^{-2} $pulse^{-1}$.

Time-resolved fluorescence (TRF) imaging was performed using a universal streak camera system from Hamamatsu (Hamamatsu Corporation) equipped with a N51716-04 streak

tube and A6365-01 Bruker spectrograph (Bruker Corporation) and coupled to an ultrafast laser system (Spectra-Physics) (43). The repetition rate of the excitation laser was set to 8 MHz (125 ns between subsequent pulses). The excitation beam was depolarized, focused on the sample in a circular spot of ~1 mm diameter and set to a wavelength of 410 nm (second order harmonics of 820 nm) and a very low photon flux of ~10^{10} photons cm^{-2} $pulse^{-1}$. Fluorescence emission was measured at 90˚ to the excitation beam with a long-pass 455 nm filter placed at the entrance slit of the spectrograph. Sample integrity was examined by observing real-time photon counts that, if constant, indicates the absence of sample photodegradation. In order to minimize sample decomposition, TRF studies were performed on the HPLC-eluted sample in the solvent mixture.

Data processing and global analysis

Prior to analysis, TA datasets were corrected for temporal dispersion using Surface Xplorer 4.0 (UltrafastSystems LLC). The datasets were globally fitted with a kinetic model assuming a sequential population of excited states/species in a cascade of nonreversible, decreasing rates (longer lifetimes). The instrument response function (IRF) was simulated by a Gaussian with a full width at half-maximum (FWHM) of ~200 fs. The fitting procedure used to TA datasets gives EADS – evolution associated difference spectra (44). According to this model, the TA signal at any time delay and wavelength, $\Delta A(t,\lambda)$, can be reconstructed from superposition of n^{th} $C_i(t)$ and $EADS_i(\lambda)$ products

$$\Delta A(t,\lambda) = \sum_{i=1}^{n} C_i(t) EADS_i(\lambda) \quad (2)$$

$C_i(t)$, is time-dependent concentration of i^{th} EADS is expressed as

$$\frac{dC_i(t)}{dt} = k_{i-1}C_{i-1}(t) - k_i C_i(t), i \neq 1, k_{i-1} > k_i \quad (3)$$

and $C_1(t)$, is populated by the excitation pulse that in spectrometer is represented as instrument response function, IRF:

$$\frac{dC_1(t)}{dt} = IRF(t) - k_1 C_1(t) \quad (4)$$

Global analysis of TRF data included customized fitting model (target analysis) which assumed more complicated (customized) interactions between compartments. The model used to fit the TRF data is provided as an insert in Figure 2B. Global analysis was performed using CarpetView 1.0 (Light Conversion Ltd.). All plots were created in Origin 2019 (OriginLab Corp.).

Modeling of the vibronic progression of fluorescence emission spectrum with Franck-Condon (FC) factors

Upon assumption that vibronic progression of the fluorescence spectrum is dominated with some average vibronic mode <ν> with average displacement <Δ> the F-C equation:

$$FC(\nu) = C\nu \sum_{\upsilon} \prod_i \frac{\Delta_i^{2\upsilon}}{2^{\upsilon}\upsilon!} \exp\left(-\frac{\Delta_i^2}{2}\right) \frac{1}{2\pi\sigma} \exp\left\{-\frac{[\nu-(\nu_{00}-\sum_i \upsilon\nu_i)]^2}{2\sigma^2}\right\} \quad (5)$$

simplifies to the following form:

$$FC(\nu) = C\nu \sum_{\upsilon} \frac{\langle\Delta\rangle^{2\upsilon}}{2^{\upsilon}\upsilon!} \exp\left(-\frac{\langle\Delta\rangle^2}{2}\right) \frac{1}{2\pi\sigma} \exp\left\{-\frac{[\nu-(\nu_{00}-\upsilon\langle\nu\rangle)]^2}{2\sigma^2}\right\} \quad (6)$$

Where C is the scaling constant, υ is the number of vibronic bands included in the progression, σ is the width of the vibronic band (in cm^{-1}) and ν_{00} is (in this case) energy of the S_1 state (in cm^{-1}) of ζ-carotene.

Data Availability

All data needed to support the conclusions of this manuscript are included in the main text and SI Appendix. Rhodobacter sphaeroides strains are available upon request.

