

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))

This is the author's final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/161092/>

Deposited on: 04 Jun 2018

## Understanding/Unraveling Carotenoid Excited Singlet States

Hideki Hashimoto <sup>1,\*</sup>, Chiasa Uragami <sup>1</sup>, Nao Yukihiro <sup>1</sup>, Alastair T. Gardiner <sup>2</sup>, and Richard J. Cogdell <sup>2</sup>

<sup>1</sup> *Department of Applied Chemistry for Environment, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan*

<sup>2</sup> *Institute of Molecular, Cell and Systems Biology, College of Medical Veterinary and Life Sciences, University of Glasgow, Avenue G12 8QQ, United Kingdom*

\* Corresponding author: e-mail, [hideki-hassy@kwansei.ac.jp](mailto:hideki-hassy@kwansei.ac.jp)

### 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  $\beta$ -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  $\alpha,\omega$ -diphenyl-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  $1750\text{ cm}^{-1}$ . 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 S<sub>2</sub> 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 S<sub>2</sub> state. The decay rate of the S<sub>1</sub> state of rhodopin glucoside was the same in the LH2 complex as in organic solvent. In this case the S<sub>1</sub> 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 S<sub>1</sub> 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 S<sub>2</sub> and S<sub>1</sub> 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 S<sub>1</sub> and S<sub>2</sub> 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 S<sub>2</sub> and S<sub>1</sub> states relative to those of the Q<sub>x</sub> and Q<sub>y</sub> states of (bacterio)chlorophyll are also important. The energy of the S<sub>2</sub> state can be determined easily by ordinary absorption measurements because the transition from the ground (S<sub>0</sub>) to S<sub>2</sub> states is optically allowed. On the contrary, the determination of the energy of the S<sub>1</sub> 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 S<sub>1</sub> state are in the order of 10<sup>-5</sup>, the 0-0 origin of the S<sub>1</sub> 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  $\beta$ -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*  $\alpha,\omega$ -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  $\beta$ -carotene, lycopene, and zeaxanthin, Wohlleben *et al.* re-examined the origin of the  $S^*$  state with the carotenoid free in solution ( $S^*_{\text{sol}}$ ) [99]. They suggested that the  $S^*_{\text{sol}}$  state is a vibrationally excited level of the electronic ground state ( $S^*_{\text{sol}} = \text{hot } S_0$ ), which is populated by a combination of impulsive Raman scattering of the pump pulse and  $S_1 \rightarrow S_0$  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  $S_2$  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  $S_1$  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  $S_1$ , from vibrationally excited levels on  $S_0$ , 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 ( $S_{\text{ICT}}$ ). 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  $S_1$ /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  $S_1$ /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  $S_1$ /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  $S_1$  ( $2^1A_g^-$ ) and/or the ICT states were observed following one-photon excitation to the optically allowed  $S_2$  ( $1^1B_u^+$ ) state in methanol. The transient absorption measurements carried out in methanol showed that the ratio of the ICT-to- $S_1$  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  $S_2$  ( $1^1B_u^+$ ) state and stimulated emission from the strongly coupled  $S_1$ /ICT state were observed following one-photon excitation to the  $S_2$  state [117]. The results showed that the amplitude of the stimulated emission from the  $S_1$ /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  $S_1$ /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  $\beta$ -carotene and its homologues, lycopene, astaxanthin and spheroidene [124-136]. As an example, the results with  $\beta$ -carotene are given here. **Figure 9(a)** shows the time evolution of a FWM signal with  $\beta$ -carotene. In this example, the abscissa gives the time interval  $T$  between pulse 2 and pulse 3 (see **Figure 8(c)**). The time interval  $\tau$  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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\text{cm}^{-1}$  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  $\beta$ -carotene with 20 fs temporal and  $10\text{ cm}^{-1}$  spectral resolutions [125, 127, 129, 147-152]. They introduced a pre-pump pulse that excites  $\beta$ -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  $1800\text{ cm}^{-1}$ . 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  $1800\text{ cm}^{-1}$  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 (I<sup>4</sup>LEC)'" (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.

### **References**

- [1] Pfander H, Gerspacher M, Rychener M, Schwabe R. 1987 *Key to carotenoids: 2nd and enlarged edition*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.
- [2] Britton G, Liaaen-Jensen S, Pfander H. 2004 *Carotenoids: Handbook*, Basel,

Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[3] Britton G, Liaaen-Jensen S, Pfander H. 1995 *Carotenoids vol.1a: Isolation and analysis*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[4] Britton G, Liaaen-Jensen S, Pfander H. 1995 *Carotenoids vol.1b: Spectroscopy*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[5] Britton G, Liaaen-Jensen S, Pfander H. 1996 *Carotenoids vol.2: Synthesis*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[6] Britton G, Liaaen-Jensen S, Pfander H. 1998 *Carotenoids vol.3: Biosynthesis and metabolism*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[7] Britton G, Liaaen-Jensen S, Pfander H. 2008 *Carotenoids vol.4: Natural functions*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[8] Britton G, Liaaen-Jensen S, Pfander H. 2009 *Carotenoids vol.5: Nutrition and health*, Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[9] Hashimoto H, Uragami C, Cogdell RJ. 2016 Carotenoids and photosynthesis. *Subcell. Biochem.* **79**, 111-139. (doi: 10.1007/978-3-319-39126-7\_4)

[10] Schalch W. 1992 Carotenoids in the retina — a review of their possible role in preventing or limiting damage caused by light and oxygen. In *Free radicals and aging* (eds I Emerit, B Chance), pp. 280-298. Basel, Switzerland, Boston, USA, Berlin, Germany: Birkhäuser Verlag.

[11] von Lintig J, Kiser PD, Golczak M, Palczewski K. 2010 The biochemical and structural basis for *trans*-to-*cis* isomerization of retinoids in the chemistry of vision. *Trends. Biochem. Sci.* **35**, 400-410. (doi: 10.1016/j.tibs.2010.01.005)

[12] von Lintig J. 2012 Provitamin A metabolism and functions in mammalian biology. *Am. J. Clin. Nutr.* **96**, 1234S-1244S. (doi: 10.3945/ajcn.112.034629)

[13] Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. 1996 Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **384**, 240-242. (doi: 10.1016/0014-5793(96)00323-7)

[14] Edge R, McGarvey DJ, Truscott TG. 1997 The carotenoids as anti-oxidants — a review. *J. Photochem. Photobiol. B: Biology* **41**, 189-200. (doi: 10.1016/s1011-1344(97)00092-4)

[15] Kurihara H, Koda H, Asami S, Kiso Y, Tanaka T. 2002 Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress. *Life Sci.* **70**, 2509-2520. (doi:

10.1016/s0024-3205(02)01522-9)

[16] Fiedor J, Burda K. 2014 Potential role of carotenoids as antioxidants in human health and disease. *Nutrients* **6**, 466-488. (doi: 10.3390/nu6020466)

[17] Krinsky NI, Mayane ST, Sies H. 2004 *Carotenoids in health and disease*, New York, USA: Marcel Dekker.

[18] Cooper DA. 2004 Carotenoids in health and disease: Recent scientific evaluations, research recommendations and the consumer. *J. Nutr.* **134**, 221S-224S.

[19] Lee J, Koo N, Min DB. 2004 Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comp. Rev. Food Sci. Food Safety* **3**, 21-33. (doi: 10.1111/j.1541-4337.2004.tb00058.x)

[20] Bendich A. 1989 Carotenoids and the immune system. In *Carotenoids: Chemistry and biology* (eds NI Krinsky, MM Mathews-Roth, RF Taylor), pp. 323-335. Boston, USA: Spriger US.

[21] Navara KJ. 2003 Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behav. Ecol.* **14**, 909-916. (doi: 10.1093/beheco/arg085)

[22] Mathews-Roth MM. 1982 Antitumor activity of  $\beta$ -carotene, canthaxanthin and phytoene. *Oncology* **39**, 33-37. (doi: 10.1159/000225601)

[23] Jyonouchi H, Sun S, Iijima K, Gross MD. 2000 Antitumor activity of astaxanthin and its mode of action. *Nutr. Cancer* **36**, 59-65. (doi: 10.1207/S15327914NC3601\_9)

[24] Soltani F, Ramezani M, Amel Farzad S, Mokhtarzadeh A, Hashemi M. 2017 Comparison study of the effect of alkyl-modified and unmodified PAMAM and PPI dendrimers on solubility and antitumor activity of crocetin. *Artif. Cells Nanomed. Biotechnol.* **45**, 1356-1362. (doi: 10.1080/21691401.2016.1236805)

[25] Hashimoto H, Uragami C. 2015 Artificial photosynthesis producing solar fuels: Natural tactics of photosynthesis. In *From molecules to materials: Pathways to artificial photosynthesis* (eds EA Rozhkova, K Ariga), pp. 57-70. Heidelberg, Switzerland, New York, USA, Dordrecht, The Netherlands, London, US: Springer International Publishing.

[26] Green BR, Parson WW. 2003 *Light-harvesting antennas in photosynthesis*, Dordrecht, The Netherlands, Boston, USA, London, US: Kluwer Academic Publishers,.

[27] Fromme P. 2008 *Photosynthetic protein complexes; a structural approach*, Weinheim, Germany: Wiley-Blackwell.

