



Hashimoto, H., Uragami, C., Yukihiro, N., Gardiner, A. T. and Cogdell, R. J. (2018) Understanding/unravelling carotenoid excited singlet states. *Journal of the Royal Society Interface*, 15(141), 20180026. (doi:[10.1098/rsif.2018.0026](https://doi.org/10.1098/rsif.2018.0026))

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Deposited on: 04 Jun 2018

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Understanding/Unraveling Carotenoid Excited Singlet States

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Abstract

Carotenoids are essential light-harvesting pigments in natural photosynthesis. They absorb in the blue-green region of the solar spectrum and transfer the absorbed energy to (bacterio-)chlorophylls, and so expand the wavelength range of light that is able to drive photosynthesis. This process is an example of singlet–singlet excitation energy transfer and carotenoids serve to enhance the overall efficiency of photosynthetic light reactions. The photochemistry and photophysics of carotenoids have often been interpreted by referring to those of simple polyene molecules that do not possess any functional groups. However, this may not always be wise since carotenoids usually have a number of functional groups that induce the variety of photochemical behaviors in carotenoids. These differences can also make the interpretation of the singlet excited states of carotenoids very complicated. In this article, we review the properties of the singlet excited states of carotenoids with the aim of producing as coherent a picture as possible of what is currently known and what needs to be learned.

1. Introduction

Carotenoids are a class of natural pigments. Over 750 species of carotenoid have been found in nature and have had their chemical structures determined [1, 2]. Different carotenoids can have a wide variety of functions [3-8]. As illustrated in **Figure 1**, they have light-harvesting and photoprotective functions in photosynthesis [9], they have pro-vitamin A activity in vision [10-12], they act as antioxidants in animals [13-16], they have an anti-aging activity [17-19], they can stimulate the immune system [20, 21], and they can exhibit an antitumor activity [22-24]. This extreme functional diversity means that what you see depends on which carotenoid you look at. There is a tendency for chemists think that all carotenoids are the same as β -carotene. They are of course not and this has led to considerable misunderstanding. Among the various functions of carotenoids listed above those found in photosynthesis are the most well studied. However, especially in the case of the involvement of carotenoid excited singlet states there still a number of open questions, debates and indeed contradictions. In this review, we especially focus on the properties of these singlet excited states and their involvement in photosynthetic light harvesting. Trying to fully understand the molecular details of photosynthetic light-harvesting so that they can be replicated in robust chemical systems is a major target of artificial photosynthesis [25].

Chlorophylls are the major light absorbing pigments in photosynthesis. However, they cannot efficiently absorb light in 450-550 nm region where the solar radiation profile (spectrum) at the surface of earth has its maximum intensity. This is precisely the region where carotenoids absorb light strongly. They are able to transfer this excitation energy to the chlorophylls by singlet-singlet excitation energy-transfer thereby making it available to power photosynthesis [26, 27]. This energy-transfer reaction allows the carotenoids to function as accessory light-harvesting pigments, broadening the spectral range over which light can support photosynthesis. This role of carotenoids is particularly significant in the cases of purple photosynthetic bacteria, heterokontophyta (*e.g.*, diatoms and brown algae), and dinoflagellates all of which tend to occupy environmental niches where light intensity is usually limiting for growth. Especially in the case of dinoflagellates, for example, in their position in the water column most of the available solar energy is in the 450 – 550 nm region [26] and so most of their photosynthesis is powered by light absorbed by carotenoids.

As illustrated in **Figure 2**, carotenoids have strong absorption of visible light in the blue and green regions of the spectrum. This is why most of the carotenoids found in

photosynthetic organisms have characteristic yellow, orange, and red colors. The lowest excited singlet (S_1) state in most pigment molecules represents the lowest energy, optically allowed one-photon transition from the ground state. The energy of this state then controls the color of that pigment molecule. However, carotenoids have a non-standard pattern of excited states. The lowest energy optically allowed excited singlet-state is not the lowest energy singlet-state. The lowest singlet excited energy state is formally a one photon forbidden state. This unusual photophysical pattern is explained classically using symmetry rules that have been developed from studies of linear polyene molecules. In what follows below we first describe the assignment of the S_1 state from an historical point of view. Then we will expand this topic by considering other possible optically forbidden singlet excited states. Finally, time-resolved and coherent spectroscopy using the ultrafast laser facilities having time-resolutions beyond 100 fs will be discussed since many of these states only exist on this ultrafast timescale. There are a number good review articles on the photophysics and photochemistry of carotenoids that have already been published [9, 25, 28-34]. Therefore, we will concentrate here on the most recent studies as well as important milestones that have set the scene our current understanding.

2. The lowest-excited singlet (S_1) state of carotenoids

The photophysics and photochemistry of carotenoids are usually explained by referring to the results from the study of polyene molecules. Polyenes are linear conjugated chains of carbon atoms joined by alternating double and single bonds. According to the ‘classical’ textbook written by Hudson, Kohler and Schulten [35], polyenes were (and continue to be) deservedly the objects of a good deal of experimental and theoretical attention. The historical importance of polyenes is due to their involvement in the development of molecular quantum theory and understanding fundamental molecular mechanisms of *cis-trans* photoisomerization. Before the pioneering work by Hudson and Kohler [36-38], polyenes were thought to be rather simple molecules, similar to that of other conjugated systems such as the polyacenes, and well described by approximate molecular orbital ideas. However, this turned out to be not the case. Referring to the exact words of Hudson, Kohler, and Schulten [35],

“Polyene electronic structures is both more complicated and more interesting than was previously thought.”

The theoretical assignment of the electronic structures of unperturbed linear polyene molecules comes from the application of symmetry rules. Planarity and the C_{2h} point symmetry are the essential properties required to allow the precise designation of the singlet excited-states of the polyene molecules to be described [38]. It is worth pointing out here that interpretations from simple linear polyenes can only be approximately applied to carotenoids due to the presence of the methyl groups perturbs the planarity of the conjugated portion of the carotenoid molecules. It also should be pointed out here that the carotenoids in the light-harvesting (LH) complexes from phototrophs are distorted significantly so that the selection rules and the standard symmetry labels ought not to be relevant. In particular, the “dark” S_x and S^* states might not be properly understood in the C_{2h} framework. The recent paper by Fiedor *et al.* (ref 154) makes this point explicitly, arguing in particular that the S_1 state does not have oscillator strength not because of the selection rules but rather because of large distortions from planar conformations. This important issue will be touched again in a later section.

The presence of an excited singlet state, nearly forbidden in absorption, below the dipole-allowed state (1^1B_u state in C_{2h} symmetry) previously thought to be the lowest lying excited singlet was first found for α,ω -dipenyl-1,3,5,7-octatetraene [36]. This finding was theoretically rationalized by Schulten and Karplus [39]. This one-photon forbidden lowest singlet excited-state was characterized as a “doubly excited” A_g state (2^1A_g state), which was only poorly described without extensive configuration interaction at that time. This ordering of electronic states, 2^1A_g below 1^1B_u , is now recognized as a general feature of polyene molecules whose number of conjugated double bonds (n) is greater than 4 [35]. The identification of the S_1 (2^1A_g) state of short polyenes ($n = 4 - 8$) is due to measurements of fluorescence and fluorescence-excitation spectra [35, 40, 41]. It is noteworthy here, however, that a subtle reinterpretation of these observations may be required, since the S_1 (2^1A_g) \rightarrow S_0 (1^1A_g) fluorescence experiments previously thought to have been carried out on pure all-*trans* isomers might actually be distorted due to the presence of *cis* isomers as impurities or formed as photochemical products from the S_1 state [42]. Nevertheless, the overall state ordering has undoubtedly been correctly predicted by theoretical work on simple all-*trans* polyenes [43, 44].

Tavan and Schulten extended their theoretical work on linear polyenes. They described the excitations within Pariser-Parr-Pople (PPP) and Hubbard models by means of a multiple-reference double-excitation expansion [43, 44]. The PPP and Hubbard Hamiltonians give rise to another symmetry, the so-called “Pariser alternancy symmetry” or “particle-hole symmetry” [45]. This symmetry classifies “-” and “+” states, *e.g.* the ground state is designated as $1^1A_g^-$ and the lowest optically allowed state as $1^1B_u^+$. The alternancy symmetry is useful for two reasons. Firstly, it allows the computational time to be reduced by taking account of the fact that the PPP and Hubbard many-electron Hamiltonians do not mix $1^1A_g^-$, $1^1A_g^+$, $1^1B_u^-$, and $1^1B_u^+$ states. Secondly, the alternancy symmetry provides a very simple classification of ionic states that are “+”, and covalent states that are “-”. The singlet excited states of carotenoids are often designated in a similar manner by referring to this classification.