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Figure Legends

Figure 1. Spectroscopic comparison of the ζ-carotene-containing LH2 antenna complex from Rba. sphaeroides and all-trans-ζ-carotene in solvent at room temperature. The chemical structure of the Car is shown above the panels. The C=C bonds highlighted in red indicate the conjugation length (N = 7) of the Car. (A) Comparison between absorptance (black) and fluorescence excitation (red) spectra shows that in LH2 ζ-carotene transfers excitation energy to BChl a essentially without any energetic loss. (B) All-trans-ζ-carotene dissolved in solvent shows pronounced fluorescence emission from the S_1 state and a much weaker emission band from the S_2 state.

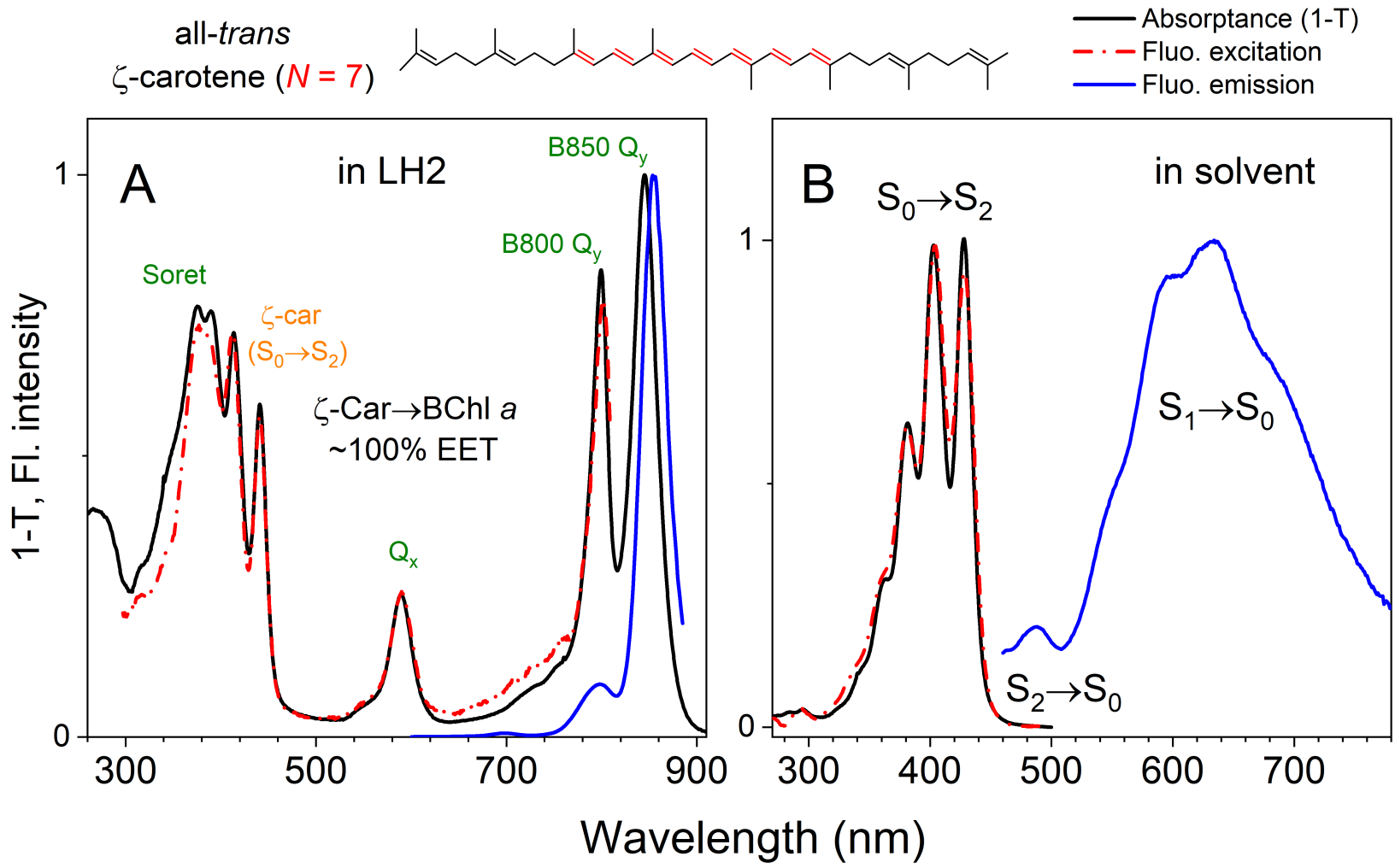
Figure 2. Time-resolved fluorescence (TRF) of all-trans-ζ-carotene in solvent upon excitation at 410 nm at room temperature. (A) Pseudo-color contour of TRF with the $S_2 \rightarrow S_0$ and $S_1 \rightarrow S_0$