- [28] Polívka T, Sundström V. 2004 Ultrafast dynamics of carotenoids excited states - from solution to natural and artificial systems. *Chem. Rev.* **104**, 2021-2071. (doi: 10.1021/cr020674n)
- [29] Hashimoto H, Yanagi K, Yoshizawa M, Polli D, Cerullo G, Lanzani G, De Silvestri S, Gardiner AT, Cogdell RJ. 2004 The very early events following photoexcitation of carotenoids. *Arch. Biochem. Biophys.* **430**, 61-69. (doi: 10.1016/j.abb.2004.04.022)
- [30] Koyama Y, Kakitani Y. 2006 Mechanisms of carotenoid-to-bacteriochlorophyll energy transfer in the light harvesting antenna complexes 1 and 2: Dependence on the conjugation length of carotenoids. In *Chlorophylls and bacteriochlorophylls* (eds B Grimm, RJ Porra, W Rüdiger, H Scheer), pp.431-443. Dordrecht, The Netherlands: Springer.
- [31] Koyama Y, Kakitani Y, Watanabe Y. 2008 Photophysical properties and light-harvesting and photoprotective functions of carotenoids in bacterial photosynthesis: Structural selections. In *Primary processes of photosynthesis - part 1: Principles and apparatus* (Ed G Renger), pp. 151-201. Cambridge, US: RSC Publishing.
- [32] Polívka T, Sundström V. 2009 Dark excited states of carotenoids: Consensus and controversy. *Chem. Phys. Lett.* **477**, 1-11. (doi: 10.1016/j.cplett.2009.06.011)
- [33] Polívka T, Frank HA. 2010 Molecular factors controlling photosynthetic light harvesting by carotenoids. *Acc. Chem. Res.* **43**, 1125-1134. (doi: 10.1021/ar100030m)
- [34] Hashimoto H, Sugisaki M, Yoshizawa M. 2015 Ultrafast time-resolved vibrational spectroscopies of carotenoids in photosynthesis. *Biochim. Biophys. Acta* **1847**, 69-78. (doi: 10.1016/j.bbabbio.2014.09.001)
- [35] Hudson BS, Kohler BE, Schulten K. 1982 Linear polyene electronic structure and potential surfaces. In *Excited states* (ed ED Lim), pp. 1-95. New York, USA: Academic Press.
- [36] Hudson BS, Kohler BE. 1972 A low-lying weak transition in the polyene  $\alpha,\omega$ -diphenyloctatetraene. *Chem. Phys. Lett.* **14**, 299-304. (doi: 10.1016/0009-2614(72)80119-2)
- [37] Hudson BS, Kohler BE. 1973 Polyene spectroscopy: The lowest energy excited singlet state of diphenyloctatetraene and other linear polyenes. *J. Chem. Phys.* **59**, 4984-5002. (doi: 10.1063/1.1680717)
- [38] Hudson BS, Kohler BE. 1974 Linear polyene electronic structure and spectroscopy.

- Ann. Rev. Phys. Chem.* **25**, 437-460. (doi: 10.1146/annurev.pc.25.100174.002253)
- [39] Schulten K, Karplus M. 1972 On the origin of a low-lying forbidden transition in polyenes and related molecules. *Chem. Phys. Lett.* **14**, 305-309. (doi: 10.1016/0009-2614(72)80120-9)
- [40] Simpson JH, Mclaughlin L, Smith DS, Christensen RL. 1987 Vibronic coupling in polyenes-high-resolution optical spectroscopy of all-*trans*-2,4,6,8,10,12,14-hexadecaheptaene. *J. Chem. Phys.* **87**, 3360-3365. (doi: 10.1063/1.452978)
- [41] Kohler BE, Spangler C, Westerfield C. 1988 The  $2^1A_g$  state in the linear polyene 2,4,6,8,10,12,14,16-octadeca-octaene. *J. Chem. Phys.* **89**, 5422-5428. (doi: 10.1063/1.455594)
- [42] Christensen RL, Galinato MGI, Chu EF, Fujii R, Hashimoto H, Frank HA. 2007 Symmetry control of radiative decay in linear polyenes: Low barriers for isomerization in the  $S_1$  state of hexadecaheptaene. *J. Am. Chem. Soc.* **129**, 1769-1775. (doi: 10.1021/ja0609607)
- [43] Tavan P, Schulten K. 1986 The low-lying electronic excitations in long polyenes: A PPP-MRD-CI study. *J. Chem. Phys.* **85**, 6602-6609. (doi: 10.1063/1.451442)
- [44] Tavan P, Schulten K. 1987 Electronic excitations in finite and infinite polyenes. *Phys. Rev. B* **36**, 4337-4358. (doi: 10.1103/PhysRevB.36.4337)
- [45] Pariser R. 1956 Theory of the electronic spectra and structure of the polyacenes and of alternant hydrocarbons. *J. Chem. Phys.* **24**, 250-268. (doi: 10.1063/1.1742461)
- [46] Hashimoto H, Koyama Y. 1989 The C=C stretching Raman lines of  $\beta$ -carotene isomers in the  $S_1$  state as detected by pump-probe resonance Raman spectroscopy. *Chem. Phys. Lett.* **154**, 321-325. (doi: 10.1016/0009-2614(89)85363-1)
- [47] Hashimoto H, Koyama Y. 1989 Raman spectra of all-*trans*- $\beta$ -carotene in the  $S_1$  and  $T_1$  states produced by direct photoexcitation. *Chem. Phys. Lett.* **163**, 251-256. (doi: 10.1016/0009-2614(89)80045-4)
- [48] Hashimoto H, Koyama Y. 1989 Raman spectra of all-*trans*- $\beta$ -apo-8'-carotenal in the  $S_1$  and  $T_1$  states: A picosecond pump-and-probe technique using ML-Qs pulse trains. *Chem. Phys. Lett.* **162**, 523-527. (doi: 10.1016/0009-2614(89)87018-6.)
- [49] Noguchi T, Kolaczowski S, Arbour C, Aramaki S, Atkinson GH, Hayashi H, Tasumi M. 1989 Resonance Raman spectrum of the excited  $2A_g$  state of  $\beta$ -carotene. *Photochem. Photobiol.* **50**, 603-609. (doi: 10.1111/j.1751-1097.1989.tb04315.x)

- [50] Hashimoto H, Koyama Y, Hirata Y, Mataga N. 1991 S<sub>1</sub> and T<sub>1</sub> species of  $\beta$ -carotene generated by direct photoexcitation from the all-*trans*, 9-*cis*, 13-*cis*, and 15-*cis* isomers as revealed by picosecond transient absorption and transient Raman spectroscopies. *J. Phys. Chem.* **95**, 3072-3076. (doi: 10.1021/j100161a022)
- [51] Hashimoto H, Miki Y, Kuki M, Shimamura T, Utsumi H, Koyama Y. 1993 Isolation by high-pressure liquid chromatography of the *cis-trans* isomers of  $\beta$ -apo-8'-carotenal. Determination of their S<sub>0</sub>-state configurations by NMR spectroscopy and prediction of their S<sub>1</sub>- and T<sub>1</sub>-state configurations by transient Raman spectroscopy. *J. Am. Chem. Soc.* **115**, 9216-9225. (doi: 10.1021/ja00073a042)
- [52] Auerbach RA, Christensen RL, Granville MF, Kohler BE. 1981 Absorption and emission of 2,12-dimethyltridecahexaene. *J. Chem. Phys.* **74**, 4-9. (doi: 10.1063/1.440857)
- [53] Kasama A, Taya M, Kamisuki T, Adachi Y, Maeda S. 1987 Resonance CARS of diphenylpolyenes in the excited states: vibronic shift of a 2<sup>1</sup>A<sub>g</sub> state vibration. In *Time-resolved vibrational spectroscopy* (Ed GH Atkinson), pp. 304-319. New York, USA: Gordon and Breach.
- [54] Lasaga AC, Aerni RJ, Karplus M. 1980 Photodynamics of polyenes: The effect of electron correlation on potential surfaces. *J. Chem. Phys.* **73**, 5230-5243. (doi: 10.1063/1.439951)
- [55] Frank HA. 2001 Spectroscopic studies of the low-lying singlet excited electronic states and photochemical properties of carotenoids. *Arch. Biochem. Biophys.* **385**, 53-60. (doi: 10.1006/abbi.2000.2091)
- [56] Macpherson AN, Arellano JB, Fraser NJ, Cogdell RJ, Gillbro T. 2001 Efficient energy transfer from the carotenoid S<sub>2</sub> state in a photosynthetic light-harvesting complex. *Biophys. J.* **80**, 923-930. (doi: 10.1016/s0006-3495(01)76071-7)
- [57] Rondonuwu FS, Yokoyama K, Fujii R, Koyama Y, Cogdell RJ, Watanabe Y. 2004 The role of the 1<sup>1</sup>B<sub>u</sub><sup>-</sup> state in carotenoid-to-bacteriochlorophyll singlet-energy transfer in the LH2 antenna complexes from *Rhodobacter sphaeroides* G1C, *Rhodobacter sphaeroides* 2.4.1, *Rhodospirillum rubrum* and *Rhodospseudomonas acidophila*. *Chem. Phys. Lett.* **390**, 314-322. (doi: 10.1016/j.cplett.2004.03.089)
- [58] Cogdell RJ, Frank HA. 1987 How carotenoids function in photosynthetic bacteria. *Biochim. Biophys. Acta* **895**, 63-79. (doi 10.1016/S0304-4173(87)80008-3)
- [59] Bondarev SL, Bachilo SM, Dvornikov SS, Tikhomirov SA. 1989 S<sub>2</sub>→ S<sub>0</sub>

- fluorescence and transient  $S_n \leftarrow S_1$  absorption of all-*trans*- $\beta$ -carotene in solid and liquid solutions. *J. Photochem. Photobiol. A: Chemistry* **46**, 315-322. (doi: 10.1016/1010-6030(89)87048-0)
- [60] Gillbro T, Cogdell RJ. 1989 Carotenoid fluorescence. *Chem. Phys. Lett.* **158**, 312-316. (doi: 10.1016/0009-2614(89)87342-7)
- [61] Cosgrove SA, Guite MA, Burnell TB, Christensen RL. 1990 Electronic relaxation in long polyenes. *J. Phys. Chem.* **94**, 8118-8124. (doi: 10.1021/j100384a026)
- [62] Bondarev SL, Knyukshto VN. 1994 Fluorescence from the  $S_1(2^1A_g)$  state of all-*trans*- $\beta$ -carotene. *Chem. Phys. Lett.* **225**, 346-350. (doi: 10.1016/0009-2614(94)87092-6)
- [63] DeCoster B, Christensen RL, Gebhard R, Lugtenburg J, Farhoosh R, Frank HA. 1992 Low-lying electronic states of carotenoids. *Biochim. Biophys. Acta* **1102**, 107-114. (doi: 10.1016/0005-2728(92)90070-I)
- [64] Fujii R, Onaka K, Kuki M, Koyama Y, Watanabe Y. 1998 The  $2A_g^-$  energies of all-*trans*-neurosporene and spheroidene as determined by fluorescence spectroscopy. *Chem. Phys. Lett.* **288**, 847-853. (doi: 10.1016/S0009-2614(98)00376-5)
- [65] Christensen RL, Goyette M, Gallagher L, Duncan J, DeCoster B, Lugtenburg J, Jansen FJ, van der Hoef I. 1999  $S_1$  and  $S_2$  states of apo- and diapocarotenes. *J. Phys. Chem. A* **103**, 2399-2407. (doi: 10.1021/jp983946s)
- [66] Frank HA, Desamero RZB, Chynwat V, Gebhard R, van der Hoef I, Jansen FJ, Lugtenburg J, Gosztola D, Wasielewski MR. 1997 Spectroscopic properties of spheroidene analogs having different extents of  $\pi$ -electron conjugation. *J. Phys. Chem. A* **101**, 149-157. (doi: 10.1021/jp962373l)
- [67] Hashimoto H, Koyama Y, Mori Y. 1997 Mechanism activating the  $2^1A_g$  state in all-*trans*- $\beta$ -carotene crystal to resonance Raman scattering. *Jpn. J. Appl. Phys.* **36**, L916-L918.
- [68] Sashima T, Shiba M, Hashimoto H, Nagae H, Koyama Y. 1998 The  $2A_g^-$  energy of crystalline all-*trans*-spheroidene as determined by resonance-Raman excitation profiles. *Chem. Phys. Lett.* **290**, 36-42. (doi: 10.1016/s0009-2614(98)00481-3)
- [69] Polívka T, Herek JL, Zigmantas D, Akerlund HE, Sundström V. 1999 Direct observation of the (forbidden)  $S_1$  state in carotenoids. *Proc. Nat. Acad. Sci. USA* **96**, 4914-4917. (doi:10.1073/pnas.96.9.4914)
- [70] Polívka T, Zigmantas D, Frank HA, Bautista JA, Herek JL, Koyama Y, Fujii R,