The lowest excited singlet (S_1) state of carotenoids is frequently designated as the one photon forbidden $2^1A_g^-$ state assuming both the planarity and C_{2h} point symmetry of their polyene backbones. Hashimoto and Koyama were first to determine the A_g character of the S_1 state of carotenoids using pico-second transient resonance Raman spectroscopy and was then confirmed by Noguchi *et al.* [46-49]. These studies were further extended using a series of mono-*cis* isomers of both symmetric and asymmetric carotenoids [50, 51]. The S_1 species of carotenoids give rise to characteristic C=C stretching Raman lines at extraordinary high frequencies above 1750 cm^{-1} . This unusual observation was explained based by the idea of vibronic coupling between S_0 ($1^1A_g^-$) and S_1 ($2^1A_g^-$) states through a_g -type C=C stretching symmetric vibration [52-54]. Therefore, the presence of the extraordinary high frequency shifted C=C stretching mode can be taken as good evidence that the S_1 electronic state of carotenoids has an A_g character that can induce vibronic coupling with the ground S_0 state. The lowest singlet excited state, S_1 has $2^1A_g^-$ symmetry and so a one photon induced transition from the ground state is optically forbidden. The lowest optically allowed state is the $1^1B_u^+$ (S_2) state. When the $1^1B_u^+$ state is induced by a short excitation pulse it decays internally into the $2^1A_g^-$ state within 100 – 300 fs. The S_1 state typically then decays back to the ground state in a few picoseconds [55]. The exact rate constant of these processes depends upon factors such as the number of conjugated double bonds (n).

When the light-harvesting role of carotenoids in pigment–protein complexes from purple bacteria is considered a simplified view is usually presented. This simplified

picture illustrated in **Figure 3**. Probably the easiest way to begin this discussion is to describe the experiments of Macpherson *et al.* [56]. These authors compared the excited state kinetics of a carotenoid in organic solvent with the same carotenoid when bound within a LH2 complex. The idea was that any carotenoid singlet excited state that was capable of transferring energy to bacteriochlorophyll *a* in LH2 would be expected to have a shorter excited state lifetime in the antenna complex than in the organic solvent. Any energy transfer pathway would open another decay channel, thereby, accelerating the overall rate of decay of that excited singlet state. Moreover, it was expected that the decay of the donor carotenoid excited singlet state should match the rate of the energy arriving at the acceptor bacteriochlorophyll *a* molecule. In the case of the carotenoid rhodopin glucoside the decay of the S₂ state in the LH2 complex from *Rbl. acidophilus* was faster than that of the carotenoid in organic solvent (56 fs in LH2 and 133 fs in benzyl alcohol). The rise time of the arrival of the energy at both B800 and B850 matched decay time of rhodopin glucoside's S₂ state. The decay rate of the S₁ state of rhodopin glucoside was the same in the LH2 complex as in organic solvent. In this case the S₁ state is not active in energy transfer to bacteriochlorophyll *a*. However, the LH2 complex from *Rba. sphaeroides* contains carotenoids with fewer conjugated double bonds and in this case the S₁ state is able to transfer energy to the bacteriochlorophyll *a* molecules and its decay is accelerated in LH2 compared with organic solvent [57]. These findings show clearly that how the S₂ and S₁ states are involved in determining the overall efficiency of excitation energy-transfer from carotenoid to bacteriochlorophyll in the bacterial light-harvesting systems. The excited-state lifetimes of the S₁ and S₂ states of carotenoids are essential pieces of information when possible mechanisms of carotenoid-to-(bacterio)chlorophyll singlet-singlet energy transfer are being considered.

The exact energies (0-0 origin) of the S₂ and S₁ states relative to those of the Q_x and Q_y states of (bacterio)chlorophyll are also important. The energy of the S₂ state can be determined easily by ordinary absorption measurements because the transition from the ground (S₀) to S₂ states is optically allowed. On the contrary, the determination of the energy of the S₁ state is not straightforward because of the optical forbiddenness. In the past, carotenoids had been thought to be non-fluorescent [58]. However, carotenoid fluorescence has clearly been demonstrated [59-65]. Although the fluorescence quantum yields from the S₁ state are in the order of 10⁻⁵, the 0-0 origin of the S₁ state has been

suggested. Location of the S_1 state has also been determined independently using the energy-gap law [66], by resonance Raman excitation profile measurements [67, 68], and by near-IR ($S_1 \rightarrow S_2$) transient absorption measurements [69, 70]. **Table 1** summarizes the singlet excited-state energies of various carotenoids reported so far. Depending on the methods that were used for determination, small but not negligible deviation of these values can be seen. Nevertheless, carotenoid to (bacterio)chlorophyll energy transfer has been discussed in most cases based on the very simple energy diagram as illustrated in **Figure 3** [71]. This, however, is probably an over simplification as theoretical studies based primarily on symmetrical polyenes have predicted other possible excited singlet states such as $3^1A_g^-$ and $1^1B_u^-$ [43, 44] (*vide infra*). The possibility of these multiple excited states, and indeed others, has made understanding carotenoid photophysics extremely complicated [72].

3. The $1^1B_u^-$ and $3^1A_g^-$ states

The efficiency of carotenoid-to-(bacterio) chlorophyll singlet energy transfer in light-harvesting complexes varies from 30 to nearly 100% depending on the species of photosynthetic bacteria [23]. Until recently it was thought that the mechanism of carotenoid-to-bacteriochlorophyll energy-transfer could be fully explained based on the energy diagram illustrated in **Figure 3**. The lifetimes of these singlet excited states depend on the extent of conjugation. In the case of β -carotene, for example, the lifetime of the S_2 state is as short as 200 fs, while that of the S_1 state is as long as 10 ps [24]. Time-resolved fluorescence spectroscopy with sub-picosecond time resolution shows clearly that energy transfer can take place from both the S_2 and S_1 states to bacteriochlorophyll [25]. The efficiency of carotenoid-to-bacteriochlorophyll energy transfer depends on how effectively the energy can be harvested from both these excited states.

However, recent suggestions that other low-lying one photon forbidden, excited singlet states of carotenoids may also be involved in light harvesting have made the story more complicated. This is illustrated in **Figure 4**, which shows the results of theoretical calculations by Tavan and Schulten [43, 44]. They have predicted the presence of another one-photon forbidden singlet-excited state, namely the $1^1B_u^-$ state,

between S_2 and S_1 in the case of shorter polyene molecules with greater than four C=C double bonds. Indeed, extrapolation of their calculations suggests the presence of yet an additional $^1A_g^-$ ($3^1A_g^-$) state between S_2 and S_1 for long polyene molecules with more than 10 C=C double bonds. Kurashige *et al.* confirmed these predictions with more modern quantum chemical computations [73]. They have applied multireference Møller–Plesset perturbation theory with complete active space configuration interaction (CASCI-MRMP) to the study of the valence $\pi \rightarrow \pi^*$ excited states of all-*trans* linear polyenes $C_{2n}H_{2n+2}$ ($n = 3-14$). This theory predicts that the $1^1B_u^-$ state becomes lower than the $1^1B_u^+$ state at $n \geq 7$ and that the $3^1A_g^-$ state also becomes lower than the $1^1B_u^+$ state at $n \geq 11$. This theoretical treatment has also been successfully expanded to the studies of all-*trans* α,ω -diphenyl polyenes and oligoacenes [74, 75].

These theoretical predictions were supported experimentally, by the group of Koyama *et al.* using resonance Raman excitation-profiles on solid crystalline carotenoids [72, 76, 77] and fluorescence, as well as steady-state absorption spectroscopies [78-80]. They further extended their studies using sub-picosecond time-resolved absorption and stimulated Raman spectroscopies [30, 31, 57, 81-95]. They have interpreted all the observed excited state dynamics of carotenoids following photoexcitation based on the ordering of the forbidden singlet excited-states ($3^1A_g^-$, $1^1B_u^-$, $2^1A_g^-$) presented by Tavan and Schulten. However, it should be noted that the spectral analyses of Koyama *et al.* probably depend too much on the application of a kinetic model that only considers the theory of Tavan and Schulten as a possibility. They also extended their analysis of their data beyond their actual detection limit of 100 fs time-resolution. The conclusions of Koyama *et al.* now need to be tested with improved time resolution to see if they are still correct.