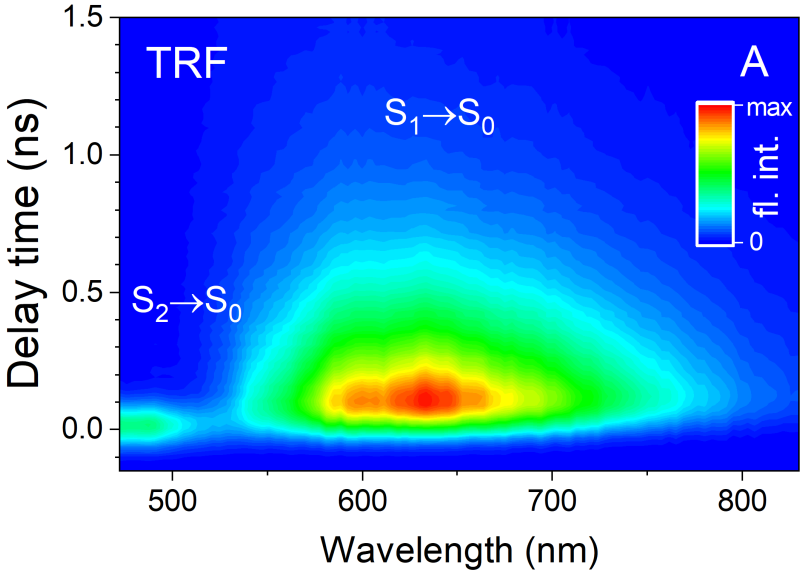
transitions indicated. (B) Global analysis results (SAS – species associated spectra) from application of fitting model depicted in the panel. The SAS are normalized to their maximal time-dependent concentrations. A small contribution of the long-lived (1.6 ns) component was also necessary to properly fit the TRF data, which most likely originates from a decomposition product that slowly accumulates during measurements. (C) Fluorescence dynamics for two exemplary wavelengths accompanied with fits. (D) Simulation of the $S_1 \rightarrow S_0$ fluorescence spectrum, approximated by the 340 ps SAS from panel B, with F-C progression. For more details please refer to the text. Simulation places the S_1 state energy at ~18,000 cm^{-1} (555 nm).

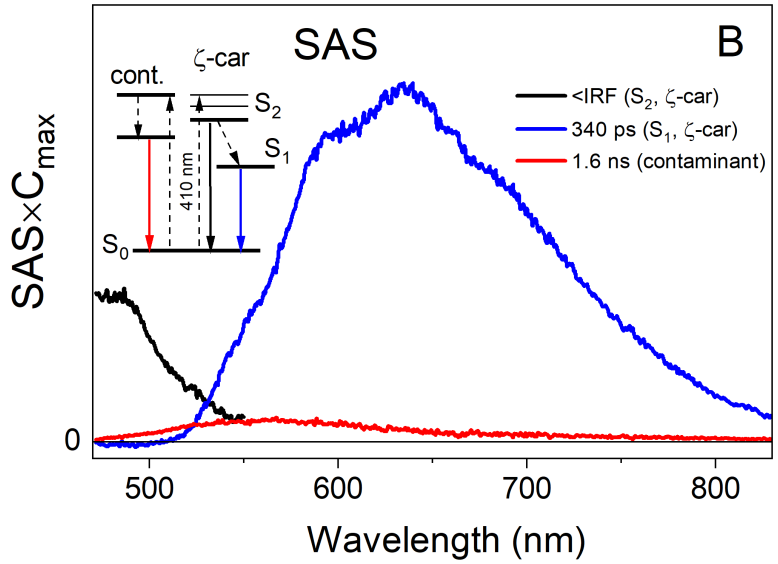
Figure 3. Room temperature transient absorption of ζ-carotene-containing LH2 from Rba. sphaeroides upon excitation of the Car absorption band. (A) 2D pseudo-color contour of TA with spectral features marked. (B) Representative TA spectra taken at various delay times after excitation at the (0-0) vibronic band of the $S_0 \rightarrow S_2$ absorption of ζ-carotene. (C) Example TA time courses recorded at selected wavelengths. (D) Evolution associated decay spectra (EADS) with application of a sequential decay model. The insert in panel D represents a zoomed-in view of the Q_x band. GSB - ground state bleaching; ESA – excited state absorption.