- Sundström V. 2001 Near-infrared time-resolved study of the  $S_1$  state dynamics of the carotenoid spheroidene. *J. Phys. Chem. B* **105**, 1072-1080. (doi: 10.1021/jp002206s)
- [71] Zhang JP, Fujii R, Qian P, Inaba T, Mizoguchi T, Koyama Y, Onaka K, Watanabe Y, Nagae H. 2000 Mechanism of the carotenoid-to-bacteriochlorophyll energy transfer via the  $S_1$  state in the LH2 complexes from purple bacteria. *J. Phys. Chem. B* **104**, 3683-3691. (doi: 10.1021/jp993970l)
- [72] Sashima T, Koyama Y, Yamada T, Hashimoto H. 2000 The  $1B_u^+$ ,  $1B_u^-$ , and  $2A_g^-$  energies of crystalline lycopene,  $\beta$ -carotene, and mini-9- $\beta$ -carotene as determined by resonance-Raman excitation profiles: Dependence of the  $1B_u^-$  state energy on the conjugation length. *J. Phys. Chem. B* **104**, 5011-5019. (doi: 10.1021/jp994185b)
- [73] Kurashige Y, Nakano H, Nakao Y, Hirao K. 2004 The  $\pi \rightarrow \pi^*$  excited states of long linear polyenes studied by the CASCI-MRMP method. *Chem. Phys. Lett.* **400**, 425-429. (doi: 10.1016/j.cplett.2004.10.141)
- [74] Mizukami W, Kurashige Y, Ehara M, Yanai T, Itoh T. 2009 *Ab initio* study of the excited singlet states of all-*trans*  $\alpha,\omega$ -diphenylpolyenes with one to seven polyene double bonds: Simulation of the spectral data within franck-condon approximation. *J. Chem. Phys.* **131**, 174313. (doi: 10.1063/1.3261729)
- [75] Kurashige Y, Yanai T. 2014 Theoretical study of the  $\pi \rightarrow \pi^*$  excited states of oligoacenes: A full  $\pi$ -valence DMRG-CASPT2 study. *Bull. Chem. Soc. Jpn.* **87**, 1071-1073. (doi: 10.1246/bcsj.20140180)
- [76] Sashima T, Nagae H, Kuki M, Koyama Y. 1999 A new singlet-excited state of all-*trans*-spheroidene as detected by resonance-Raman excitation profiles. *Chem. Phys. Lett.* **299**, 187-194. (doi: 10.1016/S0009-2614(98)01278-0)
- [77] Furuichi K, Sashima T, Koyama Y. 2002 The first detection of the  $3A_g^-$  state in carotenoids using resonance-Raman excitation profiles. *Chem. Phys. Lett.* **356**, 547-555. (doi: 10.1016/S0009-2614(02)00412-8)
- [78] Fujii R, Ishikawa T, Koyama Y, Taguchi M, Isobe Y, Nagae H, Watanabe Y. 2001 Fluorescence spectroscopy of all-*trans*-anhydrorhodovibrin and spirilloxanthin: Detection of the  $1B_u^-$  fluorescence. *J. Phys. Chem. A* **105**, 5348-5355. (doi: 10.1021/jp010150b)
- [79] Fujii R, Fujino T, Inaba T, Nagae H, Koyama Y. 2004 Internal conversion of  $1B_u^+ \rightarrow 1B_u^- \rightarrow 2A_g^-$  and fluorescence from the  $1B_u^-$  state in all-*trans*-neurosporene as probed by up-conversion spectroscopy. *Chem. Phys. Lett.* **384**, 9-15. (doi:



10.1016/j.cplett.2003.11.074)

[80] Wang P, Nakamura R, Kanematsu Y, Koyama Y, Nagae H, Nishio T, Hashimoto H, Zhang JP. 2005 Low-lying singlet states of carotenoids having 8-13 conjugated double bonds as determined by electronic absorption spectroscopy. *Chem. Phys. Lett.* **410**, 108-114. (doi: 10.1016/j.cplett.2005.05.037)

[81] Zhang JP, Inaba T, Watanabe Y, Koyama Y. 2000 Excited-state dynamics among the  $1B_u^+$ ,  $1B_u^-$  and  $2A_g^-$  states of all-*trans*-neurosporene as revealed by near-infrared time-resolved absorption spectroscopy. *Chem. Phys. Lett.* **332**, 351-358. (doi: 10.1016/S0009-2614(00)01275-6)

[82] Zhang JP, Inaba T, Watanabe Y, Koyama Y. 2000 Sub-picosecond time-resolved absorption spectroscopy of all-*trans*-neurosporene in solution and bound to the LH2 complex from *Rhodobacter sphaeroides* G1C. *Chem. Phys. Lett.* **331**, 154-162. (doi: 10.1016/S0009-2614(00)01165-9)

[83] Zhang JP, Inaba T, Koyama Y. 2001 The role of the newly-found  $1B_u^-$  state of carotenoid in mediating the  $1B_u^+$ -to- $2A_g^-$  internal conversion and the excited-state dynamics of carotenoid and bacteriochlorophyll in a bacterial antenna complex. *J. Mol. Struct.* **598**, 65-78. (doi: 10.1016/S0022-2860(01)00806-7)

[84] Zhang JP, Fujii R, Koyama Y, Rondonuwu FS, Watanabe Y, Mortensen A, Skibsted LH. 2001 The  $1B_u$ -type singlet state of  $\beta$ -carotene as a precursor of the radical cation found in chloroform solution by sub-picosecond time-resolved absorption spectroscopy. *Chem. Phys. Lett.* **348**, 235-241. (doi: 10.1016/S0009-2614(01)01157-5)

[85] Zhang JP, Inaba T, Watanabe Y, Koyama Y. 2001 Partition of carotenoid-to-bacteriochlorophyll singlet-energy transfer through two channels in the LH2 complex from *Rhodobacter sphaeroides* G1C. *Chem. Phys. Lett.* **340**, 484-492. (doi: 10.1016/S0009-2614(01)00451-1)

[86] Zhang JP, Skibsted LH, Fujii R, Koyama Y. 2001 Transient absorption from the  $1B_u^+$  state of all-*trans*- $\beta$ -carotene newly identified in the near-infrared region. *Photochem. Photobiol.* **73**, 219-222. (doi: 10.1562/0031-8655(2001)073<0219:Taftus>2.0.Co;2)

[87] Rondonuwu FS, Watanabe Y, Zhang JP, Furuichi K, Koyama Y. 2002 Internal-conversion and radiative-transition processes among the  $1B_u^+$ ,  $1B_u^-$  and  $2A_g^-$  states of all-*trans*-neurosporene as revealed by subpicosecond time-resolved Raman spectroscopy. *Chem. Phys. Lett.* **357**, 376-384. (doi: 10.1016/S0009-2614(02)00491-8)

- [88] Fujii R, Inaba T, Watanabe Y, Koyama Y, Zhang JP. 2003 Two different pathways of internal conversion in carotenoids depending on the length of the conjugated chain. *Chem. Phys. Lett.* **369**, 165-172. (doi: 10.1016/S0009-2614(02)01999-1)
- [89] Rondonuwu FS, Watanabe Y, Fujii R, Koyama Y. 2003 A first detection of singlet to triplet conversion from the  $1^1B_u^-$  to the  $1^3A_g$  state and triplet internal conversion from the  $1^3A_g$  to the  $1^3B_u$  state in carotenoids: Dependence on the conjugation length. *Chem. Phys. Lett.* **376**, 292-301. (doi: 10.1016/s0009-2614(03)00983-7)
- [90] Akahane J, Rondonuwu FS, Fiedor L, Watanabe Y, Koyama Y. 2004 Dependence of singlet-energy transfer on the conjugation length of carotenoids reconstituted into the LH1 complex from *Rhodospirillum rubrum* G9. *Chem. Phys. Lett.* **393**, 184-191. (doi: 10.1016/j.cplett.2004.06.021)
- [91] Koyama Y, Rondonuwu FS, Fujii R, Watanabe Y. 2004 Light-harvesting function of carotenoids in photo-synthesis: The roles of the newly found  $1^1B_u^-$  state. *Biopolymers* **74**, 2-18. (doi: 10.1002/bip.20034)
- [92] Nishimura K, Rondonuwu FS, Fujii R, Akahane J, Koyama Y, Kobayashi T. 2004 Sequential singlet internal conversion of  $1B_u^+ \rightarrow 3A_g^- \rightarrow 1B_u^- \rightarrow 2A_g^- \rightarrow (1A_g^- \text{ ground})$  in all-*trans*-spirilloxanthin revealed by two-dimensional sub-5-fs spectroscopy. *Chem. Phys. Lett.* **392**, 68-73. (doi: 10.1016/j.cplett.2004.04.109)
- [93] Ikuta M, Yabushita A, Rondonuwu FS, Akahane J, Koyama Y, Kobayashi T. 2006 The  $1B_u^+ \rightarrow 3A_g^- \rightarrow 1B_u^- \rightarrow 2A_g^-$  internal conversion in carotenoids following the energy-gap law identified by 5 fs spectroscopy. *Chem. Phys. Lett.* **422**, 95-99. (doi: 10.1016/j.cplett.2006.02.042)
- [94] Rondonuwu FS, Kakitani Y, Tamura H, Koyama Y. 2006 Singlet internal conversion processes in the order of  $1B_u^+ \rightarrow 3A_g^- \rightarrow 1B_u^- \rightarrow 2A_g^- \rightarrow 1A_g^-$  in all-*trans*-spheroidene and lycopene as revealed by subpicosecond time-resolved Raman spectroscopy. *Chem. Phys. Lett.* **429**, 234-238. (doi: 10.1016/j.cplett.2006.07.061)
- [95] Sutresno A, Kakitani Y, Zuo P, Li C, Koyama Y, Nagae H. 2007 Presence and absence of electronic mixing in shorter-chain and longer-chain carotenoids: Assignment of the symmetries of  $1B_u^-$  and  $3A_g^-$  states located just below the  $1B_u^+$  state. *Chem. Phys. Lett.* **447**, 127-133. (doi: 10.1016/j.cplett.2007.08.081)
- [96] Gradinaru CC, Kennis J, Papagiannakis E, van Stokkum IH, Cogdell RJ, Fleming GR, Niederman RA, van Grondelle R. 2001 An unusual pathway of excitation energy deactivation in carotenoids: Singlet-to-triplet conversion on an ultrafast timescale in a