4. The other forbidden singlet excited-states (S^* , S_X , and X)

Another type of intermediate excited state, termed as S^* has been found with carotenoids both free in solution and bound to light-harvesting complexes, revealing a further level of complication [96-100]. At the higher-energy side of the $S_1 \rightarrow S_n$ transition, a new transient absorption band was detected by means of pump–probe time-resolved absorption spectroscopy and subsequent spectral analysis using SVD (singular value decomposition) and global fitting. This newly identified absorption band

was assigned to the S* state. The lifetime of this particular state was determined to be between 5 and 12 ps depending on both the species of carotenoid and whether it was present in the light-harvesting complex or in organic solvent. The S* state decayed into the triplet state when the carotenoid was bound to the LH1 or LH2 complex. However, when the carotenoid was free in organic solvent the S* state decayed to the ground state without generating the triplet state. Applying a pump–dump and transient absorption technique for β -carotene, lycopene, and zeaxanthin, Wohlleben *et al.* re-examined the origin of the S* state with the carotenoid free in solution (S*_{sol}) [99]. They suggested that the S*_{sol} state is a vibrationally excited level of the electronic ground state (S*_{sol} = hot S₀), which is populated by a combination of impulsive Raman scattering of the pump pulse and S₁ → S₀ internal conversion. They also found the S* state of the protein-bound carotenoid and re-designated it as S*_T. These ideas have recently been supported by Hashimoto *et al.* for spirilloxanthin both free in solution and bound to light-harvesting complexes [100]. However, the debate on the characterization of the S* state is still on-going. Beck *et al.* [101] re-interpreted the radiationless decay of carotenoids after photoexcitation up to the S₂ state by referring to a model derived from studies of polymethine cyanines [102]. They suggested that the S* state can be assigned to a low lying S₁ state structure with intramolecular charge transfer character and a pyramidal conformation (see **Figure 5**). On the other hand, quite recently, the group of Hauer *et al.* have challenged this idea and presented a comprehensive and unified interpretation of S*-related features [103]. They explained the features by vibronic transitions either from S₁, from vibrationally excited levels on S₀, or from both, depending on the chain length of the carotenoid investigated (see **Figure 6**). These discrepancies in the interpretation of the observed spectral data clearly demonstrates that while it is easy to measure spectral changes it can be difficult to assign them. The problem is trying to determine from an absorption change whether it reflects different electronic states or different vibrational states. Involvement of vibrationally excited states in the relaxation process of carotenoids after photoexcitation was initially detected by time-resolved absorption spectroscopy [70, 104, 105], and has also been studied by time-resolved stimulated Raman spectroscopy [106-110].

Carotenoids that contain carbonyl groups have the possibility of forming intramolecular charge-transfer states (S_{ICT}). These states have been well documented in the case of carotenoids such as peridinin and fucoxanthin (see **Figure 7** for chemical

structures of these molecules) [106, 107, 111-118]. The importance of this charge transfer state seems to be that it allows carotenoid to chlorophyll energy transfer to be highly efficient. Readers who are interested in more details about this state should consult the excellent review by Polívka and Sundström [28]. In our recent study, it was demonstrated that a large part of excitation energy captured by fucoxanthin bound to FCP (“Mozuku FCP”) is transferred to Chl *a* *via* the coupled S₁/ICT state, resulting from a strong electronic dipole interaction between fucoxanthin and Chl *a* [116]. This strong dipole interaction was attributed to the ICT character of the excited state of fucoxanthin, enabling it to enhance the transition dipole moment of the S₁/ICT state. Indeed, the enhancement of the excitation energy-transfer efficiency from carotenoid to bacteriochlorophyll is demonstrated by incorporating fucoxanthin into the LH1 complexes from a purple photosynthetic bacterium [119]. However, the nature and origin of the S₁/ICT state of carbonyl carotenoids is yet to be fully understood. In order to try to get more information on this point the ultrafast excited state dynamics of fucoxanthin and its homologues have been investigated [114].

The spectroscopic properties of fucoxanthin in polar (methanol) and nonpolar (cyclohexane) solvents were studied [114]. Transient absorption associated with the optically forbidden S₁ (2¹A_g⁻) and/or the ICT states were observed following one-photon excitation to the optically allowed S₂ (1¹B_u⁺) state in methanol. The transient absorption measurements carried out in methanol showed that the ratio of the ICT-to-S₁ state formation increased with decreasing excitation energy. The ICT character was clearly visible in the steady-state absorption in methanol based on a Franck–Condon analysis. The results suggest that two spectroscopic forms of fucoxanthin, blue and red, exist in a polar environment. The spectroscopic properties of fucoxanthin in methanol were further studied by femtosecond pump-probe measurements in the near-infrared region, where transient absorption associated with the optically allowed S₂ (1¹B_u⁺) state and stimulated emission from the strongly coupled S₁/ICT state were observed following one-photon excitation to the S₂ state [117]. The results showed that the amplitude of the stimulated emission from the S₁/ICT state increased with decreasing excitation energy, demonstrating that the red-form of fucoxanthin exhibits a stronger ICT character. The magnitude of ICT character of carbonyl carotenoids has often been evaluated from (1) solvent polarity-dependent S₁/ICT lifetimes, (2) amplitudes of the ICT transient absorption and stimulated emission bands, and (3) the dipole moment of the ICT state.

Femtosecond pump-probe spectroscopic measurements were performed on fucoxanthin homologs with varying numbers of conjugated double bonds ($n = 4 \sim 8$) [118, 120]. The ICT properties of fucoxanthin homologs were characterized by the S_1 /ICT lifetimes and the transient absorption and stimulated emission bands due to the S_1 /ICT state.

Figure 7 shows a schematic illustration of the relative energies of the carotenoid excited singlet states discussed above together with the proposed relaxation pathways from the S_2 state as well as the energy-transfer pathways between carotenoid and bacteriochlorophyll. Since the relaxation from the S_2 state is very fast, ultrafast vibrational spectroscopies are going to be important to try to clarify further the structure–function relationship of the above singlet excited-states [34].

5. Time-resolved and coherent spectroscopies beyond 100 fs time-resolution

Recently it has become possible to use much shorter femtosecond pulses. When this was done by Cerullo *et al.* the data claimed the presence of an intermediate state between S_2 and S_1 [121]. This state was formed as S_2 decayed and gave rise to S_1 as it decayed. However, with these extremely fast reactions it was not possible to be sure that this intermediate state was another pure excited singlet state such as $1^1B_u^-$. Therefore, this dark state was tentatively designated as S_X . Since this time there have been many studies that have suggested that such an intermediate state is required to fully explain the experimental data. It has also been suggested that these results could be due the appearance of a nonlinear optical effect [122]. There have even been further studies that have not seen or required the presence of such extra intermediate state to fit the data [29]. This has led to a lot of confusion. Most recently broadband two-dimensional (2D) electronic spectroscopic measurements on light-harvesting proteins from purple bacteria and isolated carotenoids have been performed in order to characterize in more detail the excited-state manifold of carotenoids that channel energy to bacteriochlorophyll molecules. The data revealed a well-resolved signal (cross peak) consistent with a previously postulated carotenoid dark state, the presence of which was confirmed by global kinetic analysis. The most recent results therefore suggest that a carotenoid dark state does have a role in mediating energy flow from carotenoid to bacteriochlorophyll [123], and this state was designated as X. All these findings clearly suggest the presence

of a dark state in-between S_2 and S_1 . However, it is worth pointing out that bit is worth looking back at some older up-conversion experiments by the group Gillbro *et al.* [56]. Carotenoids in solution shows the decay time 150 fs for S_2 emission. If S_2 in few fs goes to another state, then one has to assume that the other intermediate state emits. If that state emits why doesn't it absorb? This old emission study should not be forgotten and strongly implies that there is more to be understood here.

Another sophisticated ultrafast spectroscopic technique called four-wave mixing (FWM) or coherent spectroscopy has been employed in order to investigate the ultrafast photophysics of carotenoids. FWM measurement is performed with an optical configuration illustrated in **Figure 8(a)**. Namely, laser light is split to three using a beam splitter and two of them are independently guided toward translational stages in order to induce time delays among three laser pulses. If these three laser pulses are well focused onto a single spot in the sample, FWM signals can be observed. This type of optical configuration, where three laser pulses excite the sample from three distinct directions, is called the BOXCARS configuration.