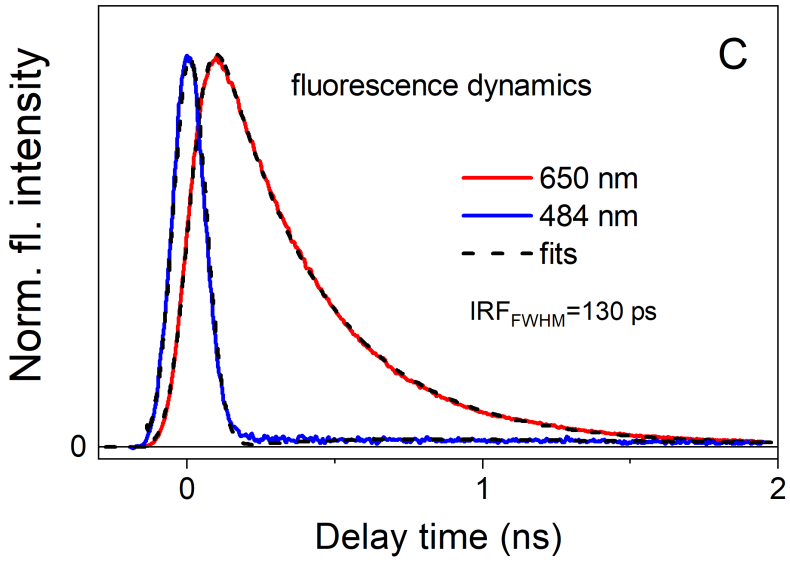
Figure 4. Transient absorption of the ζ-carotene-containing LH2 complex over a μs time range and comparison with neurosporene-containing LH2. (A, B) Pseudo-color contours of the TA for both LH2 complexes obtained after excitation of the Car band. Noticeable transient bands are marked. (C, D) Individual TA spectra recorded at various delay times after the excitation. The

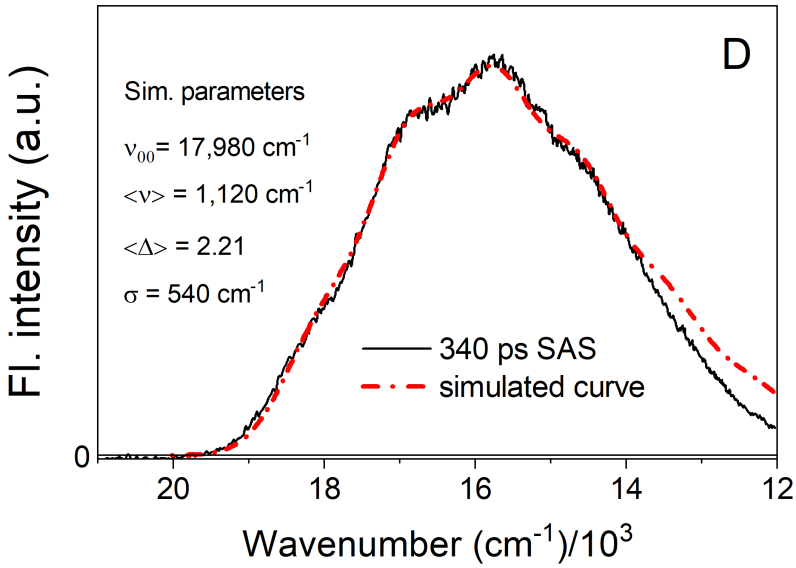
inserts show TA in the spectral range in which triplet-minus-singlet (T-S) spectra of the carotenoid appear. (E, F) Representative TA kinetic traces recorded at the bleaching of B850 Q_y band (852 and 868 nm) and at the maximum of the Car $T_1 \rightarrow T_n$ ESA band (507 nm, neurosporene-LH2 only). For clarity, time delays are plotted on a log scale and are slightly offset from t_0=0.