- photosynthetic antenna. *Proc. Natl. Acad. Sci. USA* **98**, 2364-2369. (doi: 10.1073/pnas.051501298)
- [97] Papagiannakis E, Das SK, Gall A, van Stokkum IHM, Robert B, van Grondelle R, Frank HA, Kennis JTM. 2003 Light harvesting by carotenoids incorporated into the B850 light-harvesting complex from *Rhodobacter sphaeroides* R-26.1: Excited-state relaxation, ultrafast triplet formation, and energy transfer to bacteriochlorophyll. *J. Phys. Chem. B* **107**, 5642-5649. (doi: 10.1021/jp027174i)
- [98] Papagiannakis E, van Stokkum IHM, van Grondelle R, Niederman RA, Zigmantas D, Sundström V, Polívka T. 2003 A near-infrared transient absorption study of the excited-state dynamics of the carotenoid spirilloxanthin in solution and in the LH1 complex of *Rhodospirillum rubrum*. *J. Phys. Chem. B* **107**, 11216-11223. (doi: 10.1021/jp034931j)
- [99] Wohlleben W, Buckup T, Hashimoto H, Cogdell RJ, Herek JL, Motzkus M. 2004 Pump-deplete-probe spectroscopy and the puzzle of carotenoid dark states. *J. Phys. Chem. B* **108**, 3320-3325. (doi: 10.1021/jp036145k)
- [100] Kosumi D, Maruta S, Horibe T, Nagaoka Y, Fujii R, Sugisaki M, Cogdell RJ, Hashimoto H. 2012 Ultrafast excited state dynamics of spirilloxanthin in solution and bound to core antenna complexes: Identification of the S\* and T<sub>1</sub> states. *J. Chem. Phys.* **137**, 064505. (doi: 10.1063/1.4737129)
- [101] Beck WF, Bishop MM, Roscioli JD, Ghosh S, Frank HA. 2015 Excited state conformational dynamics in carotenoids: Dark intermediates and excitation energy transfer. *Arch. Biochem. Biophys.* **572**, 175-183. (doi:10.1016/j.abb.2015.02.016)
- [102] Sanchez-Galvez A, Hunt P, Robb MA, Olivucci M, Vreven T, Schlegel HB. 2000 Ultrafast radiationless deactivation of organic dyes: Evidence for a two-state two-mode pathway in polymethine cyanines. *J. Am. Chem. Soc.* **122**, 2911-2924. (doi: 10.1021/ja993985x)
- [103] Balevicius V Jr., Abramavicius D, Polivka T, Galestian Pour A, Hauer J. 2016 A unified picture of S\* in carotenoids. *J. Phys. Chem. Lett.* **7**, 3347-3352. (doi: 10.1021/acs.jpcllett.6b01455)
- [104] Andersson PO, Gillbro T. 1995 Photophysics and dynamics of the lowest excited singlet-state in long substituted polyenes with implications to the very long-chain limit. *J. Chem. Phys.* **103**, 2509-2519. (doi: 10.1063/1.469672)
- [105] Hörvin Billsten H, Zigmantas D, Sundström V, Polívka T. 2002 Dynamics of

- vibrational relaxation in the S<sub>1</sub> state of carotenoids having 11 conjugated C=C bonds. *Chem. Phys. Lett.* **355**, 465-470. (doi: 10.1016/s0009-2614(02)00268-3)
- [106] Bautista JA, Connors RE, Raju BB, Hiller RG, Sharples FP, Gosztola D, Wasielewski MR, Frank HA. 1999 Excited state properties of peridinin: Observation of a solvent dependence of the lowest excited singlet state lifetime and spectral behavior unique among carotenoids. *J. Phys. Chem. B* **103**, 8751-8758. (doi: 10.1021/jp9916135)
- [107] Frank HA, Bautista JA, Josue J, Pendon Z, Hiller RG, Sharples FP, Gosztola D, Wasielewski MR. 2000 Effect of the solvent environment on the spectroscopic properties and dynamics of the lowest excited states of carotenoids. *J. Phys. Chem. B* **104**, 4569-4577. (doi: 10.1021/jp000079u)
- [108] Yoshizawa M, Aoki H, Hashimoto H. 2001 Vibrational relaxation of the 2A<sub>g</sub><sup>-</sup> excited state in all-*trans*-β-carotene obtained by femtosecond time-resolved Raman spectroscopy. *Phys. Rev. B* **63**, 180301(R). (doi: 10.1103/PhysRevB.63.180301)
- [109] Yoshizawa M, Aoki H, Hashimoto H. 2002 Femtosecond time-resolved Raman signals on ultrafast dynamics in all-*trans*-β-carotene. *Bull. Chem. Soc. Jpn.* **75**, 949-955. (doi: 10.1246/bcsj.75.949)
- [110] McCamant DW, Kim JE, Mathies RA. 2002 Vibrational relaxation in β-carotene probed by picosecond stokes and anti-stokes resonance Raman spectroscopy. *J. Phys. Chem. A* **106**, 6030-6038. (doi: 10.1021/jp0203595)
- [111] Zigmantas D, Polivka T, Hiller RG, Yartsev A, Sundstrom V. 2001 Spectroscopic and dynamic properties of the peridinin lowest singlet excited states. *J. Phys. Chem. A* **105**, 10296-10306. (doi :10.1021/jp010022n)
- [112] Zigmantas D, Hiller RG, Sundström V, Polívka T. 2002 Carotenoid to chlorophyll energy transfer in the peridinin-chlorophyll-*a*-protein complex involves an intramolecular charge transfer state. *Proc. Natl. Acad. Sci. USA* **99**, 16760-16765. (doi: 10.1073/pnas.262537599)
- [113] Kosumi D, Kusumoto T, Fujii R, Sugisaki M, Iinuma Y, Oka N, Takaesu Y, Taira T, Iha M, Frank HA, Hashimoto H. 2009 One- and two-photon pump-probe optical spectroscopic measurements reveal the S<sub>1</sub> and intramolecular charge transfer states are distinct in fucoxanthin. *Chem. Phys. Lett.* **483**, 95-100. (doi: 10.1016/j.cplett.2009.10.077)
- [114] Kosumi D, Kusumoto T, Fujii R, Sugisaki M, Iinuma Y, Oka N, Takaesu Y, Taira T, Iha M, Frank HA, Hashimoto H. 2011 Ultrafast excited state dynamics of

fucoxanthin: Excitation energy dependent intramolecular charge transfer dynamics.

*Phys. Chem. Chem. Phys.* **13**, 10762-10770. (doi: 10.1039/c0cp02568b)

[115] Kosumi D, Kusumoto T, Fujii R, Sugisaki M, Iinuma Y, Oka N, Takaesu Y, Taira T, Iha M, Frank HA, Hashimoto H. 2011 Ultrafast S<sub>1</sub> and ICT state dynamics of a marine carotenoid probed by femtosecond one- and two-photon pump-probe spectroscopy. *J. Lumin.* **131**, 515-518. (doi:10.1016/j.jlumin.2010.09.018)

[116] Kosumi D, Kita M, Fujii R, Sugisaki M, Oka N, Takaesu Y, Taira T, Iha M, Hashimoto H. 2012 Excitation energy-transfer dynamics of brown algal photosynthetic antennas. *J. Phys. Chem. Lett.* **3**, 2659-2664. (doi: 10.1021/Jz300612c)

[117] Kosumi D, Fujii R, Sugisaki M, Oka N, Iha M, Hashimoto H. 2014 Characterization of the intramolecular transfer state of marine carotenoid fucoxanthin by femtosecond pump-probe spectroscopy. *Photosynth. Res.* **121**, 61-68. (doi: 10.1007/s11120-014-9995-6)

[118] Kosumi D, Kajikawa T, Okumura S, Sugisaki M, Sakaguchi K, Katsumura S, Hashimoto H. 2014 Elucidation and control of an intramolecular charge transfer property of fucoxanthin by a modification of its polyene chain length. *J. Phys. Chem. Lett.* **5**, 792-797. (doi: 10.1021/jz5000287)

[119] Yukihiro N, Sugai Y, Fujiwara M, Kosumi D, Iha M, Sakaguchi K, Katsumura S, Gardiner AT, Cogdell RJ, Hashimoto H. 2017 Strategies to enhance the excitation energy-transfer efficiency in a light-harvesting system using the intra-molecular charge transfer character of carotenoids. *Faraday Discuss.* **198**, 59-71. (doi: 10.1039/c6fd00211k)

[120] Kosumi D, Kajikawa T, Yano K, Okumura S, Sugisaki M, Sakaguchi K, Katsumura S, Hashimoto H. 2014 Roles of allene-group in an intramolecular charge transfer character of a short fucoxanthin homolog as revealed by femtosecond pump-probe spectroscopy. *Chem. Phys. Lett.* **602**, 75-79. (doi: 10.1016/j.cplett.2014.04.022)

[121] Cerullo G, Polli D, Lanzani G, De Silvestri S, Hashimoto H, Cogdell RJ. 2002 Photosynthetic light harvesting by carotenoids: Detection of an intermediate excited state. *Science* **298**, 2395-2398. (doi: 10.1126/science.1074685)

[122] Kosumi D, Komukai M, Hashimoto H, Yoshizawa M. 2005 Ultrafast dynamics of all-*trans*- $\beta$ -carotene explored by resonant and nonresonant photoexcitations. *Phys. Rev. Lett.* **95**, 213601-213604. (doi: 10.1103/PhysRevLett.95.213601)