When such three laser pulses simultaneously reach to the sample (zero time delays), FWM signals are generated in the area surrounding the transmitted excitation laser light. As illustrated in **Figure 8(b)**, if the wave-vectors of excitation and signal lights are defined, respectively, as k_i ($i = 1, 2, 3$) and k_S , FWM signals appear at the direction that satisfies the relation of $k_S = \pm k_1 \pm k_2 \pm k_3$ and $k_S = 2k_i - k_j$ ($j = 1, 2, 3$ and $j \neq i$). It should be noted here that FWM signals are strong enough for carotenoids to be seen with the naked eye. This means that carotenoids are suitable molecules for investigation of their nonlinear optical responses. Information concerning the coherence can be obtained by investigating the time evolution of the FWM signals.

FWM signals in carotenoids have been reported for β -carotene and its homologues, lycopene, astaxanthin and spheroidene [124-136]. As an example, the results with β -carotene are given here. **Figure 9(a)** shows the time evolution of a FWM signal with β -carotene. In this example, the abscissa gives the time interval T between pulse 2 and pulse 3 (see **Figure 8(c)**). The time interval τ between pulse 1 and pulse 2 was set to be zero. The FWM signal that is measured under this condition is frequently called the transient grating (TG) signal. The intense signal that appears around the time origin in **Figure 9(a)** is assigned as a coherent spike. Following this spike signal, a coherent vibration signal with the very fast period of oscillation of about 20 – 30 fs can be

observed on top of the slowly decaying background that has a lifetime about 5 ps.

The origin of this coherent vibration can be clarified if the TG signal in **Figure 9(b)** is Fourier transformed. It can be readily understood, based on the comparison with the Raman spectrum of β -carotene shown in **Figure 10(b)**, that the peaks obtained by the Fourier transformation of the coherent vibration (**Figure 10(a)**) show good coincidence with those of the ground state Raman spectrum of β -carotene. Namely, the peaks that appeared at $\nu_1 = 1522 \text{ cm}^{-1}$ and $\nu_2 = 1157 \text{ cm}^{-1}$, respectively, are attributed to the totally symmetric vibration of C=C and C-C stretchings and the peak that appeared at $\nu_3 = 1007 \text{ cm}^{-1}$ is attributed to the in-plane rocking vibration of methyl groups. These vibrational modes appeared because all the β -carotene molecules under inspection start to vibrate in phase, *i.e.* coherently, following the impulsive excitation with ultrashort laser pulses. The stretching vibrations of carotenoids usually appear in the 1000 – 1500 cm^{-1} frequency domain. These frequencies correspond to 30 – 20 fs in the time domain, if the frequency to time domain conversion is performed. Therefore, coherent vibration can be induced in carotenoids, if we use the sub-20 fs ultrashort laser pulses for the FWM experiment.

The most important information that is obtained by the measurement of FWM signals is the coupling between carotenoids and their surrounding environment. This information is reflected in spectral density (see **Figure 10(c)**). It is known that there are couplings with slow vibrations of 100 fs ($\sim 300 \text{ cm}^{-1}$) or less in organic solvents [126, 130, 132]. Obtaining the spectral density, various optical responses including absorption and fluorescence spectra can be calculated and, therefore, the precise discussion on the experimental data based on theoretical models becomes feasible [137]. One of those examples, **Figure 9(c)**, shows the results of the theoretical calculation for the FWM signal. The experimental result is nicely in agreement with the theoretical calculation. As shown here spectral density includes meaningful information, however, there are few reports on carotenoids bound to pigment-protein complexes [138]. On the other hand, many studies have already been performed on the coherent vibrations that directly reflect the effect of coupling with surrounding environment in bacteriochlorophyll [139-146]. Revealing the correlation among these coherent vibrations as well as the role of coherent vibration in excitation energy transfer will be rewarding challenges for the future.

Coherent vibrations are also observed in the electronic excited state of carotenoids.

The group of Motzkus *et al.* was able to observe the coherent vibration in the S_1 state of β -carotene with 20 fs temporal and 10 cm^{-1} spectral resolutions [125, 127, 129, 147-152]. They introduced a pre-pump pulse that excites β -carotene to its S_2 state for FWM measurement (pump-FWM) to produce a populated S_1 state *via* internal conversion from the S_2 state. They concluded that the coherence of the molecular vibrations are not conserved during the process of $S_2 \rightarrow S_1$ internal conversion [125]. They also claimed that the lifetime of coherent vibration in the S_1 state is an order of magnitude smaller than that in the ground state for all the vibrational modes. Quite recently, they further extended their study to a series of open chain carotenoids with different numbers of conjugated double bonds $n = 9, 10, 11$ and 13 (neurosporene, spheroidene, lycopene and spirilloxanthin, respectively) and a closed chain carotenoid (lutein) [153]. They have interpreted their data on the relaxation from S_2 to S_1 based on the model including the forbidden singlet states ($3^1A_g^-$ and $1^1B_u^-$). They were successful to detect the frequency shift of the C=C stretching mode along the course of deactivation. They suggested that the vibrational dynamics directly after the initial excitation of carotenoids is dominated by two different vibronic couplings: (1) Diabatic mixing between 1^1B_u states takes place only for shorter open-chain carotenoids ($n = 9$ and 10), where the vibrational levels of the $1^1B_u^+$ and $1^1B_u^-$ states are energetically close (see **Figure 11**). The interaction between these states leads to a typical frequency down-shift after the deactivation of the Franck–Condon region. (2) Adiabatic vibronic coupling between the 1^1A_g states is a well-known general feature of the Raman spectra of carotenoids (see **Section 2**), which is responsible for the generation of the typical S_1 C=C stretching frequency at 1800 cm^{-1} . Nevertheless, their results suggest that it does not lead to any modification of the vibrational dynamics during $1^1B_u^+$ deactivation, since adiabatic vibronic coupling does not take place between $1^1B_u^+$ and $2^1A_g^-$ states. The evolution of the S_1 C=C stretching frequency at 1800 cm^{-1} as well as of other modes for carotenoids without diabatic mixing follows a frequency upshift due to potential anharmonicity (see **Figure 11**). This is quite a new interpretation that warrants further study both experimental and theoretical sides.

6. Conclusion

The properties of the singlet excited-state of carotenoids, that have the major role in photosynthetic light-harvesting, were reviewed extensively. The underlying photophysics to understand the forbidden singlet excited states is based on the historical work by Tavan and Schulten [43, 44] that assumes both the planarity and C_{2h} point symmetry of the polyene backbone of the carotenoids. Quite recently, Fiedor *et al.* have raised an objection to this idea [154]. This is because structures of many naturally occurring carotenoids are asymmetric due to the side groups that are coupled with the polyene backbone as has been described in the textbook by Hudson, Kohler, and Schulten [35]. They proposed that the reason for inactivity of the $S_0 \rightarrow S_1$ transition of carotenoids is not due to the symmetry but it is due to a severe molecular deformation in the S_1 state, which cannot be accessed by one-photon excitation from the ground state. This is quite an interesting new idea but a more sophisticated experimental and computational effort is needed to better understand this issue and to see what the correct interpretation really is.

Carotenoids are indeed fascinating molecules. They have remarkable photophysical and photochemical properties [155]. Though a lot of detail is known about the properties of the singlet-excited states of carotenoids, there still a lot more to be unraveled. Now is a exciting time to be involved in carotenoids research. It has been particularly notable how as physical methods have evolved and have been applied to studying carotenoids how the knowledge of the way in which carotenoids function in photosynthesis has advanced. We expect this trend to continue. One can highlight areas where we expect these developments to really help the understanding of the molecular mechanisms by which carotenoids discharge their photosynthetic functions. Examples are the further application of advanced 2D coherent time-resolved spectroscopies [156, 157] and time-resolved stimulated Raman spectroscopy [158-161]. These methods should be able to help resolve the ongoing problems of understanding the pattern of carotenoid excited singlet states and their involvement in light-harvesting. They should be able to resolve the key issues of which absorption changes reflect discrete electronic states and which come from different vibrational ones. Sorting this out will hopefully remove many of the current controversies.

Data accessibility

This article has no additional data.

Author's contribution

H.H. and R.J.C. conceived the themes of this manuscript. H.H., C.U., N.Y., A.T.G. and H.H. drafted the manuscript. All authors gave final approval for publication.

Competing interests

We declare we have no competing interests.