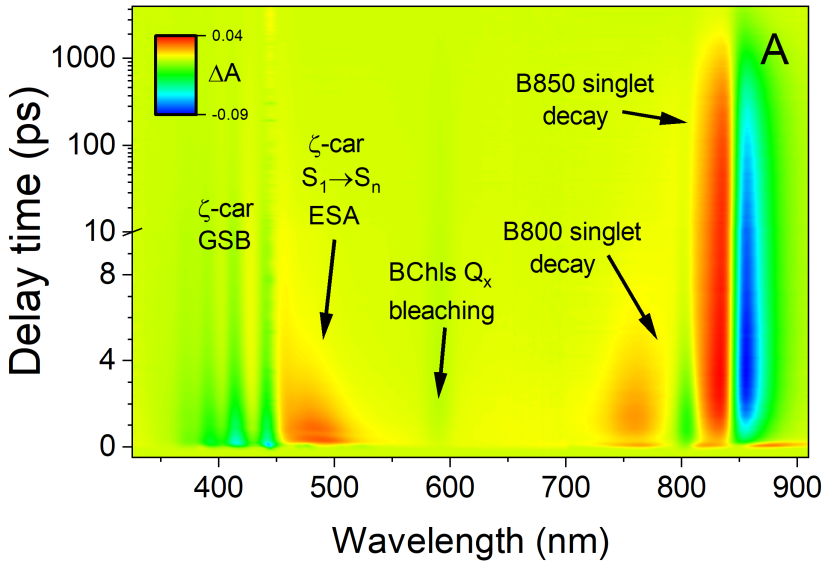

all-*trans*
ζ-carotene (*N* = 7)
Absorptance (1-T)
Fluo. excitation
Fluo. emission
A
in LH2
B850 Q_y
B800 Q_y
Soret
ζ-car
($S_0 \rightarrow S_2$)
ζ-Car→BChl *a*
~100% EET
Q_x
B
$S_0 \rightarrow S_2$
in solvent
$S_1 \rightarrow S_0$
$S_2 \rightarrow S_0$
1-T, Fl. intensity
Wavelength (nm)
0
1
300
500
700
900
400
600

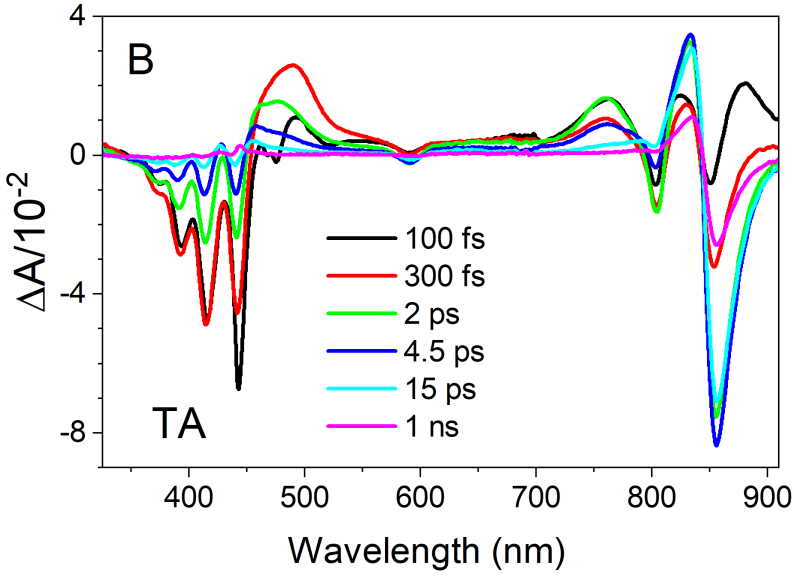

TRF
A
S1→S0
S2→S0
max
fl. int.
0
Delay time (ns)
1.5
1.0
0.5
0.0
500
600
700
800
Wavelength (nm)