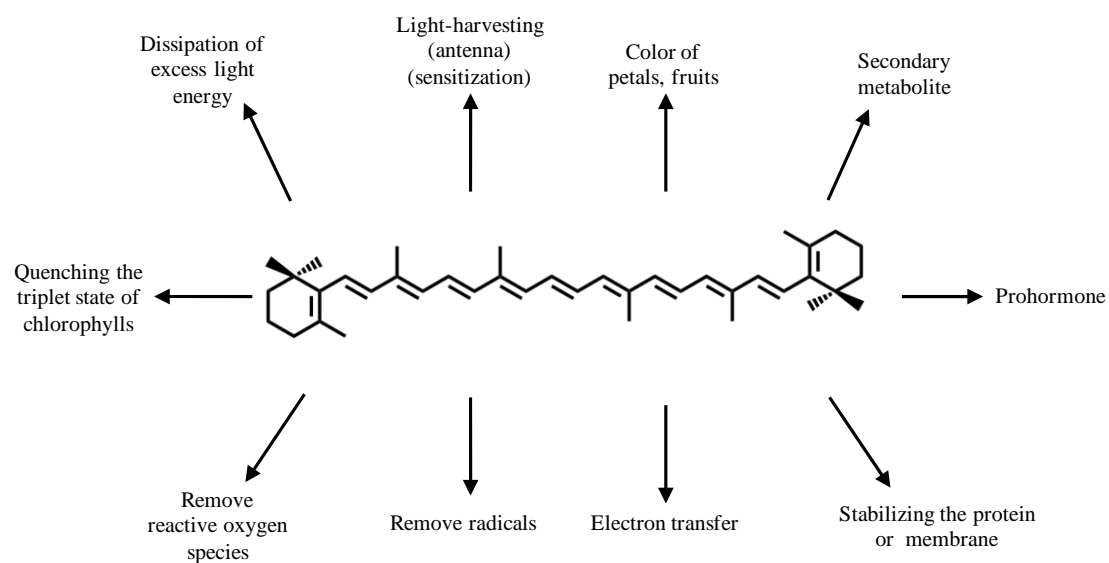
- [123] Ostroumov EE, Mulvaney RM, Cogdell RJ, Scholes GD. 2013 Broadband 2D electronic spectroscopy reveals a carotenoid dark state in purple bacteria. *Science* **340**, 52-56. (doi: 10.1126/science.1230106)
- [124] Siebert T, Engel V, Materny A, Kiefer W, Schmitt M. 2003 Probing the kinetics of a nonadiabatic transition initiating out of vibrationally excited as well as ground state modes with femtosecond time-resolved transient gratings. *J. Phys. Chem. A* **107**, 8355-8362. (doi: 10.1021/jp022650q)
- [125] Hornung T, Skenderović H, Motzkus M. 2005 Observation of all-*trans*- $\beta$ -carotene wavepacket motion on the electronic ground and excited dark state using degenerate four-wave mixing (DFWM) and pump-DFWM. *Chem. Phys. Lett.* **402**, 283-288. (doi: 10.1016/j.cplett.2004.11.135)
- [126] Sugisaki M, Yanagi K, Cogdell RJ, Hashimoto H. 2007 Unified explanation for linear and nonlinear optical responses in  $\beta$ -carotene: A sub-20-fs degenerate four-wave mixing spectroscopic study. *Phys. Rev. B* **75**, 155110. (doi: 10.1103/PhysRevB.75.155110)
- [127] Hauer J, Buckup T, Motzkus M. 2007 Pump-degenerate four wave mixing as a technique for analyzing structural and electronic evolution: Multidimensional time-resolved dynamics near a conical intersection. *J. Phys. Chem. A* **111**, 10517-10529. (doi: 10.1021/jp073727j)
- [128] Sugisaki M, Fujiwara M, Yanagi K, Cogdell RJ, Hashimoto H. 2008 Four-wave mixing signals from  $\beta$ -carotene and its  $n = 15$  homologue. *Photosyn. Res.* **95**, 299-308. (doi: 10.1007/s11120-007-9265-y)
- [129] Buckup T, Hauer J, Mohring J, Motzkus M. 2009 Multidimensional spectroscopy of  $\beta$ -carotene: Vibrational cooling in the excited state. *Arch. Biochem. Biophys.* **483**, 219-223. (doi: 10.1016/j.abb.2008.10.031)
- [130] Fujiwara M, Yamauchi K, Sugisaki M, Gall A, Robert B, Cogdell R J, Hashimoto H. 2008 Energy dissipation in the ground-state vibrational manifolds of  $\beta$ -carotene homologues: A sub-20-fs time-resolved transient grating spectroscopic study. *Phys. Rev. B* **77**, 205118. (doi: 10.1103/PhysRevB.77.205118)
- [131] Sugisaki M, Fujiwara M, Nair SV, Ruda HE, Cogdell RJ, Hashimoto H. 2009 Excitation-energy dependence of transient grating spectroscopy in  $\beta$ -carotene. *Phys. Rev. B* **80**, 035118. (doi: 10.1103/PhysRevB.80.035118)
- [132] Christensson N, Polivka T, Yartsev A, Pullerits T. 2009 Photon echo spectroscopy

- reveals structure-dynamics relationships in carotenoids. *Phys. Rev. B* **79**, 245118. (doi: 10.1103/PhysRevB.79.245118)
- [133] Fujiwara M, Sugisaki M, Gall A, Robert B, Cogdell RJ, Hashimoto H. 2009 Ultrafast optical responses of  $\beta$ -carotene and lycopene probed by sub-20-fs time-resolved coherent spectroscopy. *J. Lumin.* **129**, 1808-1812. (doi: 10.1016/j.jlumin.2009.04.098)
- [134] Sugisaki M, Fujiwara M, Kosumi D, Fujii R, Nango M, Cogdell RJ, Hashimoto H. 2010 Comparison of transient grating signals from spheroidene in an organic solvent and in pigment-protein complexes from *Rhodobacter sphaeroides* 2.4.1. *Phys. Rev. B* **81**, 245112. (doi: 10.1103/PhysRevB.81.245112)
- [135] Sugisaki M, Kosumi D, Saito K, Cogdell RJ, Hashimoto H. 2011 Control of coherent vibronic oscillations in  $\beta$ -carotene by ultrashort laser pulses. *Phys. Status Solidi C* **8**, 151-154. (doi: 10.1002/pssc.201000679)
- [136] Sugisaki M, Kosumi D, Saito K, Cogdell RJ, Hashimoto H. 2011 Strong coherent coupling of vibronic oscillations in spheroidene. *Physics Procedia* **13**, 74-77. (doi: 10.1016/j.phpro.2011.02.018)
- [137] Mukamel S. 1995 *Principles of nonlinear optical spectroscopy*, New York, USA, Oxford, US: Oxford University Press.
- [138] Sugisaki M, Fujiwara M, Fujii R, Nakagawa K, Nango M, Cogdell RJ, Hashimoto H. 2009 Transient grating spectroscopy in photosynthetic purple bacteria *Rhodobacter sphaeroides* 2.4.1. *J. Lumin.* **129**, 1908-1911. (doi: 10.1016/j.jlumin.2009.01.027)
- [139] Vos MH, Lambry JC, Robles SJ, Youvan DC, Breton J, Martin JL. 1991 Direct observation of vibrational coherence in bacterial reaction centers using femtosecond absorption-spectroscopy. *Proc. Nat. Acad. Sci. USA* **88**, 8885-8889. (doi: 10.1073/pnas.88.20.8885)
- [140] Vos MH, Rappaport F, Lambry JC, Breton J, Martin JL. 1993 Visualization of coherent nuclear motion in a membrane-protein by femtosecond spectroscopy. *Nature* **363**, 320-325. (doi: 10.1038/363320a0)
- [141] Vos MH, Jones MR, Hunter CN, Breton J, Martin JL. 1994 Coherent nuclear dynamics at room temperature in bacterial reactioncenters. *Proc. Natl. Acad. Sci. USA* **91**, 12701-12705.
- [142] Jimenez R, van Mourik F, Yu JY, Fleming GR. 1997 Three-pulse photon echo