Funding

HH thanks JSPS KAKENHI, Grant-in-Aids for Basic Research (B) (No. 16H04181) and Scientific Research on Innovative Areas “Innovations for Light-Energy Conversion (I⁴LEC)” (No. 17H06433 & No. 17H0637) for financial support. RJC and ATG wish to gratefully thank the BBSRC and Photosynthetic Antenna Research Center (PARC), an Energy Frontier Research Center funded by the DOE, Office of Science, Office of Basic Energy Sciences under Award Number DE-SC 0001035 for financial support.

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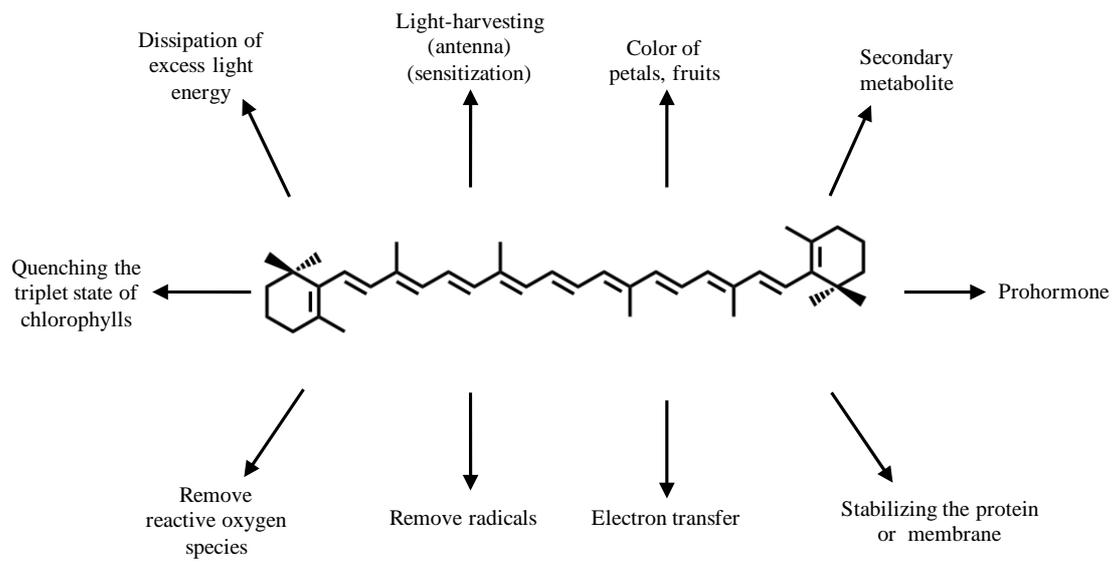


Figure 1. A schematic illustration that summarizes the functions of carotenoids in physiological systems. Chemical structure of β -carotene is shown as a representative of carotenoids.

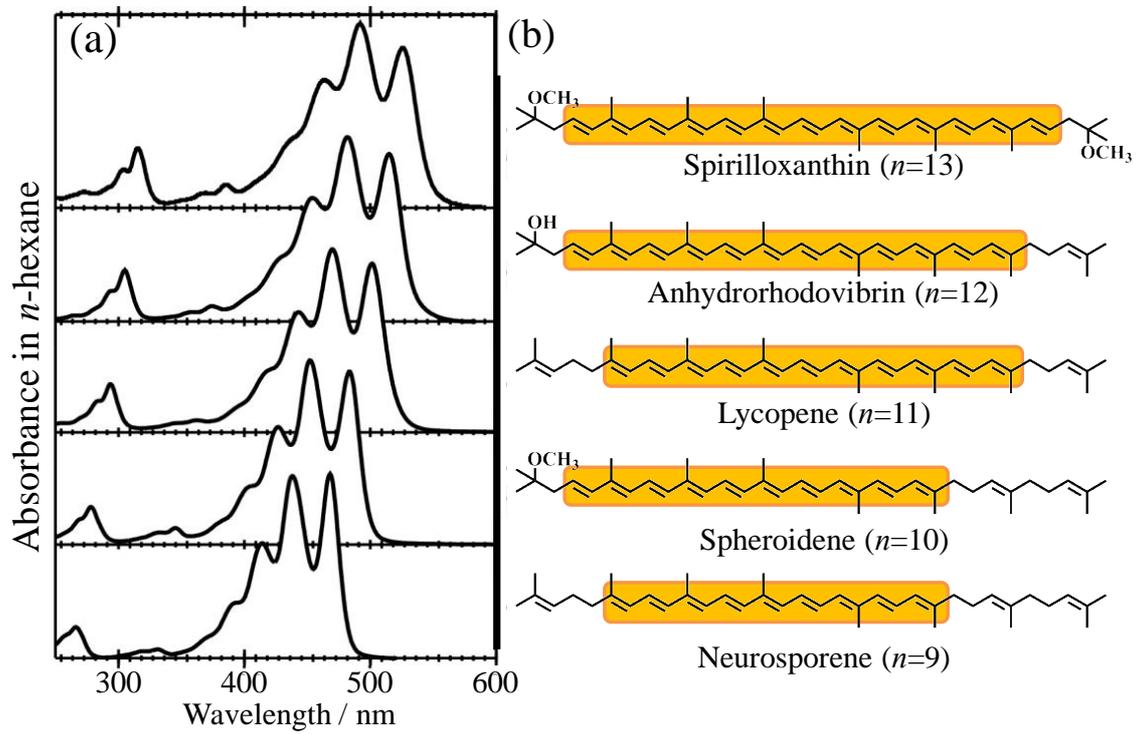


Figure 2. (a) Absorption spectra in *n*-hexane solutions and (b) chemical structures of carotenoids bound to purple photosynthetic bacteria. Conjugated polyene backbones were highlighted with orange rectangles in (b).