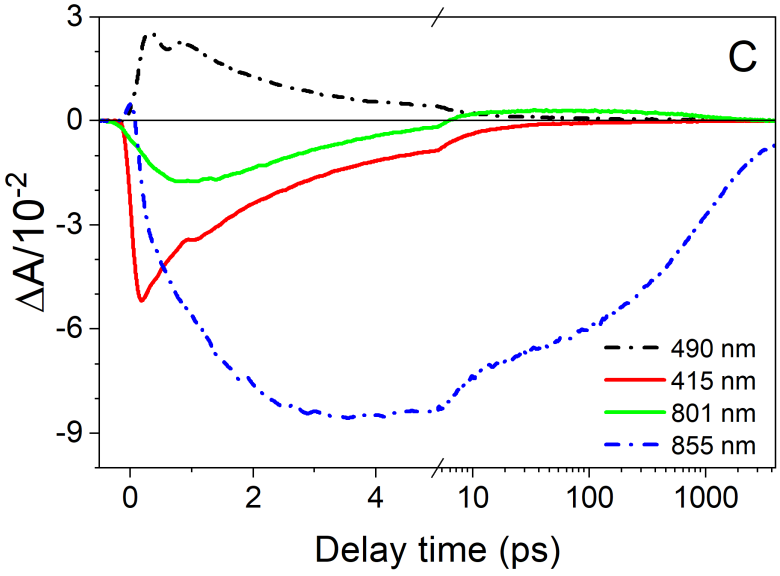

SAS
B
cont.
ζ-car
S2
S1
S0
410 nm
<IRF (S2, ζ-car)
340 ps (S1, ζ-car)
1.6 ns (contaminant)
SAS×Cmax
0
500
600
700
800
Wavelength (nm)

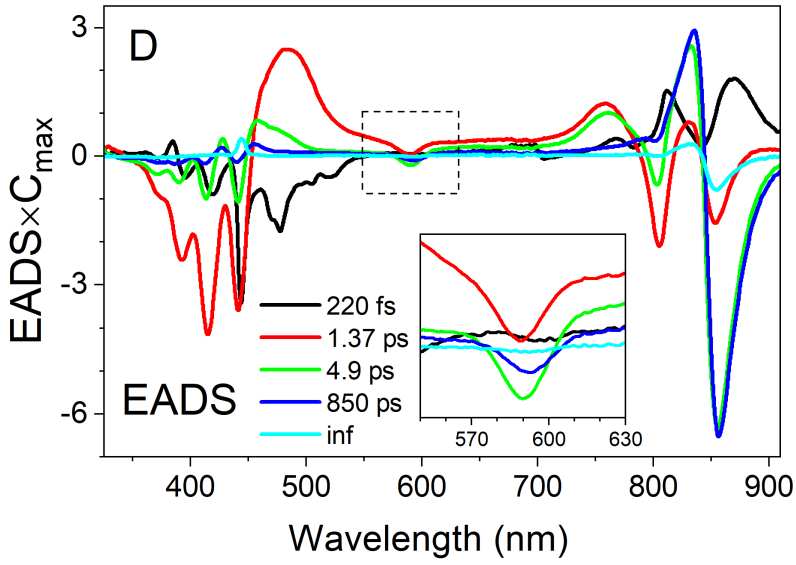

C
fluorescence dynamics
650 nm
484 nm
fits
IRFFWHM=130 ps
Norm. fl. intensity
0
0
1
2
Delay time (ns)