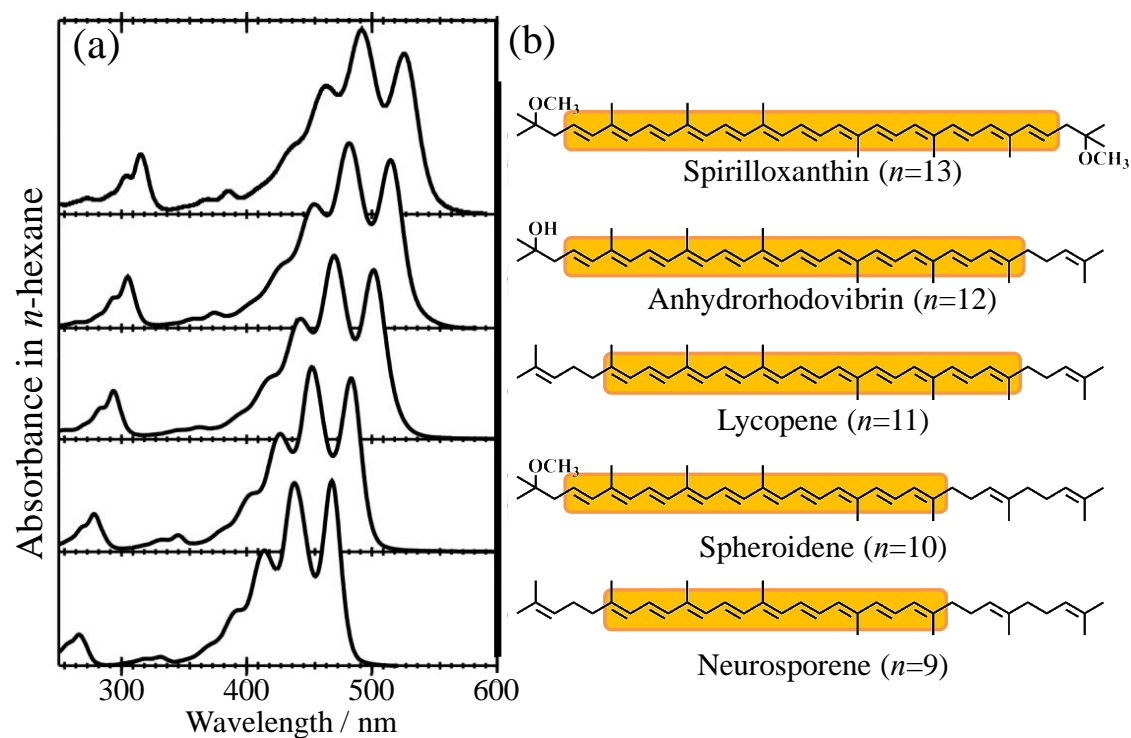
- measurements on LH1 and LH2 complexes of *Rhodobacter sphaeroides*: A nonlinear spectroscopic probe of energy transfer. *J. Phys. Chem. B* **101**, 7350-7359. (doi: 10.1021/jp970299g)
- [143] Groot ML, Yu JY, Agarwal R, Norris JR, Fleming GR. 1998 Three-pulse photon echo measurements on the accessory pigments in the reaction center of *Rhodobacter sphaeroides*. *J. Phys. Chem. B* **102**, 5923-5931. (doi: 10.1021/jp9808680)
- [144] Agarwal R, Yang M, Xu QH, Fleming GR. 2001 Three pulse photon echo peak shift study of the B800 band of the LH2 complex of *Rps. acidophila* at room temperature: A coupled master equation and nonlinear optical response function approach. *J. Phys. Chem. B* **105**, 1887-1894. (doi: 10.1021/jp0031146)
- [145] Shelly KR, Carson EA, Beck WF. 2003 Vibrational coherence from the dipyrindine complex of bacteriochlorophyll *a*: Intramolecular modes in the 10-220-cm<sup>-1</sup> regime, intermolecular solvent modes, and relevance to photosynthesis. *J. Am. Chem. Soc.* **125**, 11810-11811. (doi: 10.1021/ja0366890)
- [146] Shelly KR, Golovich EC, Beck WF. 2006 Intermolecular vibrational coherence in bacteriochlorophyll *a* with clustered polar solvent molecules. *J. Phys. Chem. B* **110**, 20586-20595. (doi: 10.1021/jp062909v)
- [147] Buckup T, Hauer J, Voll J, Vivie-Riedle R, Motzkus M. 2011 A general control mechanism of energy flow in the excited state of polyenic biochromophores. *Faraday Discuss.* **153**, 213-225. (doi: 10.1039/c1fd00037c)
- [148] Marek MS, Buckup T, Motzkus M. 2011 Direct observation of a dark state in lycopene using pump-DFWM. *J. Phys. Chem. B* **115**, 8328-8337. (doi: 10.1021/jp202753j)
- [149] Philip Kraack J, Motzkus M, Buckup T. 2011 Selective nonlinear response preparation using femtosecond spectrally resolved four-wave-mixing. *J. Chem. Phys.* **135**, 224505. (doi: 10.1063/1.3666846)
- [150] Kraack JP, Wand A, Buckup T, Motzkus M, Ruhman S. 2013 Mapping multidimensional excited state dynamics using pump-impulsive-vibrational-spectroscopy and pump-degenerate-four-wave-mixing. *Phys. Chem. Chem. Phys.* **15**, 14487-14501. (doi: 10.1039/c3cp50871d)
- [151] Marek MS, Buckup T, Southall J, Cogdell RJ, Motzkus M. 2013 Highlighting short-lived excited electronic states with pump-degenerate-four-wave-mixing. *J. Chem. Phys.* **139**, 074202. (doi: 10.1063/1.4818164)



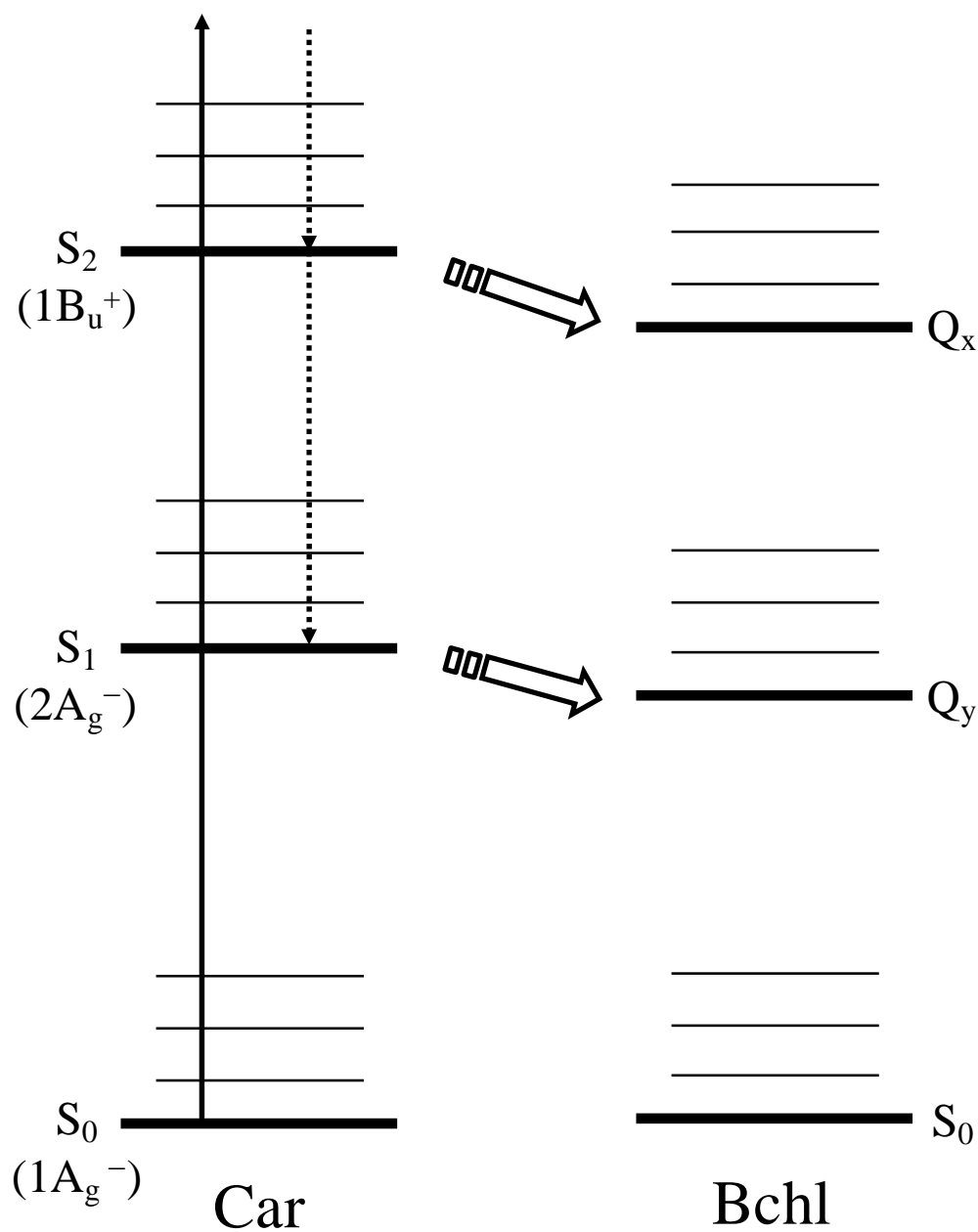
- [152] Buckup T, Motzkus M. 2014 Multidimensional time-resolved spectroscopy of vibrational coherence in biopolyenes. *Annu. Rev. Phys. Chem.* **65**, 39-57. (doi: 10.1146/annurev-physchem-040513-103619)
- [153] Miki T, Buckup T, Krause MS, Southall J, Cogdell RJ, Motzkus M. 2016 Vibronic coupling in the excited-states of carotenoids. *Phys. Chem. Chem. Phys.* **18**, 11443-11453. (doi: 10.1039/c5cp07542d)
- [154] Fiedor L, Heriyanto, Fiedor J, Pilch M. 2016 Effects of molecular symmetry on the electronic transitions in carotenoids. *J. Phys. Chem. Lett.* **7**, 1821-1829. (doi: 10.1021/acs.jpclett.6b00637)
- [155] Frank HA, Young AJ, Britton G, Cogdell RJ. 1999 The photochemistry of carotenoids. In *Advances in photosynthesis and Respiration vol.8* (ed Govindjee), pp. 235-244. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- [156] Cho M. 2008 Coherent two-dimensional optical spectroscopy. *Chem. Rev.* **108**, 1331-1418. (doi: 10.1021/cr078377b)
- [157] Cho M. 2009 *Two-dimensional optical spectroscopy*, Boca Raton, USA: CRC Press.
- [158] Yoshizawa M, Kurosawa M. 1999 Femtosecond time-resolved Raman spectroscopy using stimulated Raman scattering. *Phys. Rev. A* **61**, 013808. (doi: 10.1103/PhysRevA.61.013808)
- [159] Kukura P, McCamant DW, Mathies RA. 2007 Femtosecond stimulated Raman spectroscopy. *Annu. Rev. Phys. Chem.* **58**, 461-488. (doi: 10.1146/annurev.physchem.58.032806.104456)
- [160] Frontiera RR, Mathies RA. 2011 Femtosecond stimulated Raman spectroscopy. *Laser & Photon. Rev.* **5**, 102-113. (doi: 10.1002/lpor.200900048)
- [161] Challa JR, Du Y, McCamant DW. 2012 Femtosecond stimulated Raman spectroscopy using a scanning multichannel technique. *Appl. Spectrosc.* **66**, 227-232. (doi: 10.1366/11-06457)