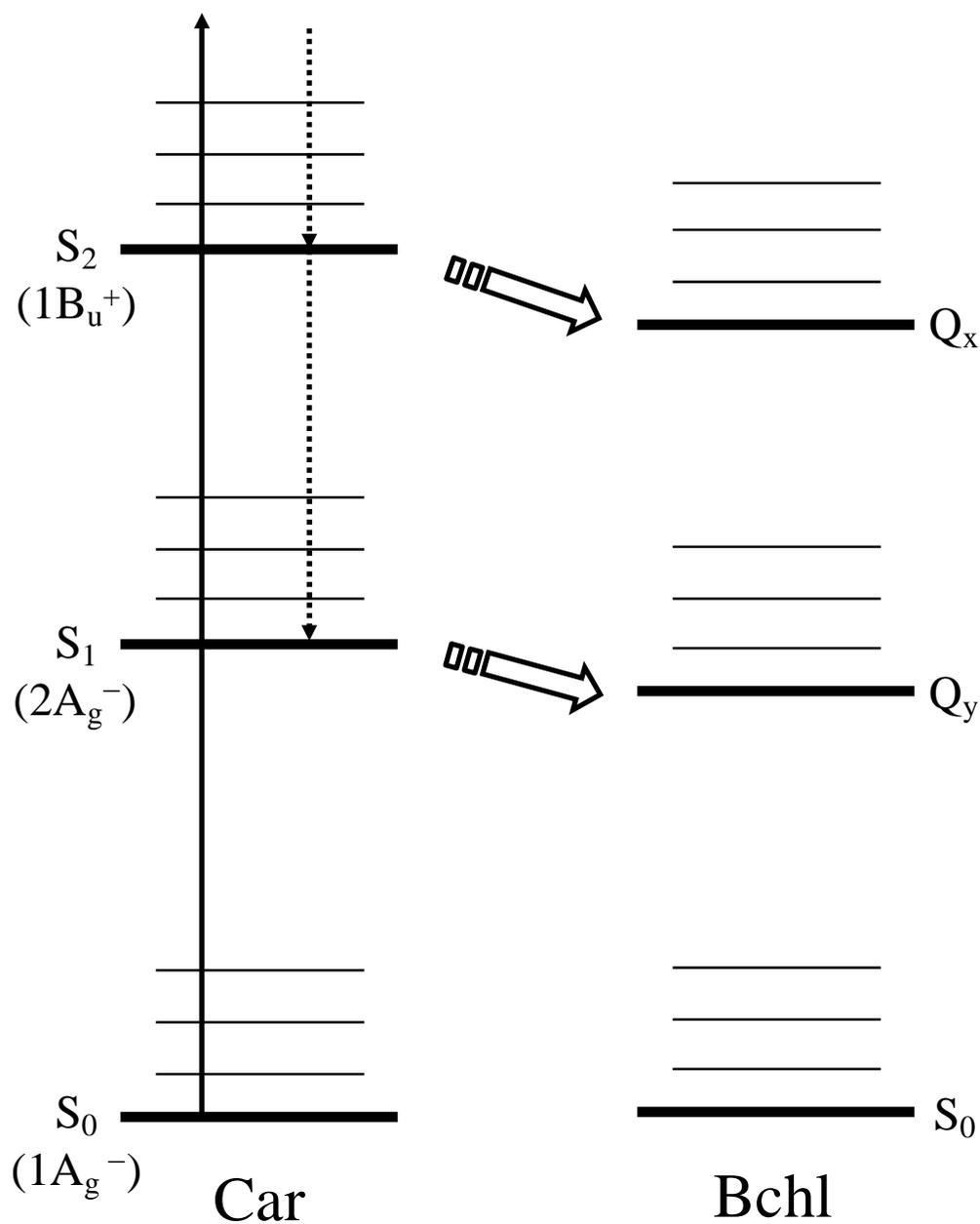


Figure 3. Relative energy levels of the S_1 and S_2 excited singlet states of a typical carotenoid relative to bacteriochlorophyll (Bchl) a Q_x and Q_y transitions. These energy levels are valid for carotenoids containing up to 10 conjugated double bonds. Thick lines show the electronic states and thin lines show the vibrational states. When carotenoid molecule is excited up to the S_2 state (solid arrow), it shows vibrational relaxation and/or internal conversion to the S_1 state (broken arrows). This process competes to the energy-transfer (box arrows) to Bchl a .

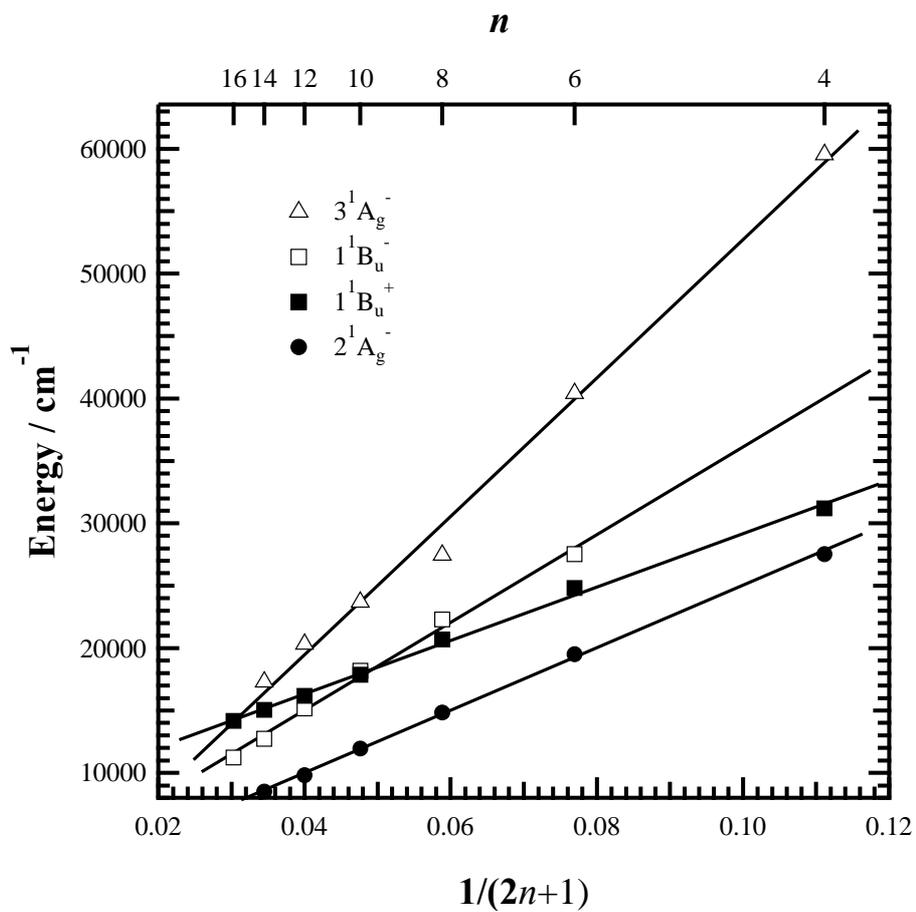


Figure 4. Energy diagram calculated by Tavan and Schulten using PPPMRD-CI method [43,44] for the low-lying singlet excited state of polyenes ($n = 4-16$).

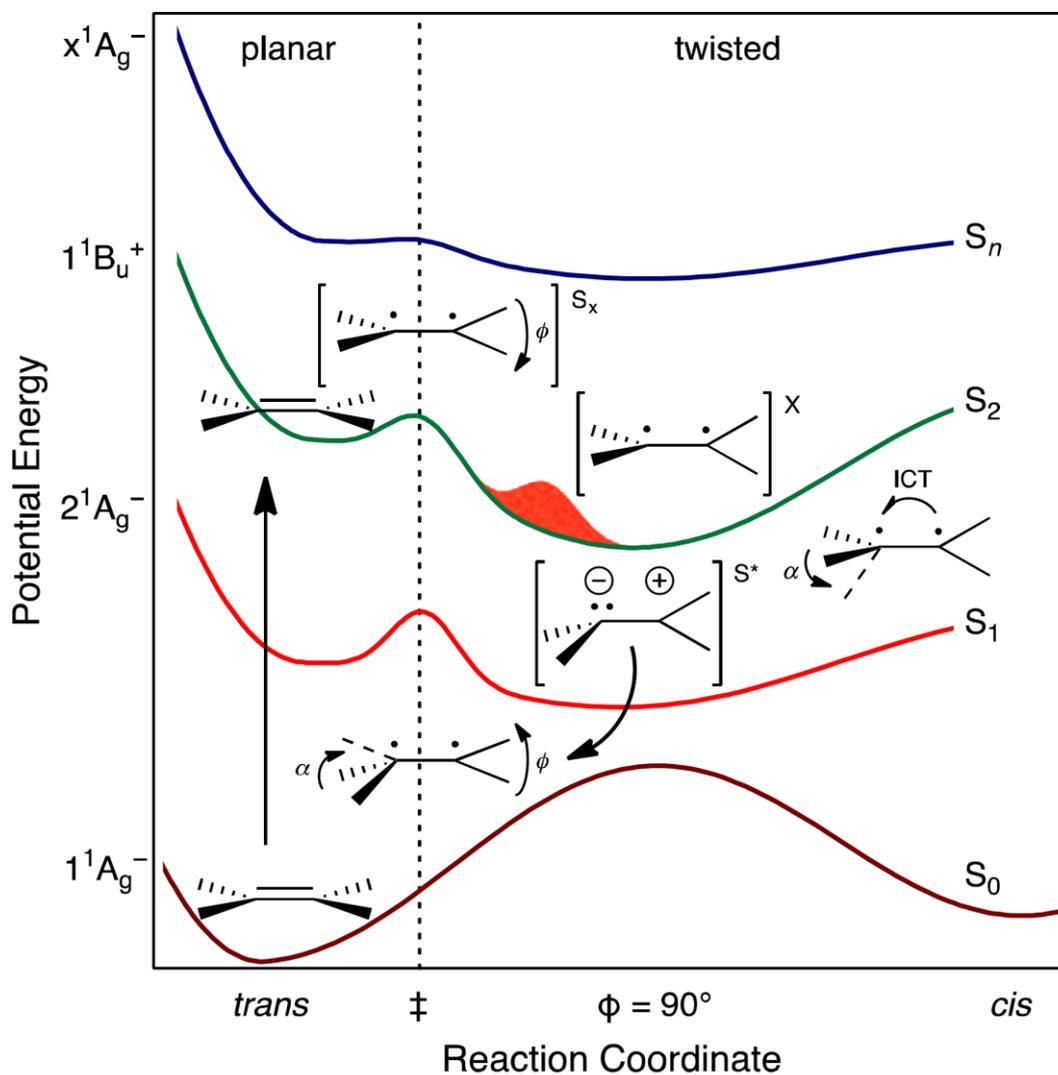


Figure 5. Proposed scheme for radiationless decay of carotenoids after optical preparation of the S_2 state. The states that apply to planar structures are indicated by symmetry labels. Key points along the path back to the initial planar ground state conformation are labeled with ethylenic structures, depicting the S_x , X , and S^* dark states as twisted structures near the S_2 transition state and the S_2 twisted minimum and pyramidal structures near the S_1 state minimum, respectively. (Transferred with permission from Ref. [101].)