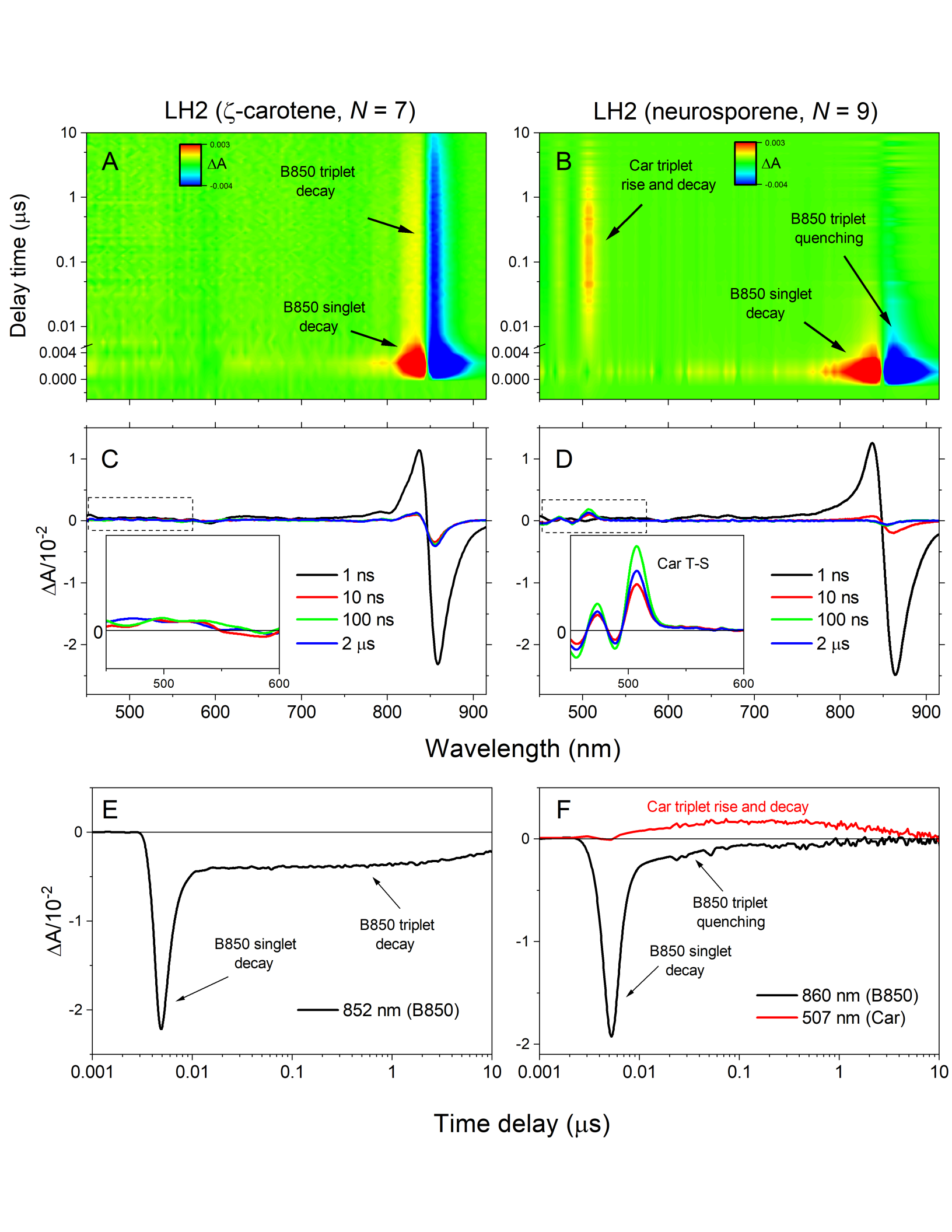

D
Sim. parameters
ν00= 17,980 cm-1
<ν> = 1,120 cm-1
<Δ> = 2.21
σ = 540 cm-1
340 ps SAS
simulated curve
Fl. intensity (a.u.)
0
20
18
16
14
12
Wavenumber (cm-1)/10^3


A
0.04
ΔA
-0.09
B850 singlet decay
ζ-car S1→Sn ESA
ζ-car GSB
B800 singlet decay
BChls Qx bleaching
Delay time (ps)
Wavelength (nm)


B
ΔA/10-2
100 fs
300 fs
2 ps
4.5 ps
15 ps
1 ns
TA
Wavelength (nm)


C
ΔA/10-2
490 nm
415 nm
801 nm
855 nm
Delay time (ps)


D
EADS×Cmax
220 fs
1.37 ps
4.9 ps
850 ps
inf
EADS
Wavelength (nm)


LH2 (ζ-carotene, N = 7)
LH2 (neurosporene, N = 9)
A
B
C
D
E
F
Delay time (μs)
10
1
0.1
0.01
0.004
0.000
0.003
ΔA
-0.004
B850 triplet decay
B850 singlet decay
Car triplet rise and decay
B850 triplet quenching
ΔA/10-2
1 ns
10 ns
100 ns
2 μs
Car T-S
500
600
700
800
900
Wavelength (nm)
852 nm (B850)
860 nm (B850)
507 nm (Car)
0.001
0.01
0.1
1
10
Time delay (μs)