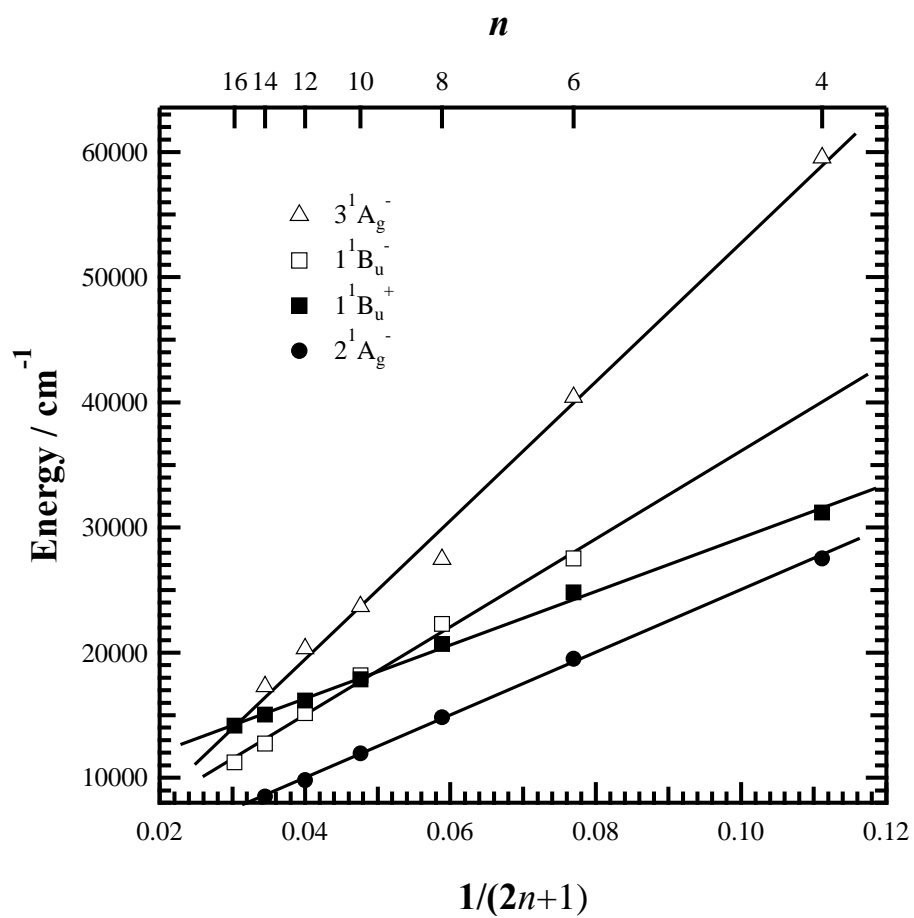
**Figure 1.** A schematic illustration that summarizes the functions of carotenoids in physiological systems. Chemical structure of  $\beta$ -carotene is shown as a representative of carotenoids.



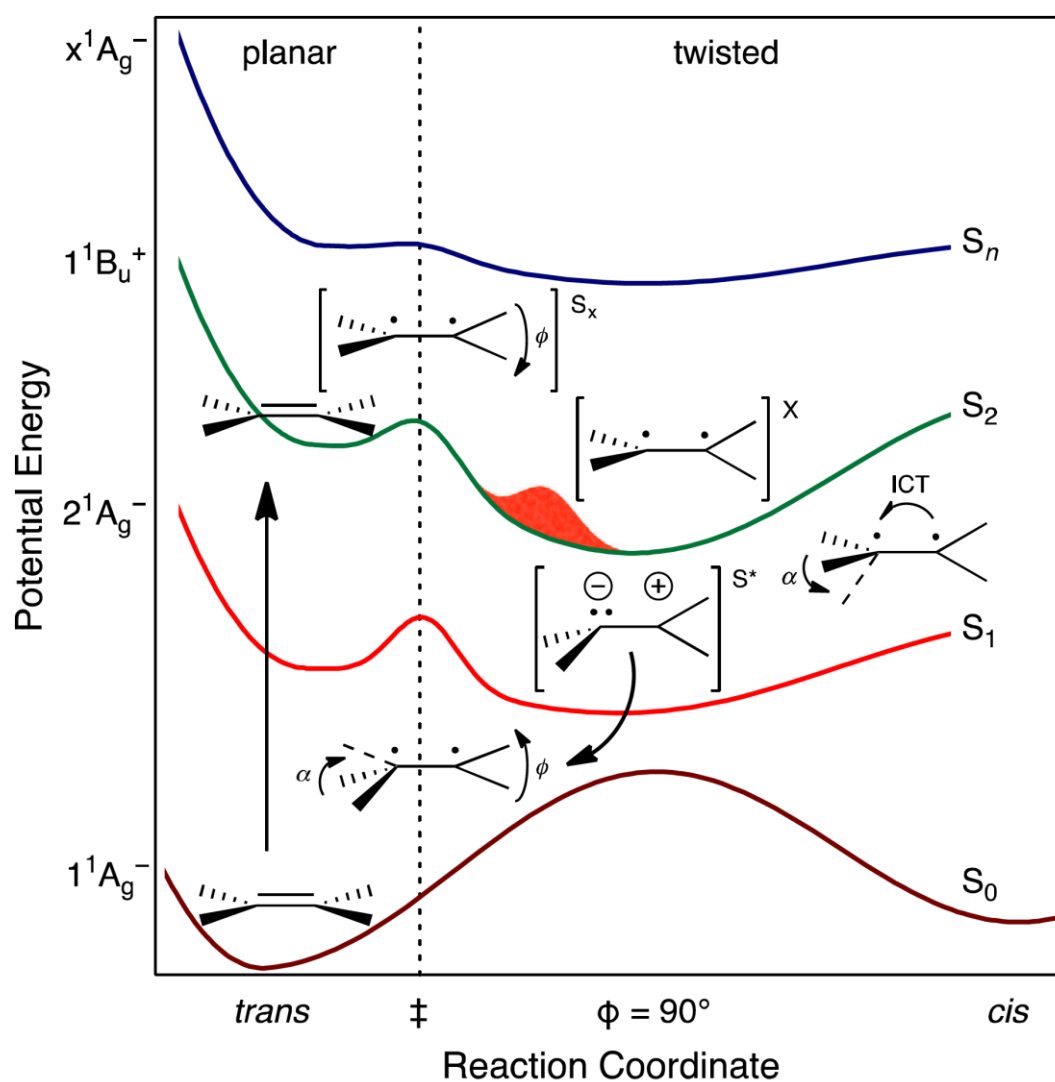
**Figure 2.** (a) Absorption spectra in *n*-hexane solutions and (b) chemical structures of carotenoids bound to purple photosynthetic bacteria. Conjugated polyene backbones were highlighted with orange rectangles in (b).



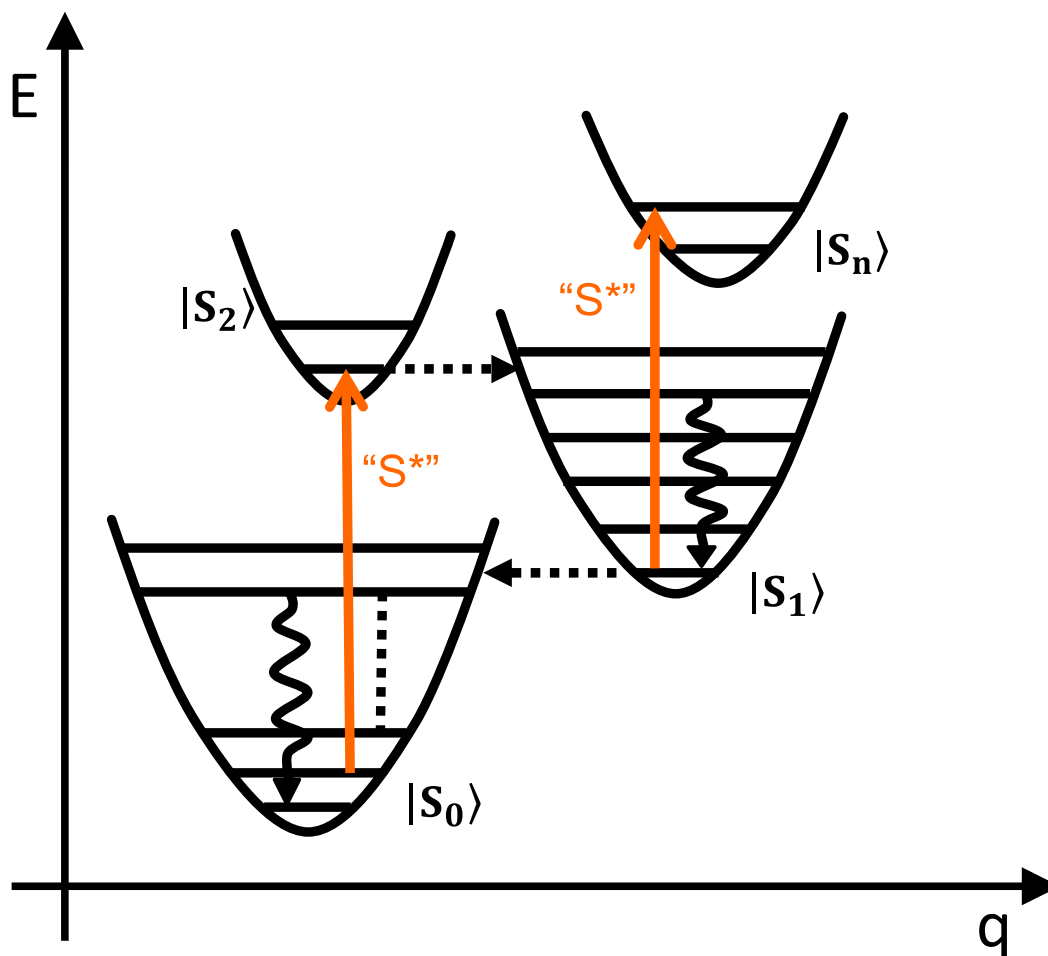
**Figure 3.** Relative energy levels of the  $S_1$  and  $S_2$  excited singlet states of a typical carotenoid relative to bacteriochlorophyll (Bchl)  $a$   $Q_x$  and  $Q_y$  transitions. These energy levels are valid for carotenoids containing up to 10 conjugated double bonds. Thick lines show the 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  $a$ .