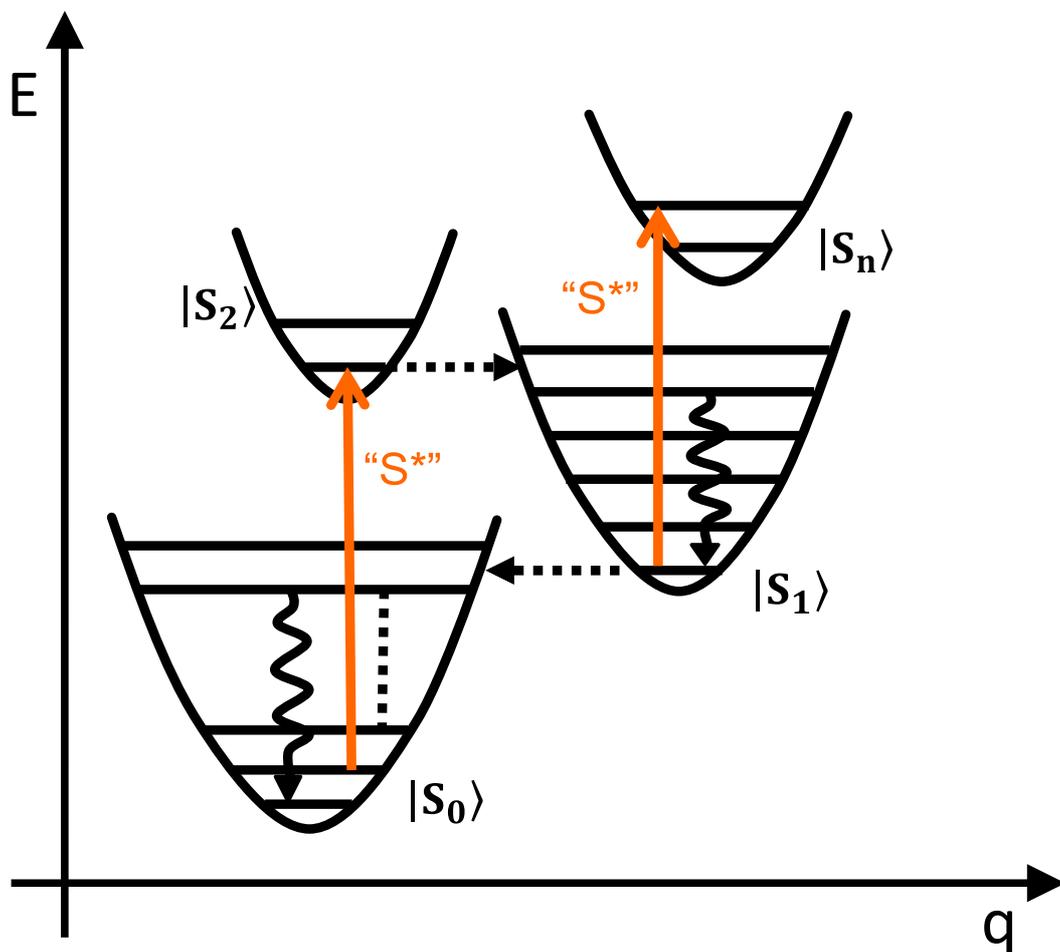


Figure 6. Energy level scheme describing electronic (dashed horizontal arrows) and vibrational (wavy vertical arrows) energy relaxation in carotenoids along a reaction coordinate. Colored vertical arrows indicate allowed electronic transitions. Electronic states are labeled in ket-notation. (Transferred with modification from Ref. [103] with permission.)

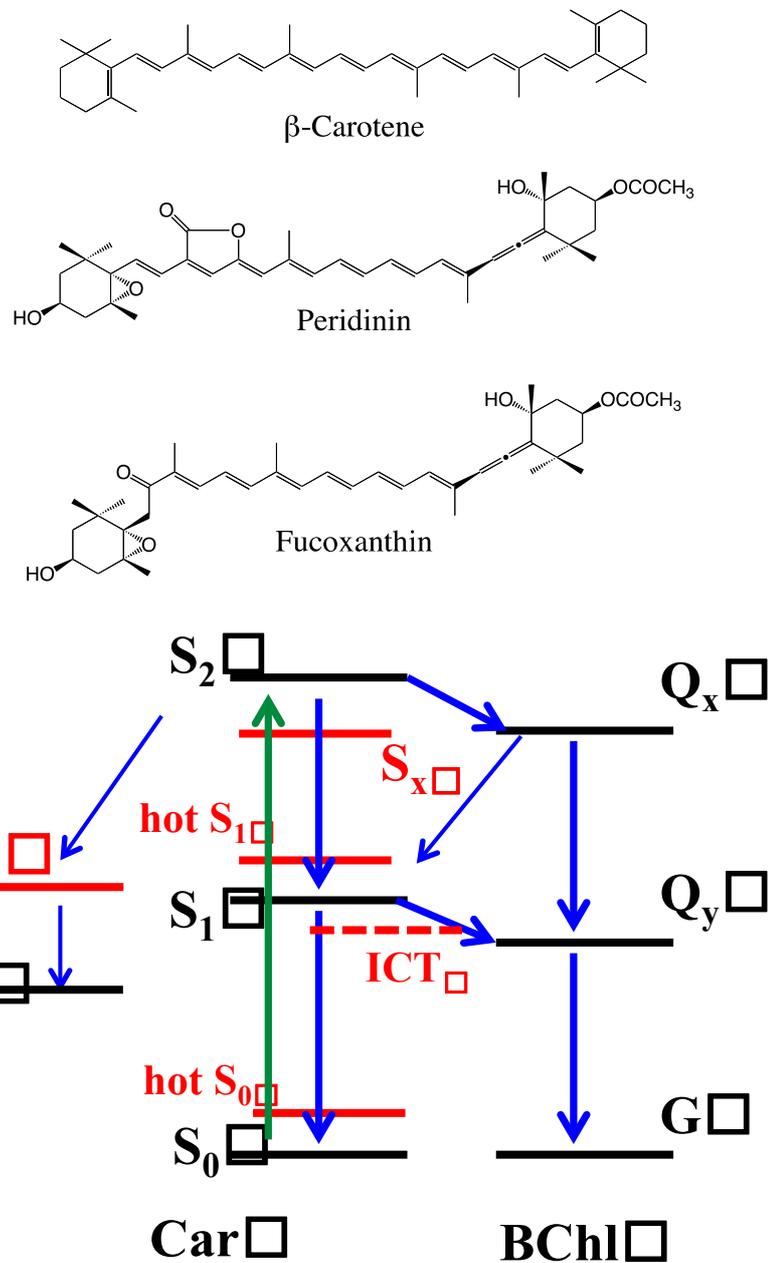


Figure 7. Chemical structures of β -carotene, peridinin, and fucoxanthin, and a schematic description of energy diagrams together with relaxation and energy-transfer pathways of carotenoids following photoexcitation up to the S_2 state.

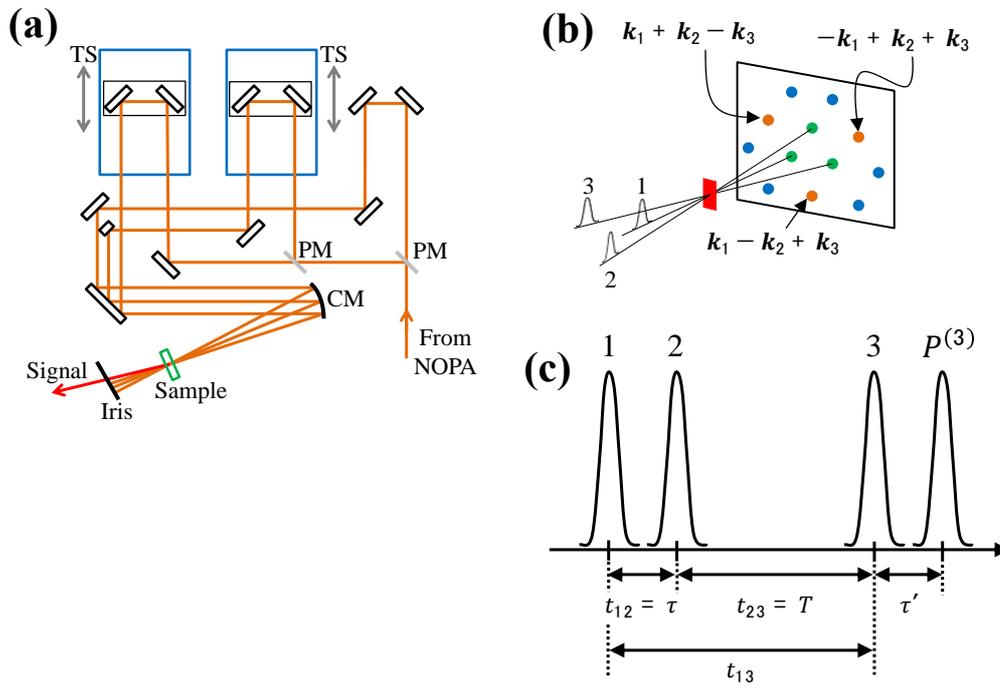


Figure 8. (a) Optical configuration of the interferometer used for the measurements of four-wave mixing (FWM) signals. The light from a non-collinearly phase-matched optical parametric amplifier (NOPA) is split into three using two pellicle mirrors (PM). Three laser pulses thus produced are then focused to excite the sample using a collimating mirror (CM). Time intervals between each of these three laser pulses were controlled using two translational stages (TS). The FWM signals are generated along the directions that satisfy the phase-match condition. One of these signals is selected using an iris diaphragm. (b) When three laser pulses are irradiated to the sample, the FWM signals can be generated along the directions that satisfy the phase-match conditions. (c) The relation between coherent time τ and population time T . t_{12} (t_{13}) is the time interval between pulses 1 and 2 (1 and 3) when they arrive at the sample.

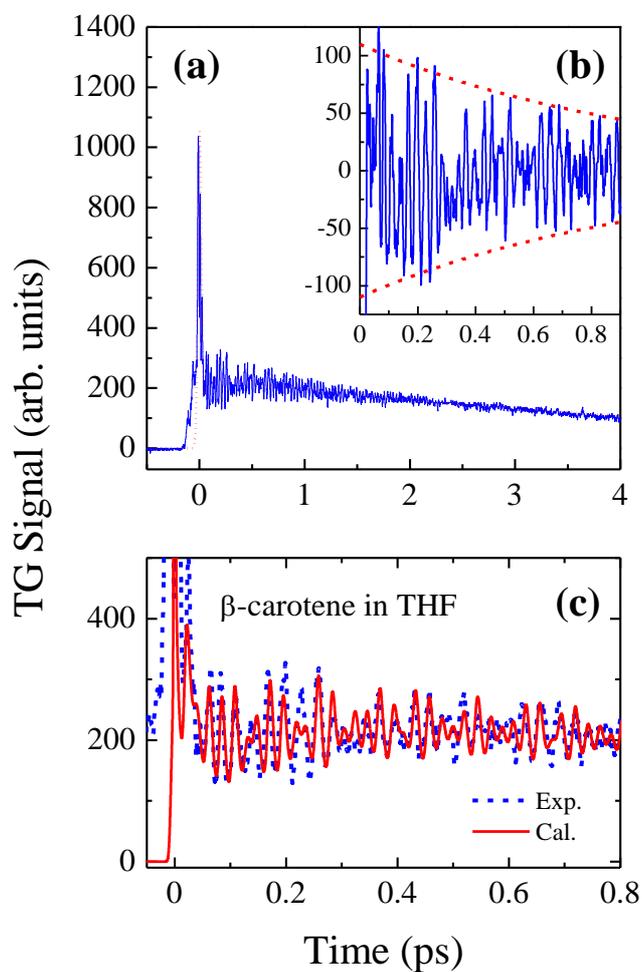


Figure 9. (a) Experimentally observed time-evolution of the transient-grating (TG) signal. A slowly varying background shown with dotted lines reflects the lifetime of the electronic excited states. If the background is subtracted from the original TG signal, coherent vibration component can be extracted as shown in (b). The decay time of coherent vibration is determined to be around 1 ps. (c) Comparison of the experimentally observed TG signal (broken line) and the result of theoretical calculation (solid line).

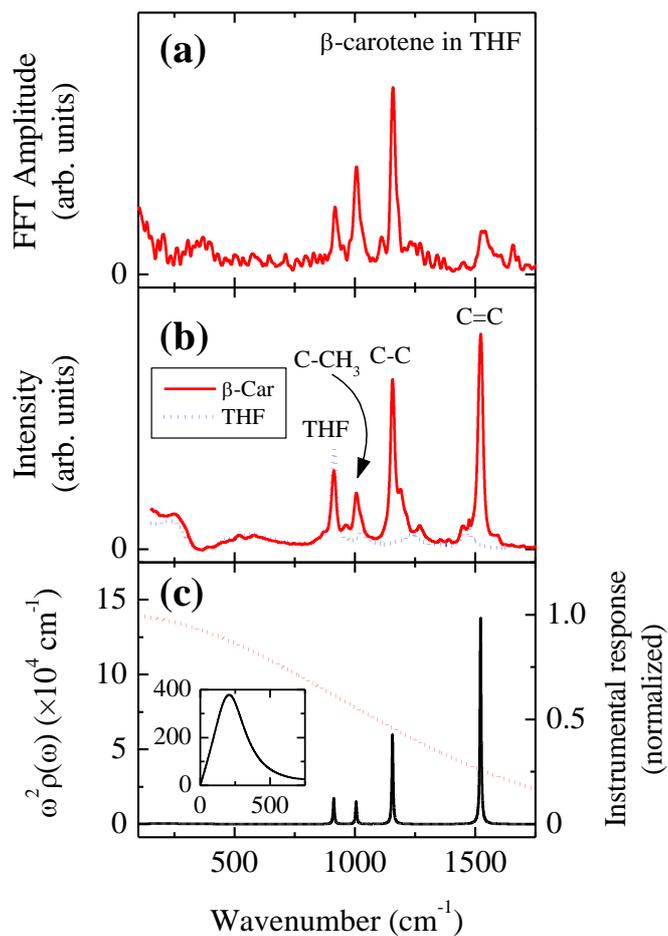


Figure 10. (a) Fourier transformed spectrum of the coherent signal component shown in Figure 9(b). (b) Raman spectra of β -carotene and solvent THF (tetrahydrofuran). (c) Spectral density (solid line) and the response function of the detecting system (dotted line). The inset shows the spectral density that reflects the system-bath (β -carotene and solvent THF) interaction in the low-frequency regime.

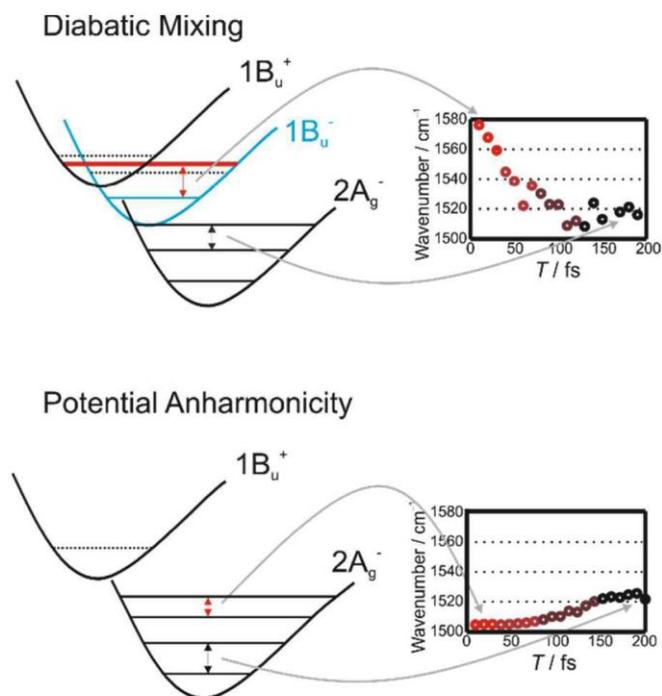


Figure 11. Scheme of vibrational frequencies shifts within the initial 200fs dynamics. Diabatic mixing between $1B_u^+$ and $1B_u^-$ leads to a frequency down-shift during the evolution from the $1B_u^+/1B_u^-$ mixed potential to the $2A_g^-$ potential. The red line in the $1B_u^+/1B_u^-$ mixed potential depicts the shifted vibrational level due to the diabatic mixing. Frequency up-shifts due to the $2A_g^-$ potential anharmonicity can be observed in the initial dynamics when diabatic mixing does not play a role. The intermediate states in the scheme for describing potential anharmonicity were omitted for clarity. (Transferred from Ref. [153] with permission).