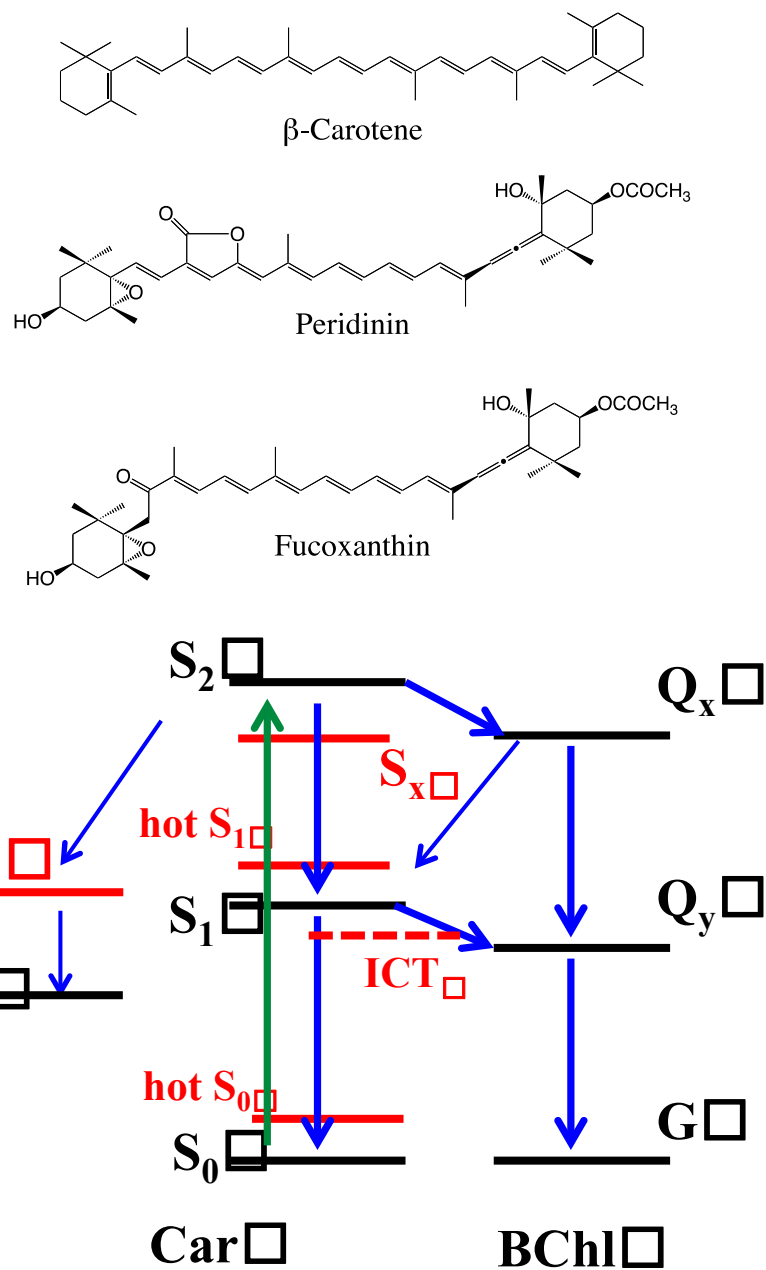
**Figure 4.** Energy diagram calculated by Tavan and Schulten using PPPMRD-CI method [43,44] for the low-lying singlet excited state of polyenes ( $n = 4-16$ ).



**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].)

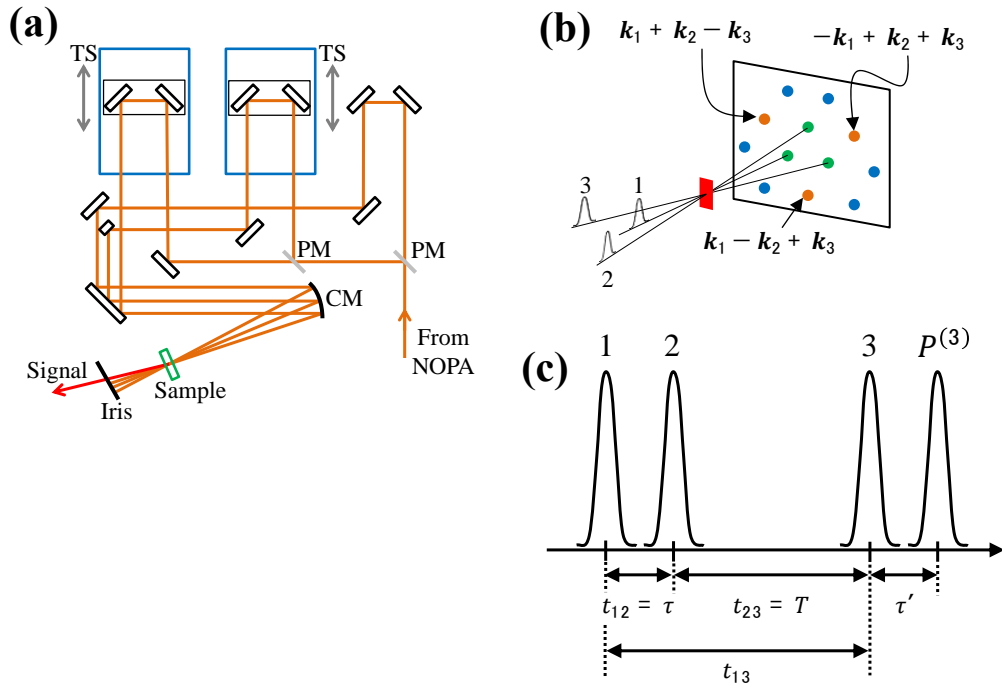


**Figure 6.** Energy level scheme describing electronic (dashed horizontal arrows) and vibrational (wavy vertical arrows) energy relaxation in carotenoids along a reaction coordinate. Colored vertical arrows indicate allowed electronic transitions. Electronic states are labeled in ket-notation. (Transferred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

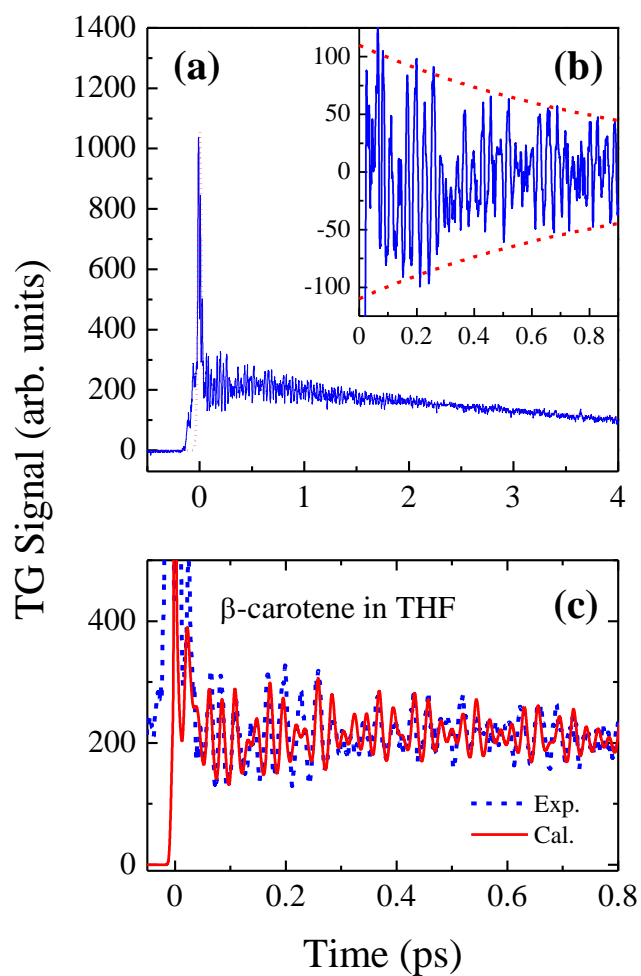


**Figure 7.** Chemical structures of  $\beta$ -carotene, peridinin, and fucoxanthin, and a schematic description of energy diagrams together with relaxation and energy-transfer pathways of carotenoids following photoexcitation up to the  $S_2$  state.

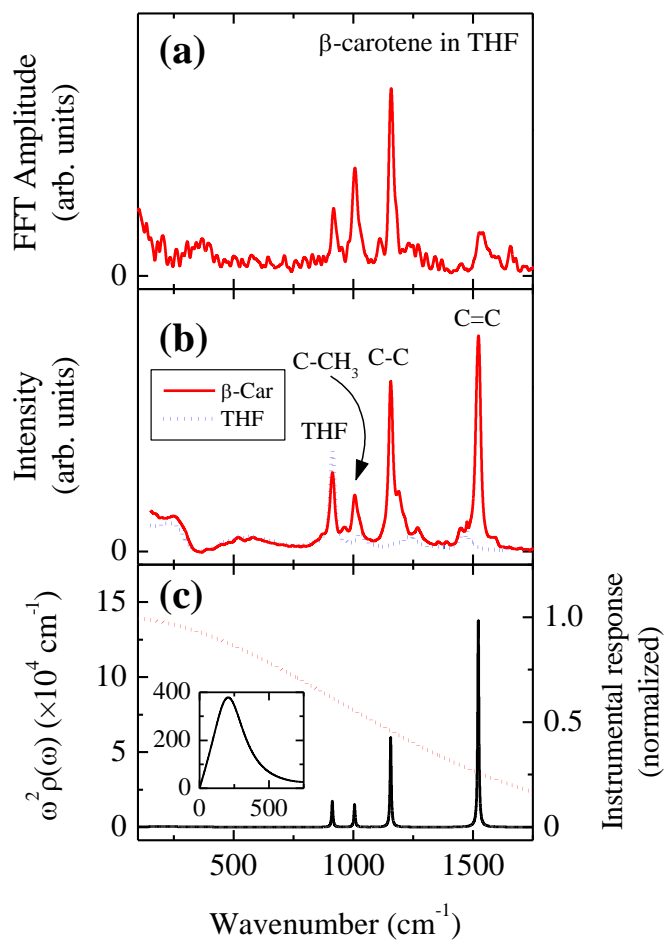




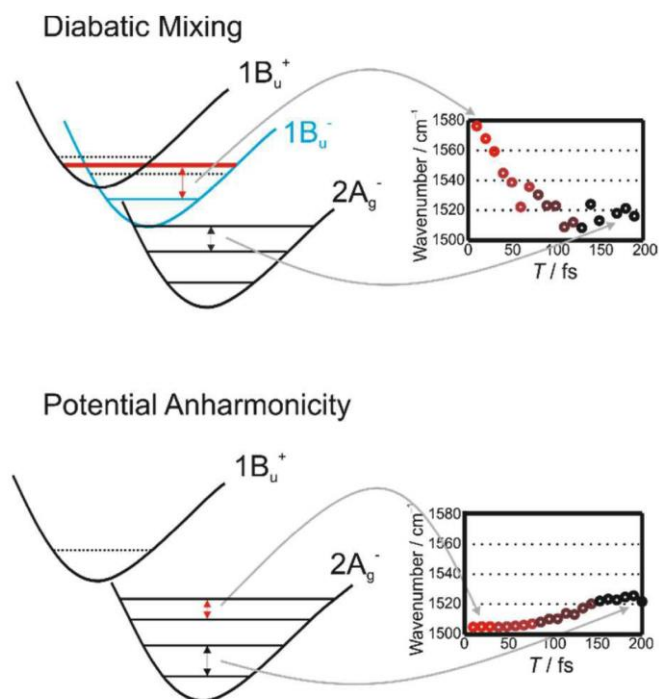
**Figure 8.** (a) Optical configuration of the interferometer used for the measurements of four-wave mixing (FWM) signals. The light from a non-collinearly phase-matched optical parametric amplifier (NOPA) is split into three using two pellicle mirrors (PM). Three laser pulses thus produced are then 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  $\tau$  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  $\beta$ -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 ( $\beta$ -